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Poster

184. Regeneration in the PNS and the CNS

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 184.01

Topic: A.04. Transplantation and Regeneration

Support: The General Research Fund Grant from the Research Grant Council of the Hong Kong Special Administrative Region Government (Ref. No.: 11101621).
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Title: A bioactive small molecule promotes axon regeneration and functional recovery after peripheral nerve injuries

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Abstract: Proximal peripheral nerve injuries (PNIs) require long-distance axon regeneration for target reinnervation and motor functional recovery. Although mature peripheral neurons can slowly regenerate their damaged axons after injuries, they often failed to form functional synapses at the motor end plates after chronic denervation resulting in incomplete motor functional recovery even after immediate surgical repairs. In the past decade, much effort has been attributed to the understanding of the molecular machinery required for successful axon regeneration after injuries. Many regeneration-associated genes (RAGs) have been identified to play an indispensable role in axon regeneration. Among these RAGs, co-ablation of PTEN and SOCS3 in injured retinal ganglion cells (RGCs) is known to induce sustained and long-distance axon regeneration after optic nerve crush injuries. Although viral-based gene delivery systems underwent rapid development in the recent years as a potential treatment option for various neurodegenerative diseases, silencing tumor suppressor genes like PTEN and SOCS3 might possess undesirable effects on tumorigenicity which limited their therapeutic use in clinical practice. Therefore, the current study aimed to identify bioactive small molecules that induced robust axon regeneration and functional recovery after nervous system injuries. We first identified differentially expressed genes in PTEN and SOCC3 co-deleted RGCs from a publicly available microarray dataset, and used this gene expression profile signature to query a drug-associated gene expression profile database LINCS for *in silico* screening of bioactive small molecules. Using a pattern-matching algorithm, 4 bioactive small molecules with high connectivity scores were shortlisted for functional validation using *in vitro* cultures of axotomized dorsal root ganglion (DRG) neurons. Of these, one small molecule was found potent in promoting neurite outgrowth from *in vitro* cultured DRG neurons, and *in vivo* axon regeneration in a mouse model of PNI. Mice treated with the small molecule showed early restorations in both sensory and motor function after sciatic nerve crush injuries. These mice also demonstrated significantly larger compound muscle action potential (CMAP) amplitudes in

proximal gastrocnemius and distal interosseous muscle compared with the vehicle-treated controls. Taken together, our study highlighted the therapeutic potential of this small molecule in treating patients with proximal PNIs. Further investigation is required to assess whether this small molecule could also promote axon regeneration after CNS injuries.

Disclosures: **N.P.B. Au:** None. **T. Wu:** None. **X. Wang:** None. **C.H.E. Ma:** None.

Poster

184. Regeneration in the PNS and the CNS

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Program #/Poster #: 184.02

Topic: A.04. Transplantation and Regeneration

Support: CIHR

Title: Conditioning electrical stimulation coupled with post-operative exercise improves nerve regeneration, plasticity and bone mineral density

Authors: *J.-L. SENGER[1], K. N. RABEY[1], K. CHAN[2], S. DUIA[1], C. A. WEBBER[3]; [2]Physical medicine and rehabilitation, [3]Div. of Anat., [1]Univ. of Alberta, Edmonton, AB, Canada

Abstract: Distal nerve transfers (DNTs) are a popular reconstructive technique for peripheral nerve injuries. In these surgery, the distal motor targets of an injured nerve are reinnervated by a redundant branch of a nearby functioning nerve. A significant challenge that inhibits full recovery following a DNT, particularly in the lower limb where synergistic transfers are not possible, is cortical relearning. As DNTs require the donor nerve to innervate new targets, synaptic plasticity is integral to success. Postoperative exercise therapy improves plasticity and enhances regeneration following a nerve injury; however, its effects have never been evaluated in DNTs. We have recently shown that conditioning electrical stimulation (CES) delivered to the donor tibial nerve one week prior to DNT significantly improves reinnervation of the tibialis anterior muscle and normalization of gait kinetics and kinematics however full range of motion is never regained, and the associated bones show a reduction of mineralization, making them susceptible to future fractures. To optimize the chances of full recovery, actions targeting the surrounding muscles and bones are needed. Postoperative exercise therapy improves plasticity and enhances regeneration following a nerve injury; however, its effects have never been evaluated in DNTs. Our data (n=4/cohort) demonstrates that compared to DNT alone, CES, and Ex animals accelerate nerve regeneration, increased NMJ innervation, tibialis anterior muscle weight, compound muscle action potential, and foot dorsiflexion. Exercise animals had increased BDNF and Vglut1 expression at the spinal cord suggesting plasticity at the ventral horn. Functionally, the CES+exercise animals had superior kinetic and kinematic gait analysis compared to CES or exercise alone. Importantly, however, micro-CT analysis revealed the tibial bone mineral density recovered in the Ex animal cohorts only. Conclusions: Conditioning electrical stimulation coupled with post-operative exercise on lower limb distal nerve transfers

further accelerates nerve regeneration and functional recovery including muscle reinnervation, gait, bone mineral density and synaptic plasticity at the spinal cord.

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Poster

184. Regeneration in the PNS and the CNS

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Topic: A.04. Transplantation and Regeneration

Support: Basic Science Research (2019R1I1A2A01060115) through the National research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning
Korea Health Technology R&D Project (HI21C0572) through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare

Title: Peripheral nerve implantation to the targeted muscle provides successful functional recovery by reinnervation to denervated muscle

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Abstract: Traumatic injury of peripheral nervous system (PNS) causes neuropathic pain and denervation of target muscles, inevitably resulting in morbidity of sensory and motor functions. The formation of neuroma resulting from absence of proper target for axonal regeneration inhibits appropriate functional restoration and provides as a primary onset of pain condition. Because they cannot make spontaneous recovery without surgical intervention, a variety of surgical attempts to prevent neuroma formation and provide environments for axon sprouts have been tried. However, it has still limited clear improvement in successful functional recovery and versatile application because of reformed neuroma. In this study, we investigated the effects of peripheral nerve transfer to the targeted muscle 1) to prevent neuroma-induced neuropathic pain and 2) to restore motor after re-innervation to the target muscle. The tibial nerve was transected and transferred to the adjacent part of gastrocnemius muscle at the different time intervals after denervation (immediately or 12 days) in rats. Behavioral test for mechanical sensitivity was quantified by using paw withdrawal threshold (PWT) test and tibial nerve function index (TFI) was analyzed for confirming change of paw deformity during 8 months after nerve implantation. The expression of CGRP from dorsal part of spinal cord to compare sensitized pain transmission of CNS was analyzed. Morphological and functional formation of neuromuscular junction (NMJ)

was investigated by labeling acetylcholine receptor (AChR) at 8 months after surgery, and electromyography (EMG) was performed at the targeted muscle at 8~12 weeks after surgery. After nerve implantation, PWT was significantly increased, and muscle atrophy was less progressed in all the nerve implantation group regardless of denervation time interval. Expression of CGRP showed no significant difference between nerve implantation and normal group, while nerve injury only group was significantly increased. The structure of AChR cluster was clearly observed from targeted muscle and the results of EMG showed the contraction of targeted muscle when provide electrical stimulation to the implanted nerve. The present results suggest that implantation of injured peripheral nerve to target muscle have effects on prevention of traumatic neuroma-related neuropathic pain and reinnervation following neuromuscular reconstruction. These sensory and functional outcomes may provide preclinical evidence for clinical application of surgical reconstruction for peripheral nerve regeneration and reinnervation.

Disclosures: **J. Kwon:** None. **J. Kim:** None.

Poster

184. Regeneration in the PNS and the CNS

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Program #/Poster #: 184.04

Topic: A.04. Transplantation and Regeneration

Support: NIDCR R03 DE028637
NIDCR R56 DE029816

Title: C5l2 sirna silencing modulates bdnf production in dental pulp stem cells via p38α pathway

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Abstract: The complement system gets activated during inflammatory conditions and has been reported to have crucial roles in various tissue regeneration processes. We have recently demonstrated that the complement C5a receptor (C5aR) is involved in tissue regeneration and healing process through various pathways including nerve sprouting and hard tissue regeneration. Another C5a-like 2 receptor (C5L2) has been cloned which is still considered controversial due to limited studies. Previously, we established that C5L2 regulates brain derived neurotropic factor (BDNF) secretion in pulp fibroblasts. However, there is no study available on human dental pulp stem cells (DPSCs), especially in the inflammatory context. Stem cells therapy is incipient technique to treat and prevent several diseases, while DPSCs are an emerging option to be considered due to their great ability to differentiate into a variety of cells and secrete nerve regeneration factors. Here, we demonstrated that C5L2 modulates BDNF secretion in DPSCs. Our results stated that C5L2 silencing through siRNA can increase the BDNF production which could accelerate the nerve regeneration process. Moreover, stimulation with

lipopolysaccharide (LPS) enhanced BDNF production in C5L2 silenced DPSCs. Finally, we quantified BDNF secretion in supernatant and cell lysates using ELISA. Our results showed enhanced BDNF production in C5L2 silenced DPSCs and hampered by the $p38^{MAPK}\alpha$ inhibitor. Taken together, our data reveal that C5L2 modulates BDNF production in DPSCs via the $p38^{MAPK}\alpha$ pathway.
Key words: BDNF, complement system, C5L2, Nerve regeneration, $p38^{MAPK}\alpha$
Conflict of interest: None
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Disclosures: M. Irfan: None. **S. Chung:** None.

Poster

184. Regeneration in the PNS and the CNS

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Topic: A.04. Transplantation and Regeneration

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Title: Self-renewing macrophages in dorsal root ganglia contribute to promote nerve regeneration

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Abstract: Sensory neurons located in dorsal root ganglia (DRG) convey sensory information from peripheral tissue to the brain. After peripheral nerve injury, macrophages derived from circulating bone-marrow-derived precursors accumulate at the site of nerve injury and promote axon regeneration. An increase in macrophages in the DRG (DRGMacs) also occurs in response to nerve injury. However, only a small population of circulating bone-marrow-derived precursors are present in the DRG after nerve injury. Hence, the source and function of DRGMacs after peripheral nerve injury remains unclear. Here we mapped the fate and response of DRGMacs to nerve injury using macrophage depletion, fate-mapping and single-cell transcriptomics. In addition to a small population of circulating bone-marrow-derived precursors, we identified three other subtypes of DRGMacs after nerve injury. Self-renewing macrophages, proliferating from local resident macrophages represent the largest population of DRGMacs. The other two subtypes include microglia-like cells and macrophage-like satellite glial cells. The self-renewing macrophage population contributes to promote axon regeneration. These data uncover DRGMacs features that distinguish them from nerve macrophages and highlight that nerve injury induces

macrophage local proliferation in DRG. The heterogeneity of DRGMacs in response to nerve injury may inform future therapeutic approaches to treat nerve injury.

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Poster

184. Regeneration in the PNS and the CNS

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Topic: A.04. Transplantation and Regeneration

Support: CIHR gran MOP 142238
U of S COMRAD grant
U of S CGPS

Title: Acute Intermittent Hypoxia promotes repair in a novel precise peripheral nerve compression/decompression model

Authors: *W. A. MUSTAFA[1], E. G. N. OLSON[2], G. D. MUIR[3], V. M. VERGE[4];
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Abstract: Peripheral nerve injuries are frequent and when severe can lead to loss of function and debilitating pain states. Past clinical evidence has shown direct median nerve electrical stimulation (ES) improves functional recovery in severe carpal tunnel syndrome (sCTS), but this approach is invasive. More recently, we have shown a novel non-invasive approach called acute intermittent hypoxia (AIH) promotes regeneration of completely transected and co-apted rat peripheral nerves in a manner akin to ES. To directly compare AIH treatment to past clinical findings in a common clinical model like sCTS model and assess the repair potential that AIH treatment could hold for compression injuries, we developed a highly reproducible and precise nerve compression and decompression preclinical model. Nerve compression injuries were performed (adapted from Chen et al., 2020 J Neurosci Meth 335:108615) using mechanical force gauge sensor linked to a data acquisition system, allowing reproducible and consistent unilateral compression injuries in adult male Lewis rats delivering 14 grams of force on each of a series of 4 constriction sites over a distance of 3mm. Preliminary data supports that either a single AIH treatment (10 cycles of 5 minutes 11% O2 alternating with 5 minutes 21% O2) 7d prior to decompression and/or daily AIH treatments for 7d post-decompression effectively conditions/promotes compressed nerves to mount a heightened expression of regeneration-associated genes and regenerate significantly longer distances than Normoxia controls when assessed 7 days post-decompression. Furthermore, our compression/decompression model induces a hyposensitivity (sCTS symptom), which improved in animals given cAIH treatment evident as early as just a few days post decompression. Collectively this supports that AIH, as a

novel non-invasive therapy, can be used to effectively support improved nerve repair outcomes following nerve decompression.

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Poster

184. Regeneration in the PNS and the CNS

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Title: Integrated stress response-mediated transcription negatively regulates adult sensory axon regeneration

Authors: *M. RUDY, P. SOMASUNDARAM, T. SISROE, T. WATKINS;
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Abstract: Injury to peripheral nerves stimulates MAP kinase stress signaling, leading to a potent pro-regenerative transcriptional response that can result in significant functional recovery. However, axon regeneration is frequently inadequate and slows with age-related reduction of environmental support. Here we provide evidence that a second branch of axonal injury signaling, the Integrated Stress Response (ISR), may limit adult sensory axon regeneration, especially in growth-restrictive settings. An siRNA screen performed on adult dorsal root ganglion (DRG) neurons *in vitro* identified Activating transcription factor-4 (Atf4), a prominent mediator of the ISR, as a negative regulator of regeneration. The promotion of axon growth upon knockdown of Atf4 could be mimicked with siRNA targeting the endoplasmic reticulum (ER) stress-responsive kinase Perk, but not siRNA against other ISR-activating kinases. To elucidate the role of Atf4 in the transcriptional regenerative response, we performed bulk RNA sequencing on cultures enriched for adult sensory neurons derived from Atf4 conditional knock-out mice. The data suggest neuronal Atf4 is a substantial contributor to the injury-induced transcriptional program, with Atf4 knockout resulting in over one thousand differentially expressed genes. However, the impact of Atf4 knockout on prominent regeneration-associated genes, such as *Sprr1a, Atf3,* and *Klf6* is modest, suggesting Atf4 does not restrict regenerative capacity by direct repression of pro-regenerative response genes. Additional gene expression profiling of siRNA-treated cultures was used to further understand how the ISR contributes to the transcriptional response. Leveraging these two RNA sequencing approaches in parallel enables stratification of Atf4-dependent genes into groups that are: (1) dependent upon Perk, and (2) dependent upon the CCAAT/enhancer binding protein-gamma (C/ebpγ), a likely heterodimeric partner of Atf4. The

data suggest a model in which injury-induced Perk, activated in parallel with pro-regenerative MAPK stress signaling, limits regeneration through upregulation of Atf4, which regulates downstream targets through its interaction with C/ebpγ and through other dimeric partners. Taken together, these data reveal a previously unappreciated role for the ISR in the transcriptional response to peripheral axon injury and raise the possibility that modulating this branch of the response could enhance nerve repair.

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Poster

184. Regeneration in the PNS and the CNS

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Program #/Poster #: 184.08

Topic: A.04. Transplantation and Regeneration

Support: NIH/NINDS R21NS072955-01A1

Title: Neuregulin 1 Enhances Remyelination adn Functional Recovery in Critical Gap injuries

Authors: *X. GU[1], F. RAHMAN[1], G. BENDALE[2], B. TRAN[2], J. MIYATA[3], M. ROMERO-ORTEGA[1];
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Abstract: Introduction: The treatment of nerve gap injury longer than 30 mm using artifical nerve conduits remains a significant challenge. Our previous study has shown successful nerve regeneration across a 40 mm gap using multiluminal nerve conduits containing glial-derived neurotrophic factor (GDNF) and pleiotrophin (PTN). While this combination improved axon regeneration and functional recovery, abnormal axonal sorting and poor remyelination of the regenerated axons were observed. Neuregulin 1 type III (NRG1) plays a significant role in Schwann axon myelination during development and after injury: binding of NRG1 to the ErbB2/3 receptors instructs the conversion of Schwann cell precursors into pro-myelinating or Remak type. We propose that NRG1 combined with PTN, will provide successful re-myelination of regenerated nerves across critical gaps. **Methods:** Growth factor encapsulated microparticles (MPs) were first tested *in vitro* using dorsal root ganglia (DRG) explant culture. *In vivo* investigation was conducted on 24 adult female New Zealand White rabbits using a peroneal nerve injury model. The toe-spread reflex of the animals was recorded 5-28 weeks post-injury as a measure of functional recovery. After 28 weeks, the animals underwent a terminal surgery for electrophysiological and muscle force measurements. Tissue samples were collected for electron microscopy and histology. **Results and Conclusion:** NRG1 and NRG1+PTN MPs showed increased axon number and length in DRG culture assy. The toe-spread reflex experiment showed that the growth factor groups significantly improved functional recovery compared with the negative control with a final toe-spread index (TSI) >0.6. Electrophysiological results from

the affected muscle showed that PTN+NRG1 increase the CMAP to 353 μV and maximum evoked muscle response to 0.0.3036 N, with both growth factor groups having better results than the empty control. Histological analysis of the distal end of the peroneal nerve also showed the growth factor groups had higher numbers of myelinated axons and lower G-ratio. We also observed an increased number of $Sox10^+$ Schwann cells in the middle segment of the conduits containing NRG1. Together, these results demonstrated an improvement in the anatomical and functional regeneration of long gap nerve injury using NRG1 containing nerve conduits and supports the notion of a combination of growth-promoting and re-myelinating factors for optimizing the repair of critical nerve gap injuries using off-the shelf alternatives.

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Poster

184. Regeneration in the PNS and the CNS

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Topic: A.04. Transplantation and Regeneration

Support: Canadian Institutes of Health Research Grant 409489

Title: Outcomes using a Novel Method to Quantify Cross-over Innervation in Nerve Transfer Surgery — A Prospective Multicentre Non-randomized Cohort Study.

Authors: *S. WU[1], M. CURRAN[2], J. OLSON[2], M. MORHART[2], R. MIDHA[3], M. BERGER[4], M. CHEUK[1], K. CHAN[1];
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Abstract: ***INTRODUCTION:*** Peripheral nerve injuries are common. Unfortunately, severe high ulnar nerves injuries carry poor outcomes even after surgery. Principle reasons being the significant distance the ulnar nerve must regenerate to reach its target. End-to-end (ETE) nerve transfers by coapting the anterior interosseous nerve (AIN) to the ulnar nerve can markedly shorten the distance. Though it produces better outcomes compared to nerve reconstruction, transection of the ulnar nerve presents a dilemma in incomplete injuries, as it ablates the possibility of native nerve regeneration. To circumvent this, reverse end-to-side (RETS) transfer by coapting the AIN to the side of the ulnar nerve close to their end target would still preserve the path for the native nerve fibers to regenerate. However, whether RETS transfer would indeed enable axons from the AIN to grow down the ulnar nerve has not been tested in humans.
The purpose of this study was to (1) determine the source of regenerating axons among nerve transfers using a novel technique, and (2) compare the outcomes of high ulnar nerve patients after AIN RETS transfer to AIN ETE or nerve decompression alone.

METHODS: Patients with severe high ulnar nerve injury were divided into 3 groups: RETS, ETE or decompression at the elbow. All had nerve compression at the elbow except 10 patients in the ETE group who had high ulnar nerve laceration or traction injury. In the nerve transfer groups, decompression was also performed at Guyon's canal. Novel electrophysiology measures were used to quantify the regeneration of AIN and ulnar nerve fibres while functional recovery was evaluated using key pinch strength and von Frey filaments. The patients were followed post-surgically at regular intervals for a minimum of 2 years.
RESULTS: Sixty-two patients (RETS=25; ETE=16; decompression=21) from four centres in Western Canada were enrolled. Post-surgically, no axonal growth from the AIN to the abductor digiti minimi muscles was seen in any of the RETS patients while significant regrowth was found in all ETE patients with corresponding functional improvement. While there was no significant improvement in CMAP amplitudes in the decompression and RETS group, key pinch strength significantly improved in the RETS group ($p<0.05$) but not in the decompression group.
CONCLUSION: All reinnervation that occurs in the RETS occurs through the ulnar nerve. ETE nerve transfers demonstrated better electrophysiologic and sensory recovery compared to RETS and decompression. However, both the RETS and ETE group demonstrated significantly improved key pinch.

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Poster

184. Regeneration in the PNS and the CNS

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Program #/Poster #: 184.10

Topic: A.04. Transplantation and Regeneration

Title: Lesion conditioning requires CREB and is partially DLK-independent across several neurons in C. elegans

Authors: *N. W. F. GROOMS, A. CHOW, C. MA, N. PATEL, S. H. K. CHUNG; Bioengineering, Northeastern Univ., Boston, MA

Abstract: Despite recent progress, the genetic and cellular mechanisms functionally underlying neuronal regeneration remain incompletely defined. Even with the census of neuronal types and mounting evidence for cell-type dependency of regeneration, relatively few neuronal types have been tested for regeneration. Also, it is often unclear whether single-lesion "conventional" pathways or two-lesion "conditioned" pathways are driving regeneration. Our lab established a regeneration model in the roundworm *C. elegans* to address the issues above. *C. elegans* is a powerful *in vivo* model for studying neuron regeneration because it shares numerous conserved processes with mammals and is genetically amenable. Dual-leucine zipper kinase, *dlk-1*/DLK, functionally underlies conventional regeneration in *C. elegans* and mice. In conditioned regeneration, the central axon regenerates following injury if there is a conditioning lesion of the

peripheral sensory axon. We assess the role of cAMP response element binding protein, *crh-1*/CREB, in conditioned regeneration because cAMP has long been shown to drive conditioning. We test conventional and conditioned regeneration in sensory, inter-, and motor neurons. To test conventional regeneration, we cut the axon once. For conditioned regeneration, we cut the axon and dendrite concomitantly (bipolar) or the axon twice, 24 hours apart (unipolar). The conditioning effect is the difference between regeneration after one cut and regeneration after two cuts. We assess regeneration in wild-type, *dlk-1*, *crh-1*, and double-mutant *dlk-1; crh-1* ($n \geq 20$ animals for each condition). Mutation of *dlk-1* reduces conventional regeneration by 100%, 67%, and 37% in the ASJ, PVQ, and HSN neurons, respectively. Mutation of *crh-1* slightly lowers regeneration after axotomy suggesting a minor role of *crh-1* in conventional regeneration. Conditioned regeneration is slightly reduced in *dlk-1* indicating a small role of DLK in conditioning. Most importantly, loss of CREB eliminates the conditioning effect in 5 of 6 cases, independently of DLK. Our study establishes *dlk-1* and *crh-1* as partially independent, primary drivers of conventional and conditioned regeneration, respectively. Our results define a baseline capacity for regeneration of several neurons. Prior studies demonstrated that overactivated CREB enhances regeneration, but we show that conditioning functionally requires *crh-1*. Our future studies will target known molecules in the well-defined CREB pathways. Eventually, we can correlate our regeneration data with CeNGEN data on neuron-specific gene expression in *C. elegans* to identify candidate genes to assay in regeneration.

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Poster

184. Regeneration in the PNS and the CNS

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Program #/Poster #: 184.11

Topic: A.04. Transplantation and Regeneration

Support: NIH/NINDS Grant NS171785 (to S.C.B.)

Title: Host brain environment influences on survival and migratory patterns of transplanted medial ganglionic eminence (MGE) progenitor cells

Authors: *S. C. BARABAN, T. VU, C. HSIEH, R. PATERNO;
Univ. California San Francisco, San Francisco, CA

Abstract: GABA progenitor cell transplantation has the potential to treat neurological diseases caused by altered inhibitory neurotransmission and/or loss of GABAergic interneurons. To be effective, transplantation should achieve certain minimally necessary conditions; in particular, transplant-derived neurons must survive after transplantation, migrate to the affected host brain region, and integrate into existing host circuitry. To that end, embryonic medial ganglionic eminence (MGE) progenitors are an ideal candidate for generation of GABAergic interneurons

following transplantation. More than a decade of previous work from our laboratory has shown that embryonic MGE progenitors migrate widely and integrate into host circuits following transplantation (Alvarez-Dolado et al. 2006; Hunt et al. 2013; Sebe et al. 2014; Howard and Baraban, 2016; Casalia et al. 2021). However, MGE survival and migration can be variable across a variety of published transplantation studies. Potential cause(s) for this variability include: (i) age of the host brain and (ii) location in the host brain where progenitors are transplanted. To systematically examine these possibilities, we transplanted murine embryonic MGE GFP-expressing progenitors into mice at different stages of development (early postnatal, juvenile, or adult) and different brain locations (cortex or hippocampus). Sacrificing recipient animals at 30 days after transplantation we used double-and triple-label immunofluorescence with confocal microscopy to assess survival, migration and maturation profiles. MGE-GFP derived cells expressed GABA, parvalbumin, somatostatin and nNOS as expected. We noted higher survival percentage (15% vs. ~2%) and greater migratory ability (3 mm vs. 0.9 mm) for MGE transplantation into early postnatal compared to juvenile or adult brain (in cortex and hippocampus). Host brain region also influenced the maturation profile of MGE-derived cells and the migratory pathways used by these cells following transplantation. MGE progenitors transplanted in hippocampus showed a unique migration route depending upon hippocampal subregion where the cells were deposited. Taken together, our data suggest that host brain environment plays a major role in determining the effectiveness of MGE progenitor cell transplantation.

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Poster

184. Regeneration in the PNS and the CNS

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Program #/Poster #: 184.12

Topic: A.04. Transplantation and Regeneration

Title: Matrix metalloproteinase-II in a larval zebrafish model of retinal regeneration

Authors: B. CARTER[1,2], L. BATIS[3], J. WILKINSON[3], P. I. GARRETT[7], K. FRIEDEL[3], J. J. SAAVEDRA[1,2], K. PAYNE[4,2], B. TONKS[5], J. B. HUTCHINS[6,2], ***E. J. SANDQUIST**[3,2]; [1]Psychology, [2]Neurosci., [3]Zoology, [4]Microbiology, [5]Athletic Training, [6]Hlth. Sci., Weber State Univ., Ogden, UT; [7]Univ. of Wyoming, Laramie, WY

Abstract: Loss of sight is a major handicap that can play a large role in the overall satisfaction of life. Diseases such as retinitis pigmentosa, macular degeneration, and diabetic retinopathy are incurable at present. Further, age-related diseases of the retina are expected to increase as a large portion of the United States population grows older. Zebrafish are a current model for research of retinal regeneration. In contrast to mammals, zebrafish Muller glia fully regenerate the retina using stem cells. Many scientists have attempted to replicate this regenerative response in mammals by transplanting stem cells to the damaged retina. Stem cell transplants have shown

some success, but must increase in efficiency for future use in the general population. Currently, it is difficult to predict if and why transplanted stem cells survive, travel to the affected site, and mature into the correct cell type. Further, disease-induced scar tissue may act as an obstacle to transplanted cells as they migrate to their target destination. Mechanisms that zebrafish use for retinal regeneration may be applied to stem cell therapies. Matrix metalloproteinase-II (MMP2) is upregulated after photolesion in adult zebrafish. This enzyme may be secreted by cells and alter the surrounding matrix to promote migration. Matrix metalloproteinase-II is positively correlated to regeneration of damaged retinal tissue in mice. We hypothesized that MMP2 is released by migrating stem cells as a way to clear the environment on their way to their target destination in the zebrafish retina. Live imaging is essential to better understand how individual cells interact with their environment. Therefore, we decided to use larval zebrafish to enable these experiments in the future. Quantitative reverse transcription polymerase chain reactions (RT-qPCR) were performed to confirm increased MMP2 expression in the larval zebrafish retina following a light lesion. We also used immunohistochemistry to determine whether Muller glia are the source of stem cells in the larval retina. Our results concur with past literature demonstrating an upregulation in MMP2 in the adult zebrafish retina, as well as proliferation of Muller glia post-injury. Future experiments will use larval zebrafish to perform live imaging of the regeneration process. Time course analysis will allow us to map the route that stem cells travel to their destination and to determine how MMP2 inhibition affects stem cell migration. By understanding the role that MMP2 plays in retinal regeneration, as well as the mechanisms of stem cell migration, we hope to increase the success and efficacy of future transplantation therapies for diseases of the retina.

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Poster

184. Regeneration in the PNS and the CNS

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 184.13

Topic: A.04. Transplantation and Regeneration

Support: Research Enhanced Program 2020-2022

Title: Building cortical grafts from induced pluripotent derived stem cells for traumatic brain injuries

Authors: *M. GLADEN, Z. LYBRAND;
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Abstract: Building cortical grafts from induced pluripotent derived stem cells for traumatic brain injuries

Authors **Myles Gladen[1]**, Zane R. Lybrand[1] [1]Texas Woman's University, Division of Biology, Denton, Texas, 46204
Traumatic brain injury (TBI) is a public health crisis, that is caused by compounding physical forces exerted on brain tissue that can lead to cell death and permanent damage to the brain. The loss of brain tissue results in long-term deficits in cognitive, motor, and emotional abilities. Current potential regenerative strategies include direct stem cell replacement therapy, or cellular transdifferentiation with the goal to replace lost neurons. However, these do not sufficiently replace all essential cell types found in the cortical niche. To achieve a complete or near full recovery, strategies for complete neurological niche replacement must be developed. The goal of this study is to develop *in vitro* cortical grafts capable of integrating into host brain tissue following TBI. To accomplish this goal, we used human-induced pluripotent stem cells to grow cortical grafts using a directed 3D organoid protocol. These grafts replicate developing cortical architecture with essential cellular populations. Male NOD SCID mice (n=4-5/group) were randomly placed into either TBI + graft, TBI + no graft, 'sham' + graft, 'sham'+ no graft, or sham. TBI was administered using a controlled cortical impactor and targeted the motor cortex. The 'sham' groups did not receive impact, instead had a needle excision of brain tissue using an 18-gauge needle to replicate surgical tissue removal. The sham group received craniotomy but not needle excision. Following surgical procedures, mice were monitored daily using a modified neurological severity score to evaluate changes in motor, sensory, balance, and reflex functions. Additional motor-specific tests were performed to evaluate left vs right forelimb impairment. By 1 week following injury, mice that received TBI showed consistent deficits in motor function of contralateral arm from injury site compared to mice that received 'sham' or sham procedures. After one month from transplantation, mice were perfused, and graft integration was mapped by stereology from serial histological sections. This is an exploratory experiment in which behavioral data will be correlated to mapping of graft integration to the injury site. Information regarding specific injury types (i.e. TBI vs 'sham') will inform potential limitations to improved cortical graft success.

Disclosures: M. Gladen: None. **Z. Lybrand:** None.

Poster

184. Regeneration in the PNS and the CNS

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 184.14

Topic: A.04. Transplantation and Regeneration

Support: National Institutes of Health (NIH) - Grant R01-EY029739, to E.F.T.

Title: Developmentally regulated microRNAs play a role in retinal ganglion cell survival and axon regeneration after optic nerve injury

Authors: *A. LUKOMSKA, J. XING, M. FROST, W. THEUNE, A. DAMANIA, M. GUPTA, E. F. TRAKHTENBERG;
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Abstract: Retinal ganglion cells (RGCs) are central nervous system (CNS) projection neurons that do not spontaneously regenerate axons disrupted by optic neuropathies, such as those resulting from optic nerve trauma, ischemia, and glaucoma. RGC capacity to grow long axons declines sharply after birth, and several factors that are developmentally regulated in RGCs were discovered to contribute to the regenerative failure. No clinical treatments exist to date that could help patients with axonal injuries. Thus, the failure of RGC and other CNS axons to regenerate after injury remains a major unmet problem. Here, we investigated the roles of developmentally regulated microRNAs (miRNAs) in RGC survival and axon regeneration after optic nerve injury, using a well-established murine *in vivo* model of optic nerve crush (ONC). We used bioinformatic analysis of small-RNA-seq data, which we generated for the developing RGCs at various ages, in order to identify miRNAs that are developmentally regulated in maturing RGCs. Then, we pre-treated the RGCs with intraocularly injected AAV2 vectors, which expressed either miRNA mimics or anti-miRNA shRNAs. Next, we performed ONC and evaluated the effects on RGC survival and axon regeneration. We found several novel miRNA targets, which either promoted or inhibited RGC survival and axon regeneration. Thus, the identified developmentally regulated miRNAs play a role in RGC survival and axon growth, and present potential therapeutic targets for treating optic neuropathies and glaucoma, as well as axonal injuries in other white matter tracts of the CNS.

Disclosures: A. Lukomska: None. **J. Xing:** None. **M. Frost:** None. **W. Theune:** None. **A. Damania:** None. **M. Gupta:** None. **E.F. Trakhtenberg:** None.

Poster

184. Regeneration in the PNS and the CNS

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Program #/Poster #: 184.15

Topic: A.04. Transplantation and Regeneration

Support: The Miriam and Sheldon G. Adelson Medical Research Foundation (LB),
NEI R01EY05690 (LB),
HSCI grant on Vision Repair and Regeneration (YY),
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CZ.02.1.01/0.0./0.0/15_003/0000419 (JWF).

Title: Gene complementation in optic nerve regeneration

Authors: *Y. YIN[1], H.-Y. GILBERT[1], V. PETROVA[2], R. EVA[2], J. W. FAWCETT[2], L. I. BENOWITZ[1,3,4];
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Abstract: Although the optic nerve, like other CNS pathways, does not regenerate spontaneouslyif injured, a variety of treatments enable retinal ganglion cells (RGCs), the projection neurons ofthe retina, to regenerate axons through the injured optic nerve. Transcriptional profiling hasrevealed that one of the strongest such treatments, a combination of the neutrophil/macrophage-derived growth factor oncomodulin (Ocm) with a cAMP analog (CPT-cAMP) and deletion of thePten gene in RGCs (Ocm/cAMP/Pten^{del}), induces massive changes in RGCs' program of geneexpression[1] but does not alter expression of Protrudin, a transmembrane protein of theendoplasmic reticulum that also promotes considerable optic nerve regeneration[2]. These findingsraise the question of whether complementing the transcriptional changes induced byOcm/cAMP/Pten^{del} with a constitutively active phospho-mimic of protrudin (P-Pro) could augmentregeneration well beyond levels induced by either approach alone. With all treatments appliedafter nerve injury, optic nerve regeneration from the combination of P-Pro and Ocm/cAMP/Pten^{del}was 5-fold greater than with P-Pro expression alone, 3-fold greater than Pten deletion alone, andalmost 2-fold greater than from the combination of Ocm/cAMP/Pten^{del}. Unlike Pten deletion, whichprimarily enables regeneration from αRGCs[3], P-Pro increased regeneration primarily from non-αRGCs. Strikingly, however, the dramatic regeneration seen after combining P-Pro withOcm/cAMP/Pten^{del} retains high levels of αRGC regeneration while also greatly augmentingregeneration from non-αRGCs. Thus, this combinatorial treatment stimulates extensiveregeneration from multiple RGC subclasses, an outcome that might eventually enable thereinnervation of multiple central target areas and recovery of multiple aspects of vision. **References:** 1. Cheng, Y. Yin, Y. *et al.* Transcription factor network analysis identifies REST/NRSF asan intrinsic regulator of CNS regeneration. *Nature Communications (under reviewing)*(2022). 2. Petrova, V. *et al.* Protrudin functions from the endoplasmic reticulum to support axonregeneration in the adult CNS. *Nat Commun* **11**, 5614, doi:10.1038/s41467-020-19436-y(2020). 3. Duan, X. *et al.* Subtype-specific regeneration of retinal ganglion cells following axotomy:effects of osteopontin and mTOR signaling. *Neuron* **85**, 1244-1256,doi:10.1016/j.neuron.2015.02.017 (2015).

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Poster

184. Regeneration in the PNS and the CNS

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 184.16

Topic: A.04. Transplantation and Regeneration

Title: Emergence of task related complex spatiotemporal population dynamics in transplanted neurons

Authors: *H. GHUMAN, K. KIM, S. BARATI, K. GANGULY;
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Abstract: Neurological impairments caused by acute brain damage due to stroke or traumatic brain injury are accompanied by loss of neurons and connections. Unlike neurogenesis in rodents, the endogenous capacity of the human brain to regenerate neural tissue is very limited. As a treatment strategy, cell transplantation has been extensively evaluated to compensate for the lost tissue. During development, immature network activity is dominated by recurrent patterns of synchronized “burst” activity. Gradually, the network desynchronizes and evolves into a less correlated state. Similar persistence of burst activity is not only characteristic of cultured neurons but have also been shown in transplanted cells. Transplanted neurons have been shown to survive for extended time periods *in vivo* and synaptically integrate into surrounding host network, yet it remains unclear if they can achieve normal task-related spatiotemporal dynamics that mimic healthy cortical circuits, a key feature of neural computations in motor networks. Here, we used single-cell resolution, real-time *in vivo* imaging in behaving mice to monitor cellular and network dynamics of transplanted embryonic neurons during maturation and integration into an adult post-stroke perilesional cortical network. Genetically encoded calcium indicators and epifluorescence imaging in freely moving mice allowed us to reliably track the emerging dynamics of transplanted network from an initial uncoordinated state to a highly reliable and coordinated state. Strikingly, the observed patterns of population neural activity in the transplanted network strongly resembled that of healthy cortical circuitry. One notable finding was that not all neurons developed task-related activation. Studies in awake and anesthetized animals have found that externally applied electrical fields can boost firing rates and bias spike timing. Such stimulation can also be used to monitor transplanted neuronal viability. For the population of transplanted neurons that were not movement responsive, were those neurons viable and reliably modulated with stimulation? We found that alternating current stimulation (ACS) acutely enhanced the neural dynamics of the transplanted network to increase the network co-firing or synchrony. Interestingly, most of the neurons that were not task related were still active with ACS. This suggests that only a partial subset of transplanted neurons became task active. Together, these results highlight that transplanted neurons can indeed produce task-related complex activity patterns when injected into an injured brain and that observed activity can be reliably modulated by electrical fields.

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Poster

184. Regeneration in the PNS and the CNS

Location: SDCC Halls B-H

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Program #/Poster #: 184.17

Topic: A.04. Transplantation and Regeneration

Support: PAPIIT UNAM IA200622
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PAPIIT UNAM IN227020
CONACYT CF-214971

Title: The Krüppel-like factor 13 (KLF13) regulates the JAK/STAT signaling pathway in hippocampal neurons

Authors: *J. AVILA MENDOZA, V. URBAN-SOSA, K. DELGADO-RUEDA, J. OLIVARES-HERNÁNDEZ, M. CARRANZA-SALAS, C. G. MARTÍNEZ-MORENO, M. LUNA, C. ARAMBURO;
Inst. of Neurobiology, UNAM, Querétaro, Mexico

Abstract: The Krüppel-like factors (KLFs) have emerged as important regulators of neuronal proliferation, differentiation and axon regeneration. They constitute a family of eighteen transcription factors characterized by three C-terminal C2H2 zinc finger motifs that recognize GC/GT rich sequences in DNA. Our work focused on KLF13, which is known to act predominantly as a transcriptional repressor by associating with chromatin within proximal promoters of its target genes. Recently, it has been described that KLF13 directly represses transcription of genes involved in neurotrophic factor signaling pathways in mouse hippocampal neurons, including the JAK-STAT pathway, which mediates the actions of several cytokines, growth factors and hormones. In the nervous system, it transduces extracellular signals into transcriptional programs to regulate survival, axon regeneration, synaptic plasticity and neuroinflammation. Growth hormone (GH), in part by activating the JAK-STAT pathway, has been shown to have some of these neurotrophic activities. Therefore, here we analyzed the GH-induced JAK-STAT activity in the adult mouse hippocampus-derived cell line HT22 to test the hypothesis that KLF13 cross-talks with the JAK/-STAT pathway to regulate its activity. We used our previously engineered HT22 cell lines: the TRTO-*Klf13*, in which the *Klf13* expression is induced by addition of doxycycline; and the CRISPR/Cas9 genome edited *Klf13*-KO HT22 cell line. Our results confirmed that KLF13 directly regulates the expression of several genes involved in the JAK-STAT pathway: *Stat3*, *Stat5b*, *Socs1* and *Socs3* were repressed while *Stat5a* was induced by forced expression of *Klf13*. We also found that expression of some of these genes (*Socs1*) was dysregulated in KLF13-deficient neurons. We then analyzed the effect of GH on mRNA levels of its main mediator, IGF1, and found that KLF13 depletion led to an enhanced effect of GH on *Igf1* expression. As a proxy for analyzing JAK-STAT activity, transfection-reporter assays were conducted using two sensor plasmids to track pathway activity either by STAT3 or STAT5. We found that forced expression of KLF13 increased baseline of STAT5 activity, which was enhanced by GH treatment, while in HT22-*Klf13*-KO cells the basal and GH-induced activity of STAT5 were lower compared with control. By contrast, KLF13 blocked the GH-induced activity of STAT3, while *Klf13* depletion caused an enhanced effect of GH on STAT3-mediated pathway activity. These findings support the notion that KLF13 has a bifunctional effect on the GH-induced JAK/STAT activity by enhancing or inhibiting the STAT5 or STAT3 branches, respectively.

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Poster

184. Regeneration in the PNS and the CNS

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 184.18

Topic: A.04. Transplantation and Regeneration

Support: NIH-NEI Grant R01-EY029739, to E.F.T
BrightFocus Foundation Grant G2017204, to E.F.T

Title: Post-injury born oligodendrocytes integrate into the glial scar and inhibit growth of regenerating axons by premature myelination

Authors: *J. XING, B. A. RHEAUME, J. KIM, A. LUKOMSKA, S. MUHAMMAD, A. DAMANIA, E. F. TRAKHTENBERG;
Neurosci., UConn Sch. of Med., Farmington, CT

Abstract: The failure of mature central nervous system (CNS) projection neurons to regenerate axons over long distance often results in permanent functional deficits and limits the recovery after CNS injuries and diseases. A major barrier to CNS axonal regenerative research is that the regenerative axons triggered by experimental treatments stall growth before reaching their post-synaptic targets. Here we test the hypothesis that premature, de novo, myelination of regenerating axons stalls their growth, even after bypassing the glial scar. To test this hypothesis, we used single cell RNA-seq (scRNA-seq) and immunohistological analysis to investigate whether post-injury born oligodendrocytes integrate into the glial scar after optic nerve injury. We also used a multiple sclerosis model of demyelination concurrently with the stimulation of axon regeneration by Pten knockdown (KD) in projection neurons after optic nerve injury. We found that post-injury born oligodendrocytes integrate into the glial scar, where they are susceptible to the demyelination treatment, which prevented premature myelination, and thereby enhanced Pten KD-stimulated axon regeneration. We also present a website for comparing the gene expression of scRNA-seq-profiled optic nerve oligodendrocytes under physiological and pathophysiological conditions.

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Poster

185. Autism Diagnosis, Physiology, and Biomarkers: Human Studies

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 185.01

Topic: A.07. Developmental Disorders

Support: NRF Grant 2020M3E5D9080787

Title: Tactile assimilation effect depends on individual autistic traits in healthy population

Authors: *J. JEONG, J.-H. KIM, S.-P. KIM;
Ulsan Natl. Inst. of Sci. and Technol., Ulsan national institute of science and technology, Ulsan, Korea, Republic of

Abstract: Autism spectrum disorder is a complex neurological impairment with social communication deficits and repetitive sensory-motor behaviors. The top-down modulation in the brain, which is important not only in social interactions but also in sensory processing, is atypical in autism. While many studies have explored autistic characteristics in visual and auditory perception, those in tactile perception are relatively rare with inconsistent results. Here, we hypothesized that tactile perception in autism would be atypical in complex sensory processing because of difficulties in sensory integration, mainly seen in other sensory modalities in autism. To test the hypothesis, we employed the vibrotactile frequency comparison task, one of the psychophysical methods that evoke the assimilation effect. The assimilation effect in tactile perception refers to a perceptual bias such that a vibration stimulated to one finger is confused by another vibration to a distractor finger. Forty-seven neurotypical adults (all right-handed, 23 female, age = 25.68 ± 5.04) performed the task while their electroencephalography (EEG) was measured. Autism Spectrum Quotient (AQ) was used to measure individual autistic traits. In the behavior result, the assimilation effect decreased as the AQ score increased across participants ($r = -0.354$, $p < 0.05$). Similarly, the assimilation effect was lower in the high-AQ group (participants with top 65% or higher AQ scores) than the low-AQ group (participants with bottom 35% or lower AQ) (adjusted $p < 0.05$, N = 16/15 for low/high, respectively). The peak frequency of EEG in the electrodes over the somatosensory cortex was less changed by the assimilation effect in the high AQ group than in the low AQ group ($p = 0.039$ (not corrected)). This EEG feature also showed a marginal negative correlation with AQ score ($r = -0.285$, $p = 0.079$). Together, our findings revealed that when an individual has high autistic traits, the assimilation effect on the perception of vibrotactile stimulations decreased. Although this study was conducted on adults in non-clinical populations, it suggested that autistic traits are associated with decreases in tactile sensory integration.

Disclosures: **J. Jeong:** None. **J. Kim:** None. **S. Kim:** None.

Poster

185. Autism Diagnosis, Physiology, and Biomarkers: Human Studies

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 185.02

Topic: A.07. Developmental Disorders

Support: Ontario Brain Institute (POND)

Title: Linking the peripheral monocyte phenotype to behaviour in adolescents with neurodevelopmental disorders: A transdiagnostic approach

Authors: *S. R. CLEARY[1,4], G. TESKEY[2], R. WEKSBERG[5,6,7], E. ANAGNOSTOU[5], D. M. E. BOWDISH[2,3], J. A. FOSTER[1,4,8];
[1]Psychiatry & Behav Neurosci, [2]Dept. of Med., [3]McMaster Immunol. Res. Ctr., McMaster Univ., Hamilton, ON, Canada; [4]The Res. Inst. at St. Joe's, Hamilton, ON, Canada; [5]Div. of Clin. and Metabolic Genet. and Genet. and Genome Biol. Program, The Hosp. for Sick Children, Toronto, ON, Canada; [6]Departments of Pediatrics and Mol. Genet., [7]Institiute of Med. Sci., Univ. of Toronto, Toronto, ON, Canada; [8]Ctr. for Depression Res. and Clin. Care, UT Southwestern, Dallas, TX

Abstract: Management of neurodevelopmental disorders (NDD) is complicated by diverse within-disorder presentations and a high degree of symptom overlap between disorders. Identifying novel biomarkers will be useful in understanding the biology underlying their clinical presentations. There is evidence that the immune response contributes to the development and clinical presentation of NDDs; however, current literature assessing the immune phenotype of those with NDDs focuses primarily on young children with ASD. Therefore, the objective of this study is to examine the link between the immune phenotype and behaviour in adolescents with both autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD). Typically developing (TD), ASD, and ADHD, participants between 10-15 years old, were recruited through the Province of Ontario Neurodevelopmental Disorders Network (POND). Participants completed behaviour assessments measuring social communication, hyperactivity-impulsivity, inattention, and anxiety. Additionally, blood samples were collected, and peripheral blood mononuclear cells (PBMCs) were cryopreserved and later assessed using flow cytometry. The Mann-Whitney U test was used for between-group comparisons. Elastic net regression was used to examine the link between immune phenotype and behaviour. A total of 72 NDD participants (85% male, 20 ADHD, 52 ASD) and 19 TD (63% male) were included in the study. We found increased proportions of B cells and CD4+CD8+ T cells as a % of CD45+ cells in the NDD group compared to TD ($p < 0.05$). Intermediate monocyte CCR2 and CD115 MFIs were higher in the NDD compared to TD ($p < 0.05$). Classical monocyte CD13, CD64, CCR2, and intermediate monocyte CD64 were associated with social communication scores. Intermediate monocyte CD115, CCR2, CX3CR1, and non-classical monocyte CX3CR1 were negatively associated with social communication scores. With all monocyte surface markers, age and sex included in the elastic net regression $R^2 = 0.37$. These results suggest that B cells, CD4+CD8+ T cells, and monocyte surface markers may reflect social communication deficits in children with NDDs. Future research should explore how these biomarkers may be used in conjunction with other immune factors to identify subgroups of children with NDDs and how these biomarkers influence social communication from a mechanistic perspective.

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Poster

185. Autism Diagnosis, Physiology, and Biomarkers: Human Studies

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 185.03

Topic: A.07. Developmental Disorders

Support: NIG Grant 5R21NS070296
P50-NS22343
NSF 1640909

Title: Change blindness and eye tracking in children with autism

Authors: *J. SNIDER[1], S. HACKER[3], J. TOWNSEND[2], L. CHUKOSKIE[4];
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Abstract: The ability to detect and respond to changes in a visual scene is essential for functional orienting in our dynamic natural world. We are interested in how unique visual and social perceptual abilities affect the way people explore changing scenes. We used a change blindness task to characterize eye movements and fixations both in autistic and non-autistic adolescents. Social and non-social changes were made in each of 16 natural scenes. These changes were balanced for low-level salience, eccentricity, and size. We compared the gaze behavior and reaction time responses of autistic and non-autistic participants. We observed that autistic participants had significantly shorter fixation times than their non-autistic peers in the search portion of each trial, but that this effect weakens when the task is to look at a static image. We observed no reliable between-group differences in search success or time to success. Similarly, we observed no consistent differences in speed or success of search for social or non-social targets overall or by group. However, when the scene was divided into social versus non-social content, with respect to fixation duration, there was a consistent and large difference in fixation dwell time in both groups. The fixation dwell time for social content was longer during both the view and search phase, but the effect was stronger in the search phase of the task. We also observed an unproductive tendency by TD participants to anchor their gaze on social regions. These results reveal that the nature of the task has substantial effects on content-specific fixation times and covert attention orienting that are durable across groups.

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Poster

185. Autism Diagnosis, Physiology, and Biomarkers: Human Studies

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 185.04

Topic: A.07. Developmental Disorders

Support: Aston University Studentship

Title: Comparing visual perspective taking and belief reasoning in autism spectrum condition

Authors: *R. L. GREEN[1], D. J. SHAW[1], K. KESSLER[2];
[1]Aston Univ., Aston Univ., Birmingham, United Kingdom; [2]Psychology, Univ. Col. Dublin, Dublin, Ireland

Abstract: Comparing Visual Perspective Taking and Belief Reasoning in Autism Spectrum ConditionsRachel Green[1], Daniel Joel Shaw[1] and Klaus Kessler[1, 21] Institute of Health and Neurodevelopment, School of Psychology, Aston University, UK[2] School of Psychology, University College Dublin, Ireland
Autism Spectrum Condition (ASC) is characterised partly by differences in social interaction. It is believed that these differences arise from alterations in predicting and understanding the behaviour of others, which are supported by theory of mind processes. One of these processes is belief reasoning (BR), which refers to the human ability to understand another's mental representation of reality even if it different from one's own (1). Another process is level-2 Visual Perspective Taking (VPT), which is the human ability to understand how the world appears from another's viewpoint (2). In adults with ASC, some studies report differences in BR and VPT reasoning between adults with and without ASC, while others report no such difference. As such, it is unclear if and how these processes are altered in ASC. To investigate this further, the present preregistered, online study (3) employed an experimental task developed recently to assess the link between BR and VPT processes (4). We recruited 123 adults aged 18-40 years, 58 with self-reported ASC and 65 without (Controls). Both groups completed the novel experimental task and Raven's Progressive Matrices online. There was no difference in intelligence between the two groups, as evidenced by Raven's Progressive Matrices, and we observed no differences between the groups in BR ability. However, those with ASC took longer to make VPT judgements than Controls. Since this is the first study to compare VPT and BR directly in adults with ASC, future studies should seek to further understand this difference apparently specific to VPT. (1)Baron-Cohen et al., (2001)(2)Flavell et al., (1981)(3)https://osf.io/57wa9/(4)Green, Shaw and Kessler (2021)

Disclosures: R.L. Green: None. **D.J. Shaw:** None. **K. Kessler:** None.

Poster

185. Autism Diagnosis, Physiology, and Biomarkers: Human Studies

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Program #/Poster #: 185.05

Topic: A.07. Developmental Disorders

Support: NSU QOL 334829

Title: Decoding peaks of interest in children with autism using time series analysis

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Abstract: Introduction. Children with Autism Spectrum Disorder (ASD) generally have attention deficits, which hinder their ability to direct and preserve attention on social stimuli. Though various studies compare the attention of children with ASD respective to human faces and other social stimuli, no eye-tracking models exist to predict the onset at which disengagement begins. This study uses a time series approach by reviewing facial expressions, eye movement, and time series pupil size data to measure responses of children with ASD compared to children with neurotypical development. **Methods.** Preliminary data were collected using the Tobii Pro Nano, semi-structured surveys, computer embedded camera, and the M-CHAT tool from neurotypical children (N=14) and children diagnosed with ASD (N=9) aged 4.5 years to 6 years old. Tobii Pro lab and IBM SPSS V 27.1 were used for analysis. Areas of Interest (AOIs) were created for each object (human face, a dog, and toys) included in a 1.5 minute video designed for this study. Pupil size, number of saccades, and fixation duration on each AOI were recorded and calculated for four events of interest (baseline, initial presentation of soft stimuli, presentation of social interaction, and question prompts). **Results.** The pupil size of children with ASD were unstable and had more variance throughout the duration of the video. Time series data of children with ASD show higher peaks in pupil size during the initial presentation of stimuli, but these peaks occurred immediately before missing data. Video evaluations reveal a corresponding pattern in eye-movement behavior and higher pupil sizes for the onsets at which 4 children with ASD disengaged with the video stimuli. Considerable dips in pupil sizes were observed during an intended shift in anticipated cues from neurotypical children but not from children with ASD. Children diagnosed with ASD also demonstrated wider and less specified gaze patterns with greater variability, and more missing data. Neurotypical children averaged 12.33 ± 4.16 fixation events on the actress' face and 33.67 ± 11.55 on the dog compared to 13.00 ± 14.05 and 23.57 ± 15.95 from children diagnosed with ASD. There were no significant differences by group ($p = 0.783$) for fixation duration on the objects (alpha 0.05, 95% CI). **Conclusion**. The results suggest that eye-tracking technologies may assist in detecting and predicting shifts in interests and degrees of interests in social stimuli to benefit children with ASD.

Disclosures: G. Cavanaugh: None. **N. Sheinberg:** None. **L. Boucher:** None. **B. Lockard:** None.

Poster

185. Autism Diagnosis, Physiology, and Biomarkers: Human Studies

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 185.06

Topic: A.07. Developmental Disorders

Title: Anxiety in children with Autism Spectrum Disorder: contributions from the hippocampus and socioeconomic status

Authors: *D. SEGUIN[1], S. PAC[1], N. BRIERLEY[1], R. NICOLSON[1], J. C. MARTINEZ-TRUJILLO[3], E. ANAGNOSTOU[4], J. P. LERCH[5], C. HAMMILL[6], J. BRIAN[7], S. GEORGIADES[8], E. G. DUERDEN[2];
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Abstract: Background: Elevated anxiety is common in children with Autism Spectrum Disorder (ASD), as well as in children experiencing socioeconomic disadvantage. The hippocampus has been associated with both anxiety and ASD, although the extant literature is mixed. Socioeconomic disadvantage is associated with significant deficits in brain development in children, particularly the hippocampus. The current study provides an investigation of the intersection of these areas by exploring the differential impact of anxiety and SES on brain development in children with ASD (n = 31) relative to typically developing (TD; n = 14) children.**Methods:** Anxiety was measured by the anxious-depressed subscale of the Child Behavior CheckList (CBCL-AD) with socioeconomic status measured by a combined metric of lower vs higher income and lower vs higher parental education. Generalized linear models were used to estimate the effect of anxiety and SES on hippocampal and amygdala volumes. Hippocampal subfield volumes were extracted using FreeSurfer. **Results:** When controlling for gender, age and total cerebral volume, ASD children experienced significant higher anxiety than TD children ($p<.001$). Larger volumes of the molecular layer ($p=.020$), hippocampal tail ($p<.001$), fissure ($p=.003$) and parasubiculum ($p<.001$) subfields of the hippocampus were associated with higher anxiety scores in ASD children, an interaction not found in TD children. SES was significantly associated with anxiety ($p<.001$), with results suggesting children with ASD are more likely to have higher anxiety than TD children from higher SES households.**Conclusions:** Hippocampal subfield contributions to anxiety differ between children with and without ASD. Our findings also suggest an interaction between SES and ASD on hippocampal development and may have implications for the development of targeted interventions. Differences in the neurocircuitry underlying anxiety in ASD compared to TD children suggest differential treatment interventions may for anxiety may benefit children with ASD.

Disclosures: D. Seguin: None. **S. Pac:** None. **N. Brierley:** None. **R. Nicolson:** None. **J.C. Martinez-Trujillo:** None. **E. anagnostou:** None. **J.P. Lerch:** None. **C. Hammill:** None. **J. Brian:** None. **S. Georgiades:** None. **E.G. Duerden:** None.

Poster

185. Autism Diagnosis, Physiology, and Biomarkers: Human Studies

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 185.07

Topic: A.07. Developmental Disorders

Support: NIH R01 MH107802

Title: Maternal bacterial infection or viral illness during pregnancy is associated with neurobehavioral outcomes in young children with autism spectrum disorder

Authors: *K. WILDE, B. CHEN, L. OLSON, A. RIOS, M. SALMINA, Z. DAMON, S. PEÑA, A. LINKE, I. FISHMAN;
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Abstract: According to the maternal immune activation (MIA) hypothesis, inflammatory perturbations in utero can affect fetal neurodevelopment, with evidence indicating links between MIA and neurodevelopmental disorders in offspring. Currently, most evidence for MIA as a risk factor for autism spectrum disorder (ASD) comes from epidemiological research and animal models (with the latter not fully capturing human ASD phenotype). This study set out to examine the links between probable MIA and functional brain organization in toddlers and preschoolers diagnosed with ASD, using functional magnetic resonance imaging (fMRI) data acquired during natural sleep as part of the SDSU Toddler MRI Project. Functional connectivity patterns for 7 canonical brain networks were compared between 40 children with ASD that had a history of MIA (estimated here as history of bacterial infection, viral illness, or antibiotics course during pregnancy, based on maternal self-report; MIA+) and 34 children with ASD that did not have a history of MIA (MIA–) between the ages of 17 and 66 months (mean age ± SD = 36 ± 14 months). Analysis of covariance revealed that presence of behavioral problems, as assessed with the Child Behavior Checklist, were significantly associated with history of MIA (main effect of MIA: $F(1,1)=5.3$, $p<.05$). Further, there was a significant interaction effect of MIA and the sex of the child on adaptive developmental skills, as assessed with the Vineland Adaptive Behavior Scales (MIA by sex interaction effect: $F(1,1)=5.8$, $p<.05$), with lower developmental skills observed in all MIA+ children and MIA– male (but not female) children. Functional connectivity patterns were also significantly associated with a history of MIA, especially within the frontoparietal and dorsal attention networks, with generally weaker connectivity between the regions of these executive control networks. These results provide initial evidence of the potential contribution of maternal immune status during pregnancy to the heterogeneous neurobehavioral outcomes in children with ASD.

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Poster

185. Autism Diagnosis, Physiology, and Biomarkers: Human Studies

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 185.08

Topic: A.07. Developmental Disorders

Title: From neurons to behavior in the motor kinematic statistical properties in neurodevelopmental disorders

Authors: *J. V. JOSE[1], **N. W. PARRIS**[2];
[1]Physics, Stark Neurosci. Inst., [2]Physics, Indiana Univ., Bloomington, IN

Abstract: There is a major concern about individuals that do not reach the learning milestones of Neurotypical (NT) subjects. There is no clear source of Neurodevelopmental disorders (NDD), like Autism-Spectrum-Disorder (ASD). ASD is characterized by problems with social interactions, communication difficulties and repetitive-restrictive behaviors. It has been recognized for some time that many of these symptoms involve movement deficiencies. We developed a stochastic biomechanical model of the arm for its mechanical response to a given motor signal. This statistical model is developed from the muscles' biochemistry that produces the force and torque provided by the motor signal sent to the arm. By using this and a jerk minimization hypothesis of human movement we derive a statistical optimization hypothesis for the motor neural signals. Using a stochastic neural model for the action potential inter-spike intervals, we build a minimization hypothesis for the neural signal rate parameter and consider the possible source of the jerk minimization hypothesis. We would further like to inspect the inverse problem by considering the possible preferable optimization neuronal principles and to deriving their respective neurological optimization models. By comparing such models to the microscopic and macroscopic statistics we observe in human movements, we assess their validities across several orders of magnitude in both space and time (from mm to m and ms to seconds scales, respectively.) We compare these models to the movement statistics of patients with ASD. These statistics give a robust motor-based distribution of the maximum velocity for ASD (Torres et al., 2013) which can be related back to the stochastic movement process we have considered. We showed that these measurements for ASD are at odds with jerk minimization. Further studies will be necessary to understand the kinematic millisecond statistical fluctuations leading to the ASD biomarker (Wu et al., 2018.)

Disclosures: **J.V. Jose:** None. **N.W. Parris:** None.

Poster

185. Autism Diagnosis, Physiology, and Biomarkers: Human Studies

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 185.09

Topic: A.07. Developmental Disorders

Support: PNPC - Conacyt

Title: Relationship between symptom severity and neurophysiological activity in children diagnosed with autism spectrum disorder

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Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder with one of the greatest increases in prevalence worldwide in recent years. ASD patients present a distinct development of social communication from the first months of life, as well as restricted and repetitive behavior patterns. Symptoms manifest within a continuum of severity and significant differences are observed between low and high functioning cases. Although distinct patterns of brain activity have been found in the evaluation of individuals with ASD in comparison with typically developing individuals, there is no brain marker due to heterogeneity of symptoms, and differences in age and diagnostic criteria in the populations evaluated. The aim of this research was to know the relationship between symptom severity and neurophysiological activity in children diagnosed with ASD; seeking an approach to describe the neural mechanism underlying ASD and a potential biological marker of it, through the detailed description of symptoms and developmental abilities according to their ages. Fifteen children with suspected or diagnosed ASD, aged 2 to 5 years, recruited from the waiting list of a public center specialized in the evaluation and diagnosis of autism in Mexico, participated. The MCHAT screening test was used for children aged 2 and 3 years and the SRS screening test for children aged 4 and 5 years. The Structured Interview CRIDI-Autism Spectrum Disorders, Gold Standard for the Assessment of Autism in Latinos and Mexicans was applied, whose results yield algorithms according to DSM 5, DSM-IV, ICD 10 and ICD 11 manuals. Recording of brain electrical activity in waking state was carried out for a duration of 20 to 30 min in order to obtain an approximate 2 min of artifact-free recording for brain mapping. It was found that children with a higher level of severity in the diagnosis of ASD had a lower developmental age, mainly in the area of communication, lower relative power in the frontal alpha band, higher relative power of the frontal delta band and an increase in the coherence of the delta, theta and alpha bands of the fronto-temporal region. The results contribute to a better understanding of the brain pattern underlying ASD symptoms at different levels of severity.

Disclosures: P.A. Batiz Flores: None. **C. Vega-Michel:** None.

Poster

185. Autism Diagnosis, Physiology, and Biomarkers: Human Studies

Location: SDCC Halls B-H

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Program #/Poster #: 185.10

Topic: A.07. Developmental Disorders

Support: NIMH award F32MH122057
U01AG068057

Title: Detection of Autism using Deep Learning Models Applied to Brain MRI across Multiple Sites

Authors: *N. J. DHINAGAR, K. E. LAWRENCE, E. LALTOO, P. M. THOMPSON;
USC, Los Angeles, CA

Abstract: According to the Center for Disease Control and Prevention (CDC), 1 in 44 children is diagnosed with autism spectrum disorder (ASD). ASD occurs approximately 4 times more often in males compared to females. Here we propose deep learning methods to detect brain biomarkers that are associated with ASD using the widely used 3D T1-weighted (T1-w) MRI scans. Data-driven methods could help augment the current clinical workflow to identify ASD more efficiently and reduce wait times. We analyzed 250 scans from 4 imaging sites collected as part of Autism Brain Imaging Data Exchange (ABIDE). We partitioned 80% of this multi-site data for training (N=202, 30(15%)F, age: 5.2-64.0 yrs.), 10% for validation (N=23, 7(30%)F, age: 5.9-62.0 yrs) and 10% for testing (N=25, 9(36%)F, age: 6.1-57.0 yrs). Each of the three folds contained data from the four sites in near equal proportions. An independent cohort of 43 scans acquired at a fifth site were used for out-of-distribution testing (N=43, 0%F, 10.0-20.0). The scans from the fifth site were scaled between 0 and 1 to increase harmonization and stability. We used two main convolutional neural network (CNN) architectures: a 3D version of the Densenet121 and 2D CNN with a Long Short-Term Memory (LSTM). The 3D CNN processes the input T1-w MRI volumes with 3D kernels, whereas the 2D CNN-LSTM combines slice-wise encodings to create subject-wise predictions. The models were trained for 50 epochs. We used a binary cross-entropy loss function with the Adam optimizer with weight decay. The 3D CNN, trained from scratch, achieved an average receiver-operator characteristic curve-area under the curve (ROC-AUC) of 0.588, compared to the 2D CNN with an ROC-AUC of 0.660. We separately pre-trained the 3D CNN for sex classification on T1-w scans from the UK Biobank (UKBB). The pre-trained 3D CNN was fine-tuned end-to-end with lower learning rate for ASD classification. The pre-trained 3D CNN model achieved an average ROC-AUC of 0.747. Upon further evaluation on the single-site test set, the pretrained 3D CNN model yielded an ROC-AUC of 0.671 with pretraining, 0.682 without pretraining and 0.515 for the 2D CNN-LSTM. Based on our experiments, pretraining on sex classification improves performance on the multi-site data, which may have implications for understanding sex-based differences in ASD and improving diagnostic prediction from brain scans. The proposed models will be tested with larger datasets to improve performance. Harmonizing data across the different sites may also improve the model's ability to generalize. Our approach could also be trained to predict longitudinal changes in autism traits.

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Poster

185. Autism Diagnosis, Physiology, and Biomarkers: Human Studies

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 185.11

Topic: A.07. Developmental Disorders

Support: NIH R01 MH107802

Title: Links between early sleep problems and neurobehavioral outcome in toddlers and preschoolers with ASD

Authors: *A. RIOS[1], B. CHEN[2], L. OLSON[3], K. WILDE[2], M. SALMINA[2], Z. DAMON[2], S. PEÑA[2], A. LINKE[1], I. FISHMAN[1];
[1]Psychology, [2]San Diego State Univ., San Diego, CA; [3]Joint Doctoral Program in Clin. Psychology, SDSU / UC San Diego, San Diego, CA

Abstract: Children with autism spectrum disorders (ASD) commonly experience sleep problems. To better understand the links between sleep problems and neurocognitive development in ASD, this study set out to test if irregular sleep in the first year of life predicts neurodevelopmental outcomes at the preschool age. Data from 72 children with ASD and 41 typically developing (TD) children, between ages 1.5 and 5 years, enrolled in the longitudinal SDSU Toddler MRI Project were analyzed. The two groups were matched at group level on age and sex. Developmental and medical history and assessments of childrens' developmental skills were obtained from caregivers (including the Vineland Adaptive Behavior Scales, Mullen Scales of Early Learning Sensory Profile, Child Behavior Checklist), and functional magnetic resonance imaging (fMRI) data were acquired in children during natural sleep. Consistent with prior literature, irregular sleep during the first year of life (reported by caregivers) was more common among children with ASD when compared to their TD peers (p=0.015). In the ASD group, children with irregular first-year sleep (FYS; n=26), compared to those with regular FYS (n=46), had significantly (p=.05) greater sensory sensitivities, persisting sleep problems, and lower adaptive functioning skills during preschool years. Additionally, in a subset of children for whom longitudinal data were available (n=21, mean=15.8 months between two assessments), preliminary results revealed a similar pattern of children with ASD and irregular FYS having significantly higher sensory sensitivities on follow-up assessments. In children with ASD with usable fMRI data (n=45), functional connectivity was assessed between 30 regions of interest forming 7 canonical functional brain networks (derived from the Human Connectome Project). Analysis of covariance (ANCOVAs), controlling for age and in-scanner motion, revealed that children with ASD and irregular FYS had significantly more variable functional brain network organization in comparison to children with ASD with regular FYS (Cohen's d=1.05, p=.001), with stronger connectivity between the default mode and sensory networks, and weaker connectivity between the dorsal attention, salience, and fronto-parietal networks (; p<.05 FDR-corrected). These results highlight the need for early and targeted interventions to improve sleep in young children with ASD.

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Poster

185. Autism Diagnosis, Physiology, and Biomarkers: Human Studies

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 185.12

Topic: A.07. Developmental Disorders

Support: NRF Grant 2020R1F1A1058200

Title: The effects of facial emotion diagnostic regions on emotion recognition in typical development and children with ASD

Authors: *H. JI[1], K. KIM[2], J. KIM[1], S.-Y. KIM[1];
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Abstract: The diagnostic regions on facial emotion is defined with facial features which contain the most emotional information for each basic emotion. A recent study with neurotypical adults found that exploration of emotion-specific diagnostic regions predicted efficient facial emotion recognition. In fact, efficient facial emotion recognition is one of the critical abilities for school-aged children as this period is important for developing peer groups and social interaction skills. However, few studies investigated how the exploration on facial emotion diagnostic regions played a role in emotion recognition abilities in both typically and atypically developing children. Here, we investigated facial exploration patterns for six basic emotions in typically developing children (TD) and children with autism spectrum disorder (ASD). We also tested the relationships between the exploration patterns of emotion-specific diagnostic regions on face and emotion recognition performance in both groups. Twenty TD children and 20 children with ASD (aged 6-12) performed a facial emotion recognition task. Children were asked to choose an appropriate emotion label upon recognizing an emotion. Children's exploration patterns on the face were measured with the Moving Window Technique (MWT). The MWT presented a small window with a blurred face, and the children explored the face through a mouse-controlled window to judge emotion. Based on previous studies, three regions of interest (ROIs) were defined on the face: the left and right eyes, and the mouth. We computed two parameters as regressors, namely, *Spent time* and *Last ROI*. Spent time refers to how much each ROI has been explored, and the Last ROI was defined by the last region when emotional choice was made. Our results showed that ASD performed significantly worse than TD on the emotion recognition task (for anger, sad, fear, and happy). Also, we found that the exploration patterns of emotion-specific diagnostic regions significantly predicted effective emotion recognition performance only in TD. Specifically, the Spent time and Last ROI of the diagnostic regions explained significant variances in accurate emotion recognition performance in TD, especially for anger, disgust, and fear emotions. Overall, our findings suggest that proper exploration of emotion-specific

diagnostic regions in a face can predict efficient performance in facial emotion recognition in TD children, whereas children with ASD do not seem to gain benefits from the diagnostic regions for emotion recognition. Such findings have important implications on explaining emotional functions and dysfunctions in children with and without ASD.

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Poster

185. Autism Diagnosis, Physiology, and Biomarkers: Human Studies

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 185.13

Topic: A.07. Developmental Disorders

Title: Emotional faces and working memory in children with high autistic and anxiety traits

Authors: *T. MULLINS[1], J. HOGEVEEN[2];
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Abstract: While the impact of anxiety and autism on working memory has been investigated, conflicting findings have been reported. For both conditions, working memory has been reported to be intact, impaired, or improved, or variously modulated by other factors, including emotional content of stimuli, and neuroimaging findings have been varied. When looking at the combined effect of comorbidity, the picture is even less clear. Here, we determined whether a classic finding in the cognitive neuroscience of anxiety—increased salience and attention to negative emotional stimuli—varies across children who do or do not also have elevated autistic traits. Specifically, we used the *Adolescent Brain Cognitive Development* (ABCD) study dataset to compare threat or negative emotion bias across 4 well-matched subgroups of 9-10-year-old children: 1) high anxiety and low autistic traits (ANX; *N*=54), 2) high autistic traits and low anxiety (AUT; *N*=48), 3) high anxiety *and* autistic traits (ANX+AUT; *N*=51), and 4) low anxiety and low autistic traits (CTRL; *N*=52). We examined an n-back task that probed visual working memory and utilized emotional face stimuli, which allowed us to look at the behavioral and neural correlates of emotional faces on working memory in these groups. Behavioral Results showed no group differences in accuracy, reaction time, or d-prime scores across groups, indicating preserved working memory across groups, but main effects of stimuli were present, showing that emotional faces did alter working memory. Analysis of frontoparietal control and limbic brain network recruitment during the n-back task is ongoing, and will enable us to determine whether similar behavioral results are mediated by common or distinct neural architectures related to working memory and emotional processing across groups.

Disclosures: T. Mullins: None. **J. Hogeveen:** None.

Poster

185. Autism Diagnosis, Physiology, and Biomarkers: Human Studies

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Program #/Poster #: 185.14

Topic: A.07. Developmental Disorders

Support: NSFC Grant 31701002
HMRF Grant 07180836

Title: Investigation of anxiety-related functional connectivity of amygdala in children with autism spectrum disorder

Authors: *X. GENG[1], P. SONG[2], H. W. HIRAI[1], S. TSANG[1], Y. WANG[2], P. C. WONG[3]; [1]Brain and Mind Inst., Chinese Univ. of Hong Kong, Hong Kong, Hong Kong; [2]Zhengzhou Univ., Zhengzhou, China; [3]Linguistics & Modern Languages, The Chinese Univ. of Hong Kong, Shatin, Hong Kong

Abstract: Anxiety is considered one of the most prominent comorbid conditions in children with autism spectrum disorder (ASD), with a prevalence rate over 40%. The symptom of anxiety greatly impacts core deficits of ASD, such as social communication issues and repetitive behaviors. As a complex neurodevelopmental disorder, however, the underlying neural mechanisms of the co-existing of anxiety and autism are unclear. In the current work, we aimed to examine how functional pathways of amygdala, a critical region underlying anxiety processing and frequently reported in ASD, are correlated with anxiety and ASD core symptoms in children with ASD.

Two cohorts were included: one from ABIDE-II, including a total of 81 subjects from three sites with both ADOS and anxiety measures available and aged from 6 to 13 years old; and another one from locally collected subjects of 15 ASD children aged from 8 to 12 years. The preprocessed functional data using DPARSF (motion correction, regression of motion and global signals, bandpass filtering) were used to build connectivity matrix based on AAL parcellation. Functional connectivity (FC) between amygdala and other regions were examined. Social deficits and restricted/repetitive behaviors (RRB) measured by ADOS and anxiety levels using Child Behavior Checklist were analyzed.

Distinct association patterns were found between anxiety and social deficits: positive correlations ($r=0.25$, $p=0.02$) in children with low anxiety levels; and negative correlations ($r=-0.25$, $p=0.02$) in individuals with high anxiety. We also examined correlations between amygdala FC and behavioral measures (social deficits, RRB and anxiety scores). For the ABIDE-II data, significant correlations were found between social deficits and FC of amygdala-inferior frontal cortex (IFC) ($r=-0.29$, $p=0.003$), and FC of amygdala-supplementary motor area ($r=-0.28$, $p=0.005$); and between anxiety levels and FC of amygdala-middle cingulate ($r=-0.26$, $p=0.01$) and FC of amygdala-inferior temporal ($r=0.24$, $p=0.02$). For local data, significant correlations were found between social deficits and FC of amygdala-IFC ($r=-0.59$, $p=0.02$), which was similar to that found in ABIDE-II.

Our results show that distinct amygdala functional connections are associated with social deficits and anxiety in children with ASD, consistent with existing studies that focused on amygdala activations. Findings suggest that a hybrid of emotion and social processes may not be reconciled

to any of the process alone in ASD. Additional analyses to determine whether anxiety serves as a moderator on symptom severity are needed to further understand the neural mechanisms of the co-morbid condition.

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Poster

185. Autism Diagnosis, Physiology, and Biomarkers: Human Studies

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Program #/Poster #: 185.15

Topic: A.07. Developmental Disorders

Support: NSF Grant#: 1640909

Title: Quantitative assessments of neurodevelopmental disorders using deep learning and systems neuroscience techniques

Authors: *K. DOCTOR[1], D. WU[2], A. PHADIS[3], J. NURNBERGER, Jr[5], M. PLAWECKI[5], J. V. JOSE[4];
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Abstract: Neurodevelopmental disorders (NDD) such as Autism Spectrum Disorder (ASD), Attention Deficit Hyperactive Disorder (ADHD), and comorbid ASD+ADHD have increased significantly in recent years. It has been known for some time that NND symptoms often manifest as movement deficiencies. There are two clinical challenges: to have early screening assessments, as well as quantitative diagnostics about the severity of the NDD condition for each individual. We started by analysing the raw kinematic data generated at millisecond time scales by the high-definition sensors (www.xsens.com), while the subjects carry out repeatedly a reaching paradigm protocol. We assumed that there is important physiological information present in the way people move. To extract this information, we train a neural network with the raw kinematic data using Deep Learning (DL) techniques. We evaluated the efficacy of this type of system by training on different subsets of data to ascertain the results stability. We found high predictive accuracy in our ability to characterise the NDD classification separating them from each other and the Neuro Typical (NT) subjects. We found that DL correctly identifies NDD and NT subjects with high accuracy. These results characterise the different NDD diagnoses but not their severity. Next, we proceeded to filter out the electronic noise present in the sensors data leaving only the signal that can be attributed to the physiological motor signal. The sensors provide linear and rotational motion kinematics data. Here, we concentrate only on the angular velocity and the linear jerk. We find rapid peak time variations in these kinematic signals. We

considered their Inter-Spike Interval (ISI) nearest neighbour separations as a random variable. This allowed us to look at the probability distribution over ISIs, for each subject. We defined a biomarker given by the distributions Fano factor (FF) , the ratio of the variance to the mean. To measure the stability of the FF metric, we assessed its changes as more reaching trials were performed by the subjects. We found that the FF quickly stabilizes into a metric with minute, insignificant deviations. We proceeded to consider the ability of the FF to measure the severity as a function of age of the NDDs. Using Support Vector Machine (SVM) on a phase diagram of the FF vs Age to find different severity regions for each kinematic variable. We conclude that our DL approach can provide early screening diagnosis in a relatively short time since once the neural network has been trained it can be used as such in new subjects without modification. Our statistical analysis can provide a personalized biomarker for each individual.

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Poster

186. Developmental Disorders: Non-Genetic Models

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Program #/Poster #: 186.01

Topic: A.07. Developmental Disorders

Support: NIH R00 MH106757
CTSI-CN

Title: Early life adversity alters social dominance behavior in mice

Authors: *R. BAHR[1], A. KISNER[1], A. M. POLTER[2];
[1]The George Washington Univ., Washington, DC; [2]Dept. of Pharmacol. and Physiol., George Washington Univ., Washington, DC

Abstract: Parent-offspring interactions during the early postnatal period are critical for nervous system development. For a neonate, the relationship with a parent is the most important interaction and source of sensory input that they have during development. Disruptions of these interactions due to exposure to adverse experiences early in development can lead to long-lasting changes in brain physiology and behavior, including changes in sociability, a hallmark of several psychiatric disorders. In this study, we aimed to examine the effects of ELS in the form of fragmented maternal care on social behaviors. In this approach we utilized the limited bedding and nesting paradigm (LBN) in C57bl/6 mice. Four days following birth (PND 4) the dam and pups were transferred to cages with a wire-mesh surface and reduced bedding provided then returned to standard home cage conditions at PND 11. The weights of the animals were tracked and recorded at different stages during development. Our results show that ELS leads to decreased weight in male and female pups relative to controls (PND 21: average mass ELS males 7.12 ± 0.46g ELS females 7.01 ± 0.11g vs control males 9.46 ± 0.13g control females 7.80

± 0.87g; PND 35: average mass ELS males 17.59 ± 0.51g ELS females 14.34 ± 0.64g vs control males 20.49 ± 0.86g control females 16.28 ± 0.87g; and PND 80: average mass ELS males 23.33 ± 0.15g ELS females 17.8 ± 0.17g vs control males 30.5 ± 0.76g control females 22.38 ± 0.50g). To investigate the effects of ELS exposure on adolescent social play, we looked at the time control and ELS adolescent mice (P35-39) spent interacting with an age- and sex- matched conspecific in their home cage. Finally, we investigated social dominance behavior in adulthood (P80). To examine dominance behavior, we used the dominance test tube approach, in which mice were separated by sex and competed to exit a narrow, open-ended tube. Analyzing intra-cage dominance hierarchies showed that male mice established a more stable dominance hierarchy than females. Additionally, inter-cage dominance test results show that in both sexes, ELS mice are more likely to exhibit subordinate behavior to control mice. The results of this study suggest that mice exposed to early life adversity based on LBN protocol exhibit changes in social behavior, suggesting that early life environment programs the circuits required for later life social cognition.

Disclosures: R. Bahr: None. **A. Kisner:** None. **A.M. Polter:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.02

Topic: A.07. Developmental Disorders

Support: ORWH-U54-MH118919

Title: Not All Models Of Maternal Stress Are Created Equal

Authors: *J. A. SHENG, R. J. HANDA, S. A. TOBET;
Biomed. Sci., Colorado State Univ., Fort Collins, CO

Abstract: Exposure to adversities or stressors during fetal life influences fetal neurodevelopment and increases risk for neuropsychiatric diseases in adulthood. To determine the generality of stress effects on offspring, we are evaluating the impact of different models of maternal stress on hypothalamic development and related behaviors in mice. These models include fetal exposure to synthetic glucocorticoids (GC) to mimic a maternal corticosterone stress response, maternal nutritional stressors [caloric restriction (CR) versus maternal high fat diet (mHFD)], and maternal immune activation (MIA). Prenatal exposure to synthetic glucocorticoid, dexamethasone (DEX), resulted in decreased neonatal body weights ($p < 0.02$) and reduced social interaction behavior in male ($p < 0.05$) and female ($p < 0.05$) offspring. Maternal CR resulted in decreased body weights and social interaction behavior in males ($p < 0.02$) and females ($p < 0.05$) and increased anxiety-like behavior ($p < 0.05$) and acute stress response ($p < 0.01$) only in males. Maternal HFD resulted in altered body weight gain in male ($p < 0.02$) and female ($p < 0.05$) offspring with decreased anxiety-like behavior in a female-biased manner ($p <$

0.02). As mHFD may induce an immune inflammatory response in the mother (Ortiz-Vallardes et al., 2021; Neurosci Biobehav Rev 129:218) we are currently testing the impact of a direct agonist of a Toll-like receptor to mimic an immune response. Given that viral stressors stimulate GC release (Silverman et al., 2005; Viral Immunol 18:41) and immune stress therapy is often synthetic GC (e.g., DEX), it will be important to test an immune stress crossed with DEX in a factorial design. These studies will help determine if late gestation exposure to anti-inflammatory DEX can partially or fully recover alterations in fetal neurodevelopment influenced by MIA.

Disclosures: J.A. Sheng: None. **R.J. Handa:** None. **S.A. Tobet:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.03

Topic: A.07. Developmental Disorders

Title: Whole litter phenotyping in a rat maternal immune activation model

Authors: S. E. GRANT, M. MATSON, A. M. BACHMAN, R. F. BERMAN, J. VAN DE WATER, *M. D. BAUMAN;
UC Davis, Davis, CA

Abstract: Women exposed to infections during pregnancy have an increased risk of having a child with a neurodevelopmental disorder, including autism and schizophrenia. The maternal immune response has been identified as a risk factor for NDDs using preclinical maternal immune activation (MIA) models. MIA studies using rodents often select individual animals from a litter from which to run behavior studies, which may impact the variability seen in behavioral performance. In addition, MIA studies typically only use one dosage of an immune stimulant, limiting our ability to interpret how the strength of an immune response during pregnancy can impact offspring behavior. Here, we examined the behavior of whole litters in a rat model of MIA after exposure to two dosages of the immune stimulant lipopolysaccharide (LPS). Our research objectives were to identify behavioral and biological differences between dosages in a MIA rat model. Specifically, we aimed to examine early offspring communication, social behavior, anxiety, spatial working memory and cognitive flexibility, as well as a measure of cytokine changes in the whole brains of animals at birth. Sprague-Dawley females were time mated and injected with either saline, 50µg/kg LPS, or 100 µg/kg LPS at gestational day 14.5. Sera was taken from the dams before and 2 hours after injections. After birth, over-large litters were culled to 10 animals each. Offspring from the saline (N=82), 50µg/kg LPS (N=74), and 100 µg/kg (N=82) exposed dams were then tested on ultrasonic vocalizations (USVs) and early developmental milestones at post-natal days (PND) 4, 8, and 12. Following weaning, litters were tested on the elevated plus maze at PND26, juvenile social dyads at PND36, and spontaneous alternation on a Y-maze at PND55. After PND90, one male from each litter was chosen for the attentional set shifting task, which was designed to assess cognitive flexibility without relying on

visual acuity. In a separate study, neonatal animals (N=52/treatment group) were culled at PND0 to assess immune system differences. MIA animals showed alterations in early communication, with differing call number, length, duration, and frequency compared to controls. MIA offspring had altered reflexes shortly after birth. Post-weaning, MIA animals spent less time in the open arms of the elevated plus maze. Different MIA dosages resulted in different behavioral phenotypes in the offspring. 50 but not 100 µg/kg LPS showed significant increases in whole-brain cytokines at birth.

Disclosures: S.E. Grant: None. **M. Matson:** None. **A.M. Bachman:** None. **R.F. Berman:** None. **J. Van de Water:** None. **M.D. Bauman:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.04

Topic: A.07. Developmental Disorders

Support: Eugene Cota Robles Fellowship
Learning, Memory and Plasticity NIMH T32 (T32-MH112507)
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UC Davis Conte Center (P50 MH106438)
MIND Institute IDDRC Grant (U54HD079125)

Title: Factors predicting risk and resilience in offspring following maternal immune activation

Authors: *K. PRENDERGAST[1], E. C. CONNOLLY[2], C. N. MCCORMACK[2], C. A. KELLAND[3], J. D. SCHAUER[3], L. HAAPANEN[3], M. PARK[4], C. CHEN[4], M. AGUILAR[4], B. CHOJOLAN[4], C. S. CARTER[2,5,6], J. VAN DE WATER[3,7], M. D. BAUMAN[8,5], A. K. MCALLISTER[2];
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Abstract: Viral infections during pregnancy are associated with increased risk of neurodevelopmental (NDD) and psychiatric disorders in offspring. The viral mimic, poly(I:C), causes maternal immune activation (MIA) in mouse models which leads to neuroanatomical and behavioral phenotypes in offspring in domains similar to those found in human disorders. Despite the potential of MIA rodent models to identify new biomarkers and therapeutic interventions for a range of NDD and psychiatric disorders, current approaches ignore two of the most important aspects of this risk factor for human disease: (i) most pregnancies are resilient to maternal viral infection and (ii) susceptible pregnancies can lead to different combinations of behavioral phenotypes in offspring. Previously, we discovered that isogenic female mice exhibit

a wide range of baseline immunoreactivity (BIR) prior to pregnancy, as determined by the level of serum interleukin-6 collected 2.5 hours following a low dose poly(I:C) injection (5mg/kg). Notably, BIR before pregnancy and the poly(I:C) dose used during pregnancy to induce MIA together predict resilience and susceptibility of offspring to repetitive and exploratory behavioral alterations. In this study, we sought to identify factors that can predict resilience and susceptibility of offspring to affective, social and cognitive phenotypes following MIA. First, a time-course analysis of changes in 23 cytokines and chemokines was conducted at 2.5, 6 and 24-hours following intraperitoneal injection of 5mg/kg poly(I:C) (n = 36) or saline (n = 18) into young virgin female C57BL/6J mice from Charles River. Then, MIA was induced in BIR- and age-matched female mice by injecting 78 dams during mid-gestation (E12.5) with saline or poly(I:C). An extended behavioral battery to assess anxiety, repetitive behavior, cognition, memory, social behavior, sensorimotor gating, and conditioned fear was conducted in juvenile and young adult offspring (n = 330). We found that BIR as defined by serum IL-6 levels at 2.5hrs post-injection, together with the poly(I:C) dose to induce MIA, predicts susceptibility of male offspring to elevated repetitive behavior and social deficits and susceptibility of female offspring to working memory and recognition deficits. Ongoing research is focused on defining the full immune response that comprises BIR and correlates with specific deficits in MIA offspring. In summary, we identify the BIR of female mice before pregnancy as a putative biomarker that predicts which offspring will be at risk for distinct clusters of behavioral perturbations following MIA.

Disclosures: K. Prendergast: None. **E.C. Connolly:** None. **C.N. McCormack:** None. **C.A. Kelland:** None. **J.D. Schauer:** None. **L. Haapanen:** None. **M. Park:** None. **C. Chen:** None. **M. Aguilar:** None. **B. Chojolan:** None. **C.S. Carter:** None. **J. Van de Water:** None. **M.D. Bauman:** None. **A.K. McAllister:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.05

Topic: A.07. Developmental Disorders

Support: Robert and Donna Landreth Foundation

Title: Early-life resource deprivation induces sex-biased microglia-dependent behavioral alterations in mice

Authors: I. M. BANKOWSKI, H. A. MOYA, A. A. OBENG-MARNU, ***E. A. BORDT**; Massachusetts Gen. Hospital/Harvard Med. Sch., Boston, MA

Abstract: Early-life adversity is a major risk factor for neuropsychiatric disorders such as major depressive disorder, anxiety, and autism spectrum disorders. Differing types of adversity at various critical developmental timepoints can impact neurodevelopment and behavioral

vulnerabilities in a sex-specific manner. In this study, we employed a widely used form of early-life adversity, resource deprivation in the form of limited bedding and nesting (LBN) material, to induce these neural and behavioral alterations. Our LBN paradigm consisted of placing mothers in a cage with wire mesh flooring, without bedding material, and with restricted amounts of a cotton nestlet, as compared to control housing conditions with adequate bedding and a full cotton nestlet. Consistent with previous literature, maternal exposure to this LBN paradigm significantly altered maternal behavior towards offspring. In this study, we assessed the impact of two variables on offspring behavior and neurodevelopment: i) timing of LBN exposure and ii) necessity of microglial immune signaling. We have found sex-specific alterations in ultrasonic vocalizations (number of calls, syllable composition, and similarity scores), anxiety-like behaviors (elevated zero maze and light-dark box assays) as well as social motivation (sociability) behaviors. Previous studies have demonstrated that LBN induces behavioral and synaptic alterations through microglial dynamics. Here, we examined sex-specific regulation of excitatory neurons by microglia in the paraventricular hypothalamic nucleus (PVN) in response to early-life LBN exposure. We have also generated mice lacking the toll-like receptor 4 (TLR4), specifically in microglia, and assessed the necessity of microglial immune signaling to LBN-induced sex-biased behavioral alterations and synaptic regulation. We are currently determining the impact of LBN timing (pre- or post-natal) on offspring health outcomes. These data indicate sex-biased alterations in behaviors that are relevant to clinical manifestations of neurodevelopmental disorders.

Disclosures: I.M. Bankowski: None. **H.A. Moya:** None. **A.A. Obeng-Marnu:** None. **E.A. Bordt:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.06

Topic: A.07. Developmental Disorders

Support: FAPESP # 2019/21980-0
CNPq # 302608/2019-2
AFIP
CAPES, Finance code 001

Title: Underwater trauma induces sex-dimorphic behavioural profile independent of early life stress

Authors: *D. SUCHECKI[1], N. C. ZANTA[2], C. E. GIRARDI[2], G. RICHTER-LEVIN[3];
[1]Univ. Federal de Sao Paulo, [2]Psychobiology, Univ. Federal de Sao Paulo, Sao Paulo, Brazil;
[3]Univ. Haifa, Univ. Haifa, Haifa, Israel

Abstract: Early life adversity is a major risk for PTSD. Maternal deprivation (DEP) for 24 h on postnatal day (PND) 3 (DEP3), but not on PND 11 (DEP11), induces anxiety- and depressive like behaviours and reduction of neuropeptide Y (NPY) in both ages and sexes. Because NPY is considered a resilience factor for stress-related disorders, in the present study we investigated whether DEP-induced NPY decrease is a risk factor for underwater trauma (UWT)-induced PTSD in adulthood, employing a behavioural profiling system in male and female rats. Eighteen litters of Wistar rats (4-5 pups/sex/litter) were equally distributed to control (CTL), DEP3 or DEP11 groups. On PND 60, 2 rats/sex in each litter were not exposed to the UWT (UWT-) and 3 rats/sex were (UWT+). On PNDs 89 and 90, rats were tested in the Sucrose Splash Test (assessment of depressive-like behaviour) and elevated plus maze (EPM), respectively. The behavioural profile was determined by comparing individual values in 6 behaviours expressed in the EPM with the mean ± 1 s.d. of the CTL/UWT- group for each sex, such that rats were classified as affected (at least 75% of altered behaviours in the EPM), intermediary (50% of alterations) or unaffected (less than 50% change) (Ethics Committee approval CEUA/UNIFESP: 8374120619). MD had no impact in the incidence or severity of behavioural changes, but we found that a greater proportion of females were more affected than males (3 females:1 male), although, in general, females explored more the open areas of the EPM than males. In both sexes, rats classified as intermediary and affected displayed greater level of anxiety-like behaviour but no co-occurrence with depressive behaviours. The present results indicate that MD at either age did not increase the incidence of PTSD-like behaviours. Instead, sex was a major risk factor, with females being more vulnerable, without comorbid behaviours relevant to depression.

Disclosures: D. Suchecki: None. **N.C. Zanta:** None. **C.E. Girardi:** None. **G. Richter-Levin:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.07

Topic: A.07. Developmental Disorders

Support: R01 AA028406
F32 AA029866

Title: Exposure to alcohol and/or cannabinoids during the second trimester impairs arterial resistance and increases cardiac stroke volume in fetal cerebral and umbilical arteries.

Authors: *R. KUMAR[1], S. ROUZER[2], A. SREERAM[1], R. MIRANDA[1];
[1]Texas A&M Univ. Syst. Hlth. Scien Neurosci. and Exptl. Therapeut., Bryan, TX; [2]Texas A&M Col. of Med., Bryan, TX

Abstract: Background: Children prenatally exposed to alcohol or cannabinoids can demonstrate growth deficits and increased expression of neurological disorders, however investigations into the effects of simultaneous alcohol-and-cannabinoid exposure (SAC) on developing neurobiology are currently minimal. During the second trimester, fetal brain vasculature emerges during peak periods of neurogenesis, supporting fetal nutrition, growth, and neural development. Therefore, our research group wanted to investigate whether prenatal polysubstance exposure alters fetal-directed blood during second-trimester exposure.
Methods: We performed high resolution *in vivo* ultrasound imaging in C57Bl/6J pregnant mice. After pregnancy confirmation, dams were assigned to one of four groups: drug-free control, alcohol-exposed, cannabinoid-exposed or SAC-exposed. Drug exposure occurred daily between Gestational Days (G)12-15. For cannabinoid exposure, dams received an i.p injection of cannabinoid agonist CP-55940 (750µg/kg), with controls receiving volume-equivalent saline. For ethanol exposure, dams were placed in ethanol vapor chambers for 30min of inhalation (95% ethanol), and controls were placed in identical chambers without ethanol administration. Dams underwent ultrasound imaging on three days of pregnancy: G11 (pre-exposure), G13.5 (peri-exposure) and G16 (post-exposure).
Results: Preliminary ultrasound data suggest that both alcohol and cannabinoid exposures reduce blood flow acceleration, a measure of arterial resistance, and Velocity-Time Integral (VTI), a metric of stroke volume, in the middle cerebral and internal carotid arteries on G16, 24hrs after drug exposure has ended. Notably, SAC fetuses exhibit an augmented reduction in VTI within these same arteries. In contrast, drug-induced effects on umbilical arterial blood flow begin acutely, with all exposures reducing measures of arterial acceleration and VTI on G13.5. Importantly, no group differences in these measures exist prior to drug exposure (G11).
Conclusions: Our results indicate that prenatal drug exposure may lead to both acute and delayed reductions in fetal-directed blood flow, which can disrupt normal fetal growth and neural development, and SAC may augment deficits specifically in cerebral arterial blood flow.

Disclosures: R. Kumar: None. **S. Rouzer:** None. **A. Sreeram:** None. **R. Miranda:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.08

Topic: A.07. Developmental Disorders

Support: R01 AA028406
F32 AA029866
T32GM135748

Title: Prenatal alcohol and/or cannabinoid exposure during the second-trimester-equivalent in mice leads to sex-specific behavioral impairments in adult offspring.

Authors: *R. FIGUEROA, S. ROUZER, R. KUMAR, R. MIRANDA;
Texas A & M Univ., Bryan, TX

Abstract: Background: Children prenatally exposed to alcohol or marijuana are at greater risk of developing motor impairments, hyperactivity, and drug-seeking behaviors. However, we know little about the effects of simultaneous alcohol and cannabinoid (SAC) exposure, particularly given the increased commonality of SAC in human populations. We therefore investigated whether SAC augments behavioral deficits in exposed offspring compared to alcohol or cannabinoid exposure alone.**Method:** Pregnant C57Bl/6J mice were assigned to one of four groups: drug-free control, alcohol-exposed, cannabinoid-exposed, or SAC-exposed. Drug exposure occurred daily between Gestational Days 12-15. For cannabinoid exposure, dams received an i.p injection of cannabinoid agonist CP-55940 (750µg/kg) or volume-equivalent saline (controls). For ethanol exposure, dams were then placed in ethanol vapor chambers for 30min of inhalation (95% ethanol) or identical chambers without ethanol (controls). Adult male and female offspring (Postnatal Days 90-120) were assessed for a) motor deficits in a Rotarod performance test, b) hyperactivity in a 10min Open Field test, and c) alcohol preference via a 3hr self-administration assessment in their home-cage. **Results:** In male offspring, alcohol exposure reduced time balanced on the Rotarod, distance traveled, and rotations-per-minute achieved. Notably, SAC augmented these motor deficits, impeding rotarod performance further. Interestingly, prenatal exposure had no effect on Rotarod performance in female offspring. Offspring were also assessed in an Open Field assay. Compared to controls, SAC imposed opposite effects between sexes, decreasing time spent in the center of the open field in males, but increasing center time in females. Notably, exposure to alcohol-only in females, and cannabinoids-only in males, increased hyperactivity, including increased speed, distance travelled within the open field, and total times entering the center, while SAC groups demonstrated no differences from controls. Ongoing experiments of ethanol preference in the home-cage demonstrate no differences in self-administration between any exposure groups.**Conclusions:** Our preliminary data suggest that SAC may impose distinct, sex-specific motor impairments in adult offspring. Ongoing experiments will further determine how each exposure influences operant chamber ethanol-seeking behaviors in these offspring.

Disclosures: R. Figueroa: None. **S. Rouzer:** None. **R. Kumar:** None. **R. Miranda:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.09

Topic: A.07. Developmental Disorders

Title: Impaired extinction in female gestational ethanol exposure model mice

Authors: *N. REUVENI, S. BARISELLI, Y. MATEO, D. M. LOVINGER;
Lab. for Integrative Neurosci., Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD

Abstract: Fetal Alcohol Spectrum Disorder (FASD) is caused by the consumption of alcohol during pregnancy. FASD is a heterogenous lifelong disorder characterized by a variety of cognitive deficits. Our research aims to model this disorder in mice to potentially identify novel molecular targets for FASD patients. In this study, we implemented a gestational ethanol exposure (GEE) mouse model to mimic binge-like alcohol exposure from postnatal day 0 (P0) to P10. In mice, this period corresponds to the third trimester of human pregnancy, when mothers recovering from Alcohol Use Disorder tend to relapse. We tested the GEE progeny with an assortment of tasks for cognitive abilities to identify behavioral deficits during early adulthood (P60). We trained adult GEE offspring with an instrumental conditioning task to form action-outcome associations followed by reversal learning to assess their behavioral flexibility. Then, we used a random ratio reinforcement schedule to bias adult GEE mice towards goal-directed actions and tested extinction, when action execution was no longer being reinforced. Although we did not detect deficits in learning and reversal, we observed a sex-specific reduction in the rate of extinction in female GEE mice suggesting behavioral perseverance. A reinstatement session was conducted to exclude motivation deficits. We plan to characterize the neural circuit changes underlying the reduced rate of extinction in female GEE mice to then design strategies to prevent or reverse the behavioral perseveration.

Disclosures: **N. Reuveni:** None. **S. Bariselli:** None. **Y. Mateo:** None. **D.M. Lovinger:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

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Program #/Poster #: 186.10

Topic: A.07. Developmental Disorders

Support: NIH P20GM139762
NIH R21 AA025751

Title: Auditory processing alterations in a mouse model of Fetal Alcohol Spectrum Disorders

Authors: M. DOUCHEY, ***P. RAGUNATHAN**;
Neurolog. Sci., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Fetal alcohol spectrum disorders (FASD) are one of the leading causes of developmental abnormalities worldwide. Individuals with FASD have altered sensory processing and impaired auditory processing in particular. Atypical auditory behaviors have been reported in the majority of FASD children. In this study, we use a model of maternal voluntary alcohol consumption throughout gestation in a mouse model to investigate the effects of prenatal alcohol exposure (PAE) on auditory processing. Longitudinal investigation of auditory brainstem response (ABR) in male and female mice was performed throughout development and adulthood. ABR waves were analyzed to assess hearing sensitivity, neural responsivity and speed of neurotransmission across development. We found that PAE males exhibited increased hearing

thresholds, reduced response amplitudes and prolonged latencies of the ABR during the juvenile period. Although hearing thresholds normalized during the late adolescent period, altered response amplitudes and prolonged latencies were observed. We found increased ratios of amplitudes of ABR waves 2 to 4 relative to wave 1 suggesting that the central auditory system exhibits hyperactivity relative to peripheral activity. Analysis is currently ongoing to investigate the effects of PAE on female offspring. The effects of PAE on acoustic reactivity, sensory filtering, and sensorimotor gating was examined during the late adolescent period. We found that PAE males exhibited increased reactivity to acoustic stimuli and PAE females showed disrupted short-term habituation. Sensorimotor gating was not found to be altered in PAE offspring. Our results show that chronic exposure to alcohol during fetal development can result in perturbations in auditory processing.

Disclosures: M. Douchey: None. **P. Ragunathan:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.11

Topic: A.07. Developmental Disorders

Support: NIH Grant RO1 AA02711
NIH Fellowship F31AA030445

Title: Effects of developmental ethanol exposure on adult cerebellar microglia and Purkinje cells

Authors: *M. Y. CEALIE[1], P. D. DREW[2], A. K. MAJEWSKA[1];
[1]Neurosci., Univ. of Rochester, Rochester, NY; [2]Neurobio. and Developmental Sciences; Neurol., Univ. of Arkansas for Med. Sci., Little Rock, AR

Abstract: Fetal alcohol spectrum disorders (FASD) are the most common cause of non-heritable, preventable mental disability. They occur in almost 5% of births in the U.S, leading to a wide range of cognitive, behavioral, and physical impairments, including deficits related to the cerebellum. There is no known cure for FASD, underscoring the importance of research to elucidate the biological mechanisms that translate developmental alcohol exposure to neuropathology. We examine the effects of ethanol on a cellular level to better understand these mechanisms. Microglia, the immune cells of the Central Nervous System, as well as Purkinje cells, the sole output of the cerebellum, are both impacted by developmental ethanol exposure. Alterations in Purkinje cell firing, immune activation of microglia, and reduced numbers of both cell types have been reported. Microglia are known to shape neuronal circuit development and connectivity in the cerebellum, and these functions of microglia are linked to their structural dynamics. How ethanol affects these dynamics and how that impacts microglia-Purkinje cell interactions in the long term is unknown. When ethanol was given to adult mice, cerebellar microglia displayed reductions in motility and surveillance, particularly in the Purkinje cell layer

of the cerebellum. Here in our exploratory study, we examine the dynamics of cerebellar microglia and their interactions with Purkinje cells in adult animals of both sexes that were developmentally exposed to ethanol, in order to understand how ethanol impacts these cells in the long-term. We used a mouse model of human third trimester exposure, whereby L7-cre/Ai9/CX3CR1-GFP mice (in which microglia and Purkinje cells are fluorescently labeled) were treated with ethanol or saline from P4-9. Mice underwent in vivo two-photon imaging in adulthood to visualize cerebellar microglia morphology, microglia dynamics, including microglia motility and surveillance, as well as microglia interactions with Purkinje cells. We found no significant changes in cerebellar microglia motility or surveillance in adult animals exposed to ethanol in development. We are currently exploring cerebellar microglia morphology, which may show larger effects related to an immune response. We are also examining if microglial interactions with Purkinje neurons are affected by developmental ethanol. Elucidating how ethanol induces changes in microglial dynamics, morphology, and interactions may be critical for understanding the long-term effects of ethanol exposure.

Disclosures: **M.Y. Cealie:** None. **P.D. Drew:** None. **A.K. Majewska:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

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Program #/Poster #: 186.12

Topic: A.07. Developmental Disorders

Support: University of Illinois Research Board
NIH P01 ES002848-Project 3

Title: Effects of developmental phthalate exposure on hippocampal-influenced behaviors in juvenile and adult rats

Authors: *E. P. SELLINGER[1], A. S. BRINKS[1], T. BOZIC[2], J. M. JURASKA[2];
[1]Neurosci. Grad. Program, [2]Psychology, Univ. of Illinois Urbana-Champaign, Champaign, IL

Abstract: The extensive neurodevelopment during the perinatal period is sensitive to environmental influence including that of endocrine-disrupting chemicals, phthalates. These plasticizers are widely used in consumer products, most prominently in food packaging/processing, personal care products, and medical tubing leading to ubiquitous human exposure. In humans, there is a correlation between developmental phthalate exposure and impaired cognitive and emotional processing outcomes in children (Radke et al., 2020), however establishing causation in humans is not possible. Using a rat model, our lab has shown exposure to an environmentally relevant mixture and dose of phthalates during the perinatal period results in elevated developmental cell death in the medial prefrontal cortex (mPFC) (Sellinger et al., 2021), fewer neurons in this region in adulthood and impaired cognitive flexibility (Kougias et al. 2018). More recently, we observed similar changes in developmental apoptosis as a result of

perinatal phthalate exposure in the hippocampus, particularly the ventral region (unpublished observations). The mPFC sends direct connections to dorsal hippocampus while receiving direct projections from ventral hippocampus (Sigurdsson and Duvarci, 2016) and together, these regions mediate several cognitive behaviors including social memory (Sun et al., 2020), anxiety-like behavior (Ciocchi et al., 2015), and working spatial memory (Sigurdsson and Duvarci, 2016). The current study therefore asks if phthalate exposure during neurodevelopment leads to immediate and/or lasting changes in behaviors involving these regions.To expose developing pups to the phthalate mixture, dams were fed 0, 0.2, or 1 mg/kg/day on a cookie from gestational day 2 through 25 days post-partum. One male and one female offspring per litter were tested on the elevated plus maze (EPM) on P27 followed by the Morris Water Maze (MWM) beginning on P29. In the EPM, a significant effect of phthalate exposure was seen only in males (n=5-6/group) where those exposed to 1mg/kg spent a higher percentage of time in the open arms ($p = 0.04$) and had a decreased latency to enter the open arm for the first time compared to controls ($p = 0.02$). However, no apparent effects of phthalate exposure were seen in preliminary analyses (n=5-6/group) of the MWM. To better understand the potential lasting effects of early phthalate exposure on behaviors mediated by mPFC and hippocampal connectivity, we are currently running behavioral tests of social memory along with the MWM beginning on P70.

Disclosures: E.P. Sellinger: None. **A.S. Brinks:** None. **T. Bozic:** None. **J.M. Juraska:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.13

Topic: A.07. Developmental Disorders

Support: University of Illinois Research Board
NIH P01 ES002848-Project 3

Title: Specificity of increased apoptosis following developmental phthalate exposure in the rat medial prefrontal cortex and hippocampus

Authors: *M. KING[1], T. BOZIC[1], E. P. SELLINGER[2], J. M. JURASKA[3];
[1]Univ. of Illinois Urbana-Champaign, Champaign, IL; [2]Neurosci. Grad. Program, Univ. of Illinois Urbana-Champaign, Urbana, IL; [3]Psychology, Univ. of Illinois Urbana-Champaign, Champaign, IL

Abstract: Everyone comes into contact with phthalates, a class of endocrine- disrupting chemicals that comprise many consumer products like food packages and hygiene products. Using a rat model, our lab has shown perinatal exposure to an environmentally relevant mixture and dose of phthalates in rats leads to fewer neurons in the adult mPFC and deficits in cognitive flexibility (Kougias et al. 2018). Furthermore, perinatal phthalate exposure leads to higher levels of developmental cell death in the medial prefrontal cortex (mPFC) measured on postnatal (P)

day 10 (Sellinger et al., 2021). Therefore, we are examining phthalate-induced cell death in the mPFC at a second earlier timepoint to see if the effects of phthalates generalize to a different postnatal age as well as measuring these effects in a second neural structure associated with cognition, the hippocampus, to explore the regional specificity of phthalate action.To expose developing offspring, pregnant dams were dosed orally with 0, 0.2, or 1mg/kg of the phthalate mixture from gestational day 2 through 10 days after birth. One male and one female per litter were sacrificed on P5, and mPFC and hippocampal tissue was stained for TUNEL, a marker of apoptotic cells. Preliminary data shows no significant effect of phthalates on cell death in the mPFC in either sex (n=4-5/group). However, there was a significant effect of phthalates and a phthalate by subregion interaction within the hippocampus in both sexes (n= 6-8/group). Phthalates altered cell death only within dorsal CA1 (p=0.0008) where both doses of phthalates caused more cell death than controls. Both dorsal and ventral CA3, as well as ventral CA3, were unaffected. More subjects will be added to all groups to better understand our findings. At this point, the effects of phthalates on apoptosis are specific to age in the mPFC and region within the hippocampus.

Disclosures: **M. King:** None. **T. Bozic:** None. **E.P. Sellinger:** None. **J.M. Juraska:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.14

Topic: A.07. Developmental Disorders

Support: R01-AA027269

Title: Discovering the potential for an adolescent intervention to restore corpus callosum myelination and functionalconnectivity in the adult brain using a rodent model of Fetal Alcohol Spectrum Disorders

Authors: *K. MILBOCKER[1], P. P. KULKARNI[2], D. ATHREYA[2], E. BRENGEL[1], S. KEDHARNATH[2], C. F. FERRIS[2], A. Y. KLINTSOVA[3];
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Abstract: Fetal Alcohol Spectrum Disorders (FASD) is a class of developmental disorders that may result from prenatal alcohol exposure (AE) and affects 1 in 9 infants annually (CDC, 2019). FASD-affected youth suffer from impairments to executive function due to abnormal functional connectivity of the attention networks, increasing the risk for breaking the law (Ware et al. 2021; McLachlan et al., 2020). Importantly, diminished executive function capacity is linked to disruptions in corpus callosum growth and myelination during adolescence (Jacobson et al., 2017; Kar et al., 2021; Treit et al., 2013, Newville et al., 2017 and 2021; Creeley et al., 2013).

However, targeted interventions that support neurodevelopment in FASD-affected youth are nonexistent. This preclinical study investigates the potential for a myelin-stimulating adolescent intervention to restore the development of corpus callosum. Female Long-Evans rat pups underwent either intragastric intubation of alcohol in milk substitute (5.25 g/kg/day) or intubation without AE (sham-controls) from postnatal days (PD) 4 through 9 in a model of third-trimester equivalent AE (Milbocker & Klintsova, 2020). Adolescent rats from treatment and control groups were randomly selected to receive voluntary exercise intervention or remain as sedentary controls on PD 30-42. Previous work from our lab has shown that this intervention mitigates neuroanatomical impairments in gray matter regions when applied to this model of FASD (Klintsova et al., 2013). Lasting changes to brain volume using voxel-based morphometry, white matter microarchitecture using diffusion weighted imaging and functional connectivity with connectomics were assessed in adulthood. The data for each imaging modality was registered to a 3D MRI rat atlas with over 170 segmented brain areas providing site specific information on altered brain structure and function. Indices of water diffusion showed significant differences between AE sedentary and intervention-exposed rats in brain areas associated with memory (hippocampus), emotion (central amygdala) and motivation (nucleus accumbens). Changes in brain region volumes were nominal. Moreover, preliminary analysis of ultrastructural changes to myelin ensheathment via electron microscopic analysis demonstrates that AE significantly increases the number of atypically-myelinated axons ($F(3,8) = 7.1$, $p = 0.03$, $\eta 2 = 0.47$) in rostral corpus callosum in all rats. Further, the interaction between postnatal treatment group and intervention exposure is approaching significance ($p = .05$), suggesting that exercise intervention reduces the number of atypically-myelinated axons in the AE brain.

Disclosures: K. Milbocker: None. **P.P. Kulkarni:** None. **D. Athreya:** None. **E. Brengel:** None. **S. Kedharnath:** None. **C.F. Ferris:** None. **A.Y. Klintsova:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

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Program #/Poster #: 186.15

Topic: A.07. Developmental Disorders

Support: NIH/NIAAA Grant R01AA027269

Title: Reduced oligodendrocyte-related gene expression and epigenetic markers in mPFC and corpus callosum in a rodent model of third trimester alcohol exposure

Authors: *I. F. SMITH, K. A. MILBOCKER, T. L. ROTH, A. Y. KLINTSOVA;
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Abstract: Fetal Alcohol Spectrum Disorders (FASDs) are a group of prevalent but preventable developmental disorders that result from alcohol exposure (AE) *in utero*. FASDs are a major concern for public health, as an estimated 1.1 - 5% of children born in the United States are

affected (May et al., 2018). Some morphological changes in the brain brought on by AE are improper myelination and growth of white matter tracts connecting cortical structures to deeper brain regions (Wilhelm and Guizzetti, 2016; Mathews et al., 2021). Indeed, myelination of white matter is disrupted in children and adolescents with AE (Jacobson et al., 2017; Kar et al., 2021; Treit et al., 2013). Deficits in the proliferation and differentiation of oligodendrocyte precursor cells (OPCs) have been linked to many neurological disorders, including developmental disorders (Berry & Lu, 2020). Patterns of epigenetic modifications have been closely associated with cell fate specification and differentiation, suggesting a crucial role for epigenetic mechanisms in the regulation of such cellular functions. Of note, class I histone deacetylases (HDACs) such as HDAC1 and HDAC3 are highly involved in regulating OPC development (Berry and Lu, 2020). This study investigated the effect of binge AE (5.25 g/kg/day, two doses 2 hours apart) during postnatal days (PD) 4-9 on *Hdac1* and *Hdac3* expression, as well as gene expression of the OPC marker platelet-derived growth factor receptor A (*Pdgfra*) and the mature oligodendrocyte product myelin basic protein (*Mbp*) in male and female Long Evans rat pups at both neonatal and juvenile timepoints. On PD10 or PD15, brains were extracted and flash frozen for later biochemical procedures. RNA was isolated from medial prefrontal cortex (mPFC) and rostral corpus callosum (rCC), and RT-PCR was used to assess quantitative expression of gene targets. Data were analyzed using two-way ANOVAs which revealed a significant main effect of postnatal treatment on *Mbp* expression in rCC at PD10 [$F(2, 45) = 3.428$, $p = 0.0411$]. *Post hoc* analyses revealed significantly lower gene expression in AE males compared to suckle control males, suggesting an effect of the combined intubation procedure and AE in males at PD10. Additionally, there was a significant main effect of postnatal treatment on *Hdac3* expression in mPFC at PD10 [$F(2, 46) = 7.093$, $p = 0.0021$]. *Post hoc* analyses revealed significantly higher gene expression in suckle control animals compared to either AE or sham intubated animals, suggesting an effect of intubation stress on *Hdac3.* This ongoing study will help us understand the early onset mechanisms by which AE affects OPC development and subsequent gray and white matter myelination.

Disclosures: I.F. Smith: None. **K.A. Milbocker:** None. **T.L. Roth:** None. **A.Y. Klintsova:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

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Program #/Poster #: 186.16

Topic: A.07. Developmental Disorders

Support: John Templeton Foundation.

Title: Paternal Cannabis Exposure Prior to Conception Causes Transgenerational Neurobehavioral Impairment

Authors: A. B. HAWKEY, A. GONDAL, M. JONES, A. H. REZVANI, S. K. MURPHY, *E. D. LEVIN;
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Abstract: Previously, we found that in male rats, chronic exposure to a moderate dose of cannabis extract (4 mg/kg/day delta9-THC) for 28 days prior to mating causes significant alterations in the methylation of their sperm DNA and persistent neurobehavioral effects in their offspring. This study progressed to the F2 generation, mating male offspring of studs exposed to cannabis extract (4 mg/kg/day THC, daily or 2 consecutive days per week) or vehicle treated controls to produce an additional generation. Interestingly, F1 offspring from the cannabis exposed groups, showed behavioral effects that were not seen in the F2 offspring, and vice versa. In pre-weanling rats, F1 offspring showed no treatment effects on righting reflex (PND 2-6) or negative geotaxis (PND 7-13), but F2 offspring from the daily cannabis group showed initial impairments in the righting reflex and both cannabis groups showed overall deficits in the negative geotaxis response. By contrast, F1 offspring of males exposed to cannabis daily showed altered physical development. These F1 offspring showed elevated body weights from PN17-28, and altered anogenital distances, with both males and females showing increased AGD at PN17 and males showing increases at PN21. Furthermore, F1 females showed more rapid habituation of locomotor behavior in adolescence, but not adulthood. F2 offspring did not show this locomotor effect, but preliminary data indicate that grand-paternal cannabis exposure disrupts typical sex differences in locomotion in adulthood (female levels decrease, male levels increase). Further behavioral assessment of locomotor activity, emotional function and cognition are ongoing. Paternal cannabis exposure can affect intergenerational and transgenerational neurobehavioral development, including effects that attenuate or emerge in the later generation.

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Poster

186. Developmental Disorders: Non-Genetic Models

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Program #/Poster #: 186.17

Topic: A.07. Developmental Disorders

Support: Institute for Cannabis Research
Colorado Clinical and Translational Science TL1 TR002533
University of Colorado Diabetes Research Center

Title: Fetal cannabidiol (CBD) exposure impacts brain development and postnatal behavior

Authors: *K. S. SWENSON[1], L. E. GOMEZ WULSCHNER[2], W. C. OH[2], E. A. BATES[1];
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Abstract: Pregnant people consume cannabidiol (CBD) to help with nausea because it is readily available and public perception is that it is safe. CBD is the non-psychoactive component of marijuana that is federally legal and sold commercially across the United States. **Consumption of marijuana during pregnancy is increasing in the U.S., with umbilical cord tissue showing marijuana exposure rates as high as 22% in Colorado.** Maternally consumed CBD and THC diffuse across the placenta to the fetus and accumulate in the fetal brain, liver, and other fatty tissues. Clinical studies suggest that fetal marijuana exposure is associated with poor birth outcomes, and increased rates of anxiety and attention deficit and hyperactivity disorder (ADHD) at puberty. However, these studies are confounded by inadequate dosing information and inability to distinguish the impact of CBD from THC**.** Little is known about how **CBD exposure affects brain development and behavior.** CBD activates the Transient Potential Villanoid 1 Receptor (TRPV1) and the Serotonin 1A Receptor (5HT1AR) which are expressed in the developing brain. Excessive activation of TRPV1 and 5HT1AR by fetal CBD exposure could have adverse effects on brain development. For example, dysregulation of these receptors in-utero can induce physiological and behavioral changes across a lifetime. Postnatally, these receptors mediate critical central nervous system (CNS) functions and can impact behavior, including anxiety, obsessive compulsive disorder, cognition, and thermal pain sensitivity. We administered CBD (50mg/kg) or vehicle to mice daily throughout pregnancy. To elucidate the impact of CBD exposure on development of the hypothalamus, we conducted single cell RNA sequencing on the hypothalamuses from the postnatal day (P)1.5 pups. This data revealed differences in gene expression within the hypothalamus based on CBD exposure and offspring sex that predicted changes in neuronal function. To determine if CBD affects postnatal behavior, we measured cognition, memory, anxiety, pain, and compulsivity in female and male mice exposed to CBD or vehicle during fetal development. We show fetal CBD exposure reduces cognition in female offspring. Fetal CBD exposure increases sensitivity to thermal pain in male offspring, an effect that is dependent on TRPV1. Preliminary electrophysiology data supports the hypothesis that fetal CBD exposure renders layer 2/3 pyramidal neurons in the prefrontal cortex less excitable through 5HT1AR activation, though they have a similar resting membrane potential. Combined, these data suggest that intra uterine CBD exposure could be harmful to fetal brain development.

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Poster

186. Developmental Disorders: Non-Genetic Models

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Program #/Poster #: 186.18

Topic: A.07. Developmental Disorders

Support: ReBUILDetroit Bridge Award

Title: Opioid administration during pregnancy: Effects of morphine compared to buprenorphine exposure on offspring neurodevelopmental outcomes in a translational rodent model

Authors: *A. M. MYERS[1], L. RICHARDSON[1], S. NEOLE[1], N. KULAGLIC[1], C. M. WALLIN[1], C. J. DAVIDSON[2], S. A. PERRINE[2], S. E. BOWEN[1], S. BRUMMELTE[1]; [1]Psychology, [2]Psychiatry and Behavioral Neurosciences, Wayne State Univ., Detroit, MI

Abstract: Opioid use during pregnancy has increased drastically within the last several years. To avoid harmful opioid-induced effects on the fetus, pregnant women who use opioids are often prescribed Medications for Opioid Use Disorder (MOUDs), including buprenorphine (BUP) or methadone. However, exposure to synthetic opioids during pregnancy is known to negatively impact offspring neurodevelopment. Importantly, it is not yet understood how gestational BUP exposure may affect fetal brain development. In the current study, we used a translational rodent model to investigate offspring neurodevelopmental outcomes following discontinued or continued administration of morphine (MS; to mimic opioid use disorder) or BUP (to mimic MOUD treatment). Seven days prior to pregnancy, female rats were administered either MS (3-6.0mg/kg, b.i.d., s.c.) or BUP (1.0mg/kg, q.d., s.c.) until gestational day (GD) 19 ('discontinued', mimicking withdrawal before parturition) or until postnatal day 2 (PN2, 'continued' through parturition). Pups were sacrificed on PN2, and brains and truck blood were collected for subsequent analysis. Both continued and discontinued BUP exposure resulted in higher pup mortality, reduced body weight, and increased neonatal withdrawal symptoms compared to MS-exposed pups and controls. On PN2, discontinued BUP exposure resulted in fewer visible milk bands in pups compared to other groups, while discontinuation of both opioids resulted in an increase in righting latency compared to the continued groups. Preliminary results indicate that BUP levels were very low in pups on PN2, especially in the discontinued group; while pups exposed to continued MS seemed to accumulate drug in their system (i.e., elevated serum levels compared to dams'). Offspring brains are currently being analyzed for neurotransmitter levels (dopamine, serotonin, norepinephrine, and common metabolites DOPAC, 5-HIAA, HVA & 3-MT) in the prefrontal cortex, hypothalamus, and hippocampus. This will allow us to measure the impact that gestational exposure to these opioids has on neurotransmitters during early fetal development. Taken together, our results suggest that BUP use during pregnancy negatively influences pup survival despite very low levels of BUP in the pups' system after birth. More research is crucial to investigate the underlying mechanisms associated with offspring outcomes following prenatal BUP exposure with the goal of improving clinical outcomes in pregnant women undergoing treatment for opioid use disorder.

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Poster

186. Developmental Disorders: Non-Genetic Models

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Program #/Poster #: 186.19

Topic: A.07. Developmental Disorders
NHMRC
PhD scholarship_Monash University
PhD Merit Scholarship_IsDB

Title: Fetal growth restriction induces damage to the dorsal motor nucleus of the vagus that is evident near-term

Authors: *E. AHMADZADEH, I. DUDINK, A. E. SUTHERLAND, Y. PHAM, V. STOJANOVSKA, G. R. POLGLASE, B. J. ALLISON, S. L. MILLER;
Dep. Obstetrics and Gynaecology, Fac. of Medicine, Nursing and Hlth. Science, Monash Univ., The Ritchie Ctr. | Hudson Inst. of Med. Res., Melbourne, Australia

Abstract: Introduction: The dorsal motor nucleus of the vagus (DMNV) is an elongated cardiorespiratory centre within the brainstem that innervates the thorax and abdomen with parasympathetic motor impulses and controls the heart rate under normal conditions. Fetal growth restriction (FGR) is caused by placental dysfunction, which limits oxygen and nutrients available to the fetus. In response to fetal hypoxia, the brainstem mediates an adaptive cardiovascular response, known as brain sparing, to favour oxygen supply to vital organs, such as the brain and heart. However, brain injury still occurs in FGR offspring. Cardiovascular dysfunction is also evident in babies born FGR, thought to be due to the vascular adaptations to hypoxia in utero. However, we hypothesise that differential development of the brainstem cardiorespiratory centres, such as the vagus nerve (CNX) nuclei, in growth restricted fetuses may contribute to cardiovascular complications postnatally. **Methods:** We induced FGR at 88 days (d) gestation in our ovine model (term is 147d). Using histopathological analysis, possible structural deficits were characterised in the three main CNX nuclei including the DMNV, nucleus ambiguus (NA), and the nucleus tractus solitarius (NTS), from FGR and control fetal sheep at gestational ages 110d (extremely preterm), 127d (preterm) and 138d (near-term). These time points were comparable to 29-, 33- and 36 weeks gestation in human. **Results:** Within the growth restricted brain tissues, there was increased oxidative stress, neuroinflammation and cell death, and decreased cell proliferation in the NA and NTS at 110, 127 and 138d. However, no histopathology was detected in the DMNV in FGR at 110 and 127d. Interestingly, neuropathology was only seen in the DMNV at 138d in the FGR group compared to the control. The sympathetic control centre of the cardiovascular tone, known as the rostral/caudal ventrolateral medulla (C/RVLM) was spared from damage. **Conclusion:** FGR is associated with altered cellular development of the critical parasympathetic control centres in the medulla oblongata, such as the CNX nuclei, including the NA and NTS in all fetal ages studied, and the DMNV only in near-term growth restricted fetuses.

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Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.20

Topic: A.07. Developmental Disorders

Title: Early IGF-1 receptor inhibition in mice mimics human preterm brain disorders

Authors: *A. POTENZIERI[1,2], S. UCCELLA[3], D. PREITI[3], S. ROSATI[1], C. LAVARELLO[3], D. DEBELLIS[1], F. CATALANO[1], R. MAROTTA[1], A. PETRETTO[3], V. TUCCI[1], L. RAMENGHI[3], A. SAVARDI[1], L. CANCEDDA[1];
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Abstract: Recent advances in neonatal care units have sharply increased the survival of preterm newborns and drastically decreased the occurrence of severe brain lesions and associated motor deficits in premature infants. Nevertheless, nowadays preterm newborns still present abnormalities in functional connectivity, myelination deficits and behavioural alterations, including cognitive and social deficits, and repetitive behaviours. Interestingly, human literature reported female gender as generally protected against preterm brain related complications, but with some emotional/anxiety difficulties.Insulin like Growth Factor 1 (IGF-1) is a major fetal growth hormone *in utero*, especially during the third trimester of pregnancy. As a consequence of the premature birth, preterm newborns fail to receive IGF-1 from the mother's placenta. Thus, serum levels of IGF-1 in preterm newborns are lower compared to the corresponding *in utero* levels. In this study, we mimicked in mice the IGF-1 deficiency of preterm newborns (*i.e.*, 23-32 weeks of human gestational age), by systemic administration of the IGF-1 receptor specific antagonist JB1 (0.018 mg/kg; subcutaneously, once daily from postnatal day (P)1 to P5). Behavioral analysis during mouse adolescence showed that only JB1 treated male mice performed poorly in a spatial and recognition memory test, and exhibited social deficits and repetitive behavior. Nevertheless, JB1 female mice showed increased anxiety behavior when evaluated in the elevated plus maze test. Furthermore, immunofluorescence and electron microscopy analysis showed hypomyelination in white matter area and reduced number of interneurons in the hippocampus and prefrontal cortex of JB1 treated mice, anatomical features described in human preterm. Finally, patch-clamp recording in acute hippocampal slices uncovered increased aberrant neuronal excitability in the hippocampus of adolescence JB1 treated male mice, suggesting a role of GABAergic circuits in the sex specific behavioural outcomes.In conclusion, our study demonstrate a causal relationship between reduced IGF-1 signaling during the critical period of prematurity and the development of preterm brain disorder like phenotype in mice. This discovery supports the rational of IGF-1 supplement in preterm newborns and establishes a reliable experimental animal model to dissect mechanisms involved in preterm brain disorder.

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Poster

186. Developmental Disorders: Non-Genetic Models

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Program #/Poster #: 186.21

Topic: A.07. Developmental Disorders

Support: R56 MH119435
R01MH122471
NIH/NIMH R01MH122471

Title: Long term axonal defects in mouse models of anti-NMDA receptor encephalitis associated with persistent behavior deficits

Authors: *J. ZHOU, A. L. GREENFIELD, R. LOUDERMILK, C. M. BARTLEY, B. T. TRAN, H. WANG, M. R. WILSON, S. J. PLEASURE;
Univ. of California San Francisco, Univ. of California San Francisco, San Francisco, CA, CA

Abstract: Anti-NMDA receptor encephalitis characterized by anti-NMDA receptor autoantibodies in cerebrospinal fluid, manifests with prominent sensory-motor coordination deficits amongst a variety of other neurologic symptoms. We have demonstrated that the NMDA receptor controls callosal projections in primary somatosensory cortex (S1), which is a key structure for integrating sensory-motor information. Here, we generated monoclonal antibodies from an anti-NMDA receptor encephalitis patient and identified antibodies against GluN1 and GluN2A subunits. In mice, both antibodies disrupt callosal circuit leading to overabundance of callosal projections in S1, with anti-GluN1 antibody provoking the more severe disruption. The disruption of the S1 circuit during development caused permanent axon morphology alterations and sensory-motor deficits in adulthood, most importantly, the severity of sensory-motor deficits was positively correlated with the severity of axon morphology alterations in S1. Our study confirms the central role of anti-GluN1 and anti-GluN2A autoantibodies in the pathogenesis of anti-NMDA receptor encephalitis and reveals that persistent anatomic changes associate with behavior deficits.

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Poster

186. Developmental Disorders: Non-Genetic Models

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Topic: A.07. Developmental Disorders

Support: Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience (project " Experimental and clinical research of hyoxic ischemic damage in

perinatal and adult brain" KK01.1.1.01.0007
Croatian Science Foundation (project "Brain extracelular matrix in development and in perinatal hypoxia" IP-2019-04-3182

Title: Quantitative in-vivo magnetic resonance imaging of moderate perinatal hypoxia-related changes in a rat brain

Authors: M. DRLJE[1], S. TRNSKI[1], A. ŠTAJDUHAR[1,3], B. NIKOLIć[4], M. DRLJE[1], M. BOBIć-RASONJA[1,2], D. HRANILOVIć[4], S. ŠKOKIć[1], *N. JOVANOV MILOSEVIC[1,2]; [1]Croatian Inst. for Brain Res., [2]Dept. of Biol., Sch. of Medicine, Univ. of Zagreb, Zagreb, Croatia; [3]Dept. of Med. Statistics, Epidemiology and Med. Informatics, "Andrija Štampar" Sch. of Publ. Hlth., Zagreb, Croatia; [4]Dept. of Biol., Fac. of Sci., Zagreb, Croatia

Abstract: AimThe in-vivo MRI study of juvenile rats aims to detect neuroradiological structural and volumetric changes consequently to a moderate perinatal hypoxic brain lesion.
MethodsSixteen male Wistar Han (RccHan: WIST) rats were subjected to moderate hypoxia (8% O2, 92% N2/2h, (8 treated and 8 control) on the first postnatal day (P1). On P15, MRI T2-weighted scans were obtained in-vivo, and brain volume was quantified after manual delineation of regions of interest, using ITK-SNAP and custom scripts in Python 3.8. In addition, 16 post-hypoxia (8 males, 8 females) rats were sacrificed on P50, brains were instantly isolated, and immediately weighed on a digital scale. Due to the sex-related difference in size, brain mass was compared separately in males and females by student t-test.
ResultsThe rats subjected to hypoxia show an indicative increase in olfactory bulb volume fraction (VF) median from the total brain volume (TBV; 4.36+- 0.78%) and 4.55+- 0.37%, p=0,0734). We found no difference between groups in the TBV or ventricular system VF medians. Post-hypoxic adult animals displayed a slight but significant increase in the brain mass in both males(1,81+- 0.06 g and 1.72 +- 0.03 g, respectively, p= 0.0032) and females (1.67+- 0.05 g and 1.54+- 0.15 g, respectively, p=0.0448), in comparison to control rats.
ConclusionFurther investigation is needed to understand the underlying mechanisms of demonstrated brain volume and mass changes after moderate perinatal hypoxia.

Disclosures: M. Drlje: None. **S. Trnski:** None. **A. Štajduhar:** None. **B. Nikolić:** None. **M. Drlje:** None. **M. Bobić-Rasonja:** None. **D. Hranilović:** None. **S. Škokić:** None. **N. Jovanov Milosevic:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.23

Topic: A.07. Developmental Disorders

Title: Gestational organophosphorus pesticide exposure alters the development of the rat somatosensory cortex

Authors: *J. A. KOENIG, C. HAGA, A. KELLER;
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Abstract: Organophosphorus (OP) pesticides are used widely in agriculture and act by inhibiting the enzyme acetylcholinesterase (AChE). As AChE is highly conserved, OP exposure additionally poses a real risk to human health. This is of particular concern when the exposures occur *in utero*, as it is correlated with an increased prevalence of neurodevelopmental disorders such as autism spectrum disorder and ADHD. The causative mechanisms remain unclear. This project uses a rat model of gestational exposure to the OP compound, chlorpyrifos, to measure structural and functional detriments imparted on the primary somatosensory cortex, a translationally relevant brain region. Rat dams were exposed daily to 5.0 mg/kg chlorpyrifos subcutaneously on gestational days 18-21. This exposure produced no overt signs of toxicity. Neonatal tissue collected on PND 0 revealed an approximately 50% reduction of AChE activity in the forebrain, hindbrain, and heart. Cytochrome oxidase staining of the somatosensory cortex on PND 5-7 showed a delay in the establishment of the barrel field pattern following chlorpyrifos exposure, with no discernable difference seen at PND 21. To measure functional alterations, *ex vivo* slice electrophysiology was utilized in conjunction with a spike-timing dependent plasticity protocol. A post-before-pre (-25ms) negative timing pairing displayed a delayed shift in the initiation of the L4 to L2/3 critical period of plasticity in the primary somatosensory cortex. There was no difference in spontaneous EPSC frequency or amplitude, or in the firing rate/current relationship (>PND 12). These results offer new evidence for a possible mechanism underlying the neurodevelopmental disorders associated with gestational OP exposure and could potentially guide the development of new therapies and improved strategies for the use of organophosphorus pesticides in and around residential communities.

Disclosures: J.A. Koenig: None. **C. Haga:** None. **A. Keller:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.24

Topic: A.07. Developmental Disorders

Support: TRDRP 28IP-0026
5R37AA012446-17

Title: Postnatal choline supplementation mitigates the effects of prenatal THC exposure on spatial memory in rats

Authors: *K. J. THOMAS, C. G. RODRIGUEZ, J. D. THOMAS;
Ctr. for Behavioral Teratology, San Diego State Univ., San Diego, CA

Abstract: Cannabis and tobacco are among the most commonly used drugs among pregnant women. Increasingly, both of these drugs are being consumed via electronic cigarettes (e-

cigarettes), which also allow for cannabis products and nicotine to be easily combined. However, prenatal exposure to either drug may place the fetus at a higher risk for developing cognitive impairments. Interestingly, clinical evidence suggests that higher maternal choline levels are associated with reduced risk to the adverse effects of marijuana on the fetus. Choline is an essential nutrient that is critical for brain development. Thus, the present study used a rodent model to investigate the ability of early choline supplementation to modify the effects of prenatal exposure to nicotine, tetrahydrocannabinol (THC), and the combination on cognitive function. Pregnant Sprague-Dawley dams were placed into a vapor inhalation chamber from gestational days (GD) 5-20. Dams were exposed to either THC (100 mg/mL), nicotine (36 mg/mL), the combination, or propylene glycol (vapor vehicle control), via e-cigarette (a 6-second puff every 5 min for 30 minutes per day). On GD 22, dams gave birth, and one sex pair/litter was assigned to treatment group. From postnatal day (PD) 10-30, male and female offspring received s.c. injections of either choline chloride (100 mg/kg) or saline vehicle. From PD 40-46, offspring were tested on a Morris Water Maze spatial learning and memory task. Prenatal nicotine did not impair spatial learning or memory. However, prenatal exposure to THC impaired spatial memory in females, with those exposed to both THC and nicotine showing the most severe deficits. Importantly, THC-exposed females who also received choline treatment exhibited improved spatial memory, not differing from control levels. Females exposed to the combination of nicotine and THC and who received choline also showed reduced thigmotaxis, indicating less anxiety behavior. These data indicate that choline can mitigate memory deficits associated with prenatal THC exposure, particularly when combined with nicotine, and may also modify emotional behavior. Importantly, these preliminary findings suggest that choline administered postnatally may reduce the severity of prenatal cannabis effects.

Disclosures: K.J. Thomas: None. **C.G. Rodriguez:** None. **J.D. Thomas:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.25

Topic: A.07. Developmental Disorders

Support: AA012446

Title: Choline supplementation mitigates alterations in hippocampal neurotrophic support caused by developmental ethanol exposure

Authors: *S. LIANG, C. RODRIGUEZ, Z. KELSO, J. D. THOMAS;
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Abstract: Fetal Alcohol Spectrum Disorders (FASD) refer to the range of outcomes observed in individuals exposed to alcohol prenatally and constitute a serious, but preventable, public health concern. In fact, FASD affects an estimated 0.77% of the global population, with much higher

rates in many areas, including the U.S. where estimates range from 1-5%. As a potent teratogen, ethanol (EtOH) can negatively affect the sensitive fetal environment, leading to neurotoxicity and a range of cognitive deficits. Interestingly, both clinical and preclinical studies have shown that supplementation with the essential nutrient choline can reduce the severity of neurobehavioral and cognitive alterations associated with prenatal alcohol exposure, particularly those that depend on the functional integrity of the hippocampus. Importantly, the recovery in behavioral deficits occur even when choline is provided postnatally, after the alcohol insult. Although postnatal choline supplementation appears to improve FASD outcomes, the molecular mechanism of choline in conferring these benefits remains unclear. Prenatal alcohol exposure is known to disrupt neurotrophic support; thus, choline could be improving function by enhancing neurotrophic factors. Using a third-trimester equivalent model of EtOH exposure, we examined the effects of early alcohol and postnatal choline supplementation on brain-derived neurotrophic factor (BDNF) signaling. Sprague-Dawley rats were exposed to EtOH on Postnatal Days (PD) 4-9 via intubation, at a dose of 5.25 g/kg/day. Controls were given sham intubations. From PD 10-30, half of the subjects received 100 mg/kg s.c. injections of choline chloride and the other half received saline vehicle. Hippocampal tissue was microdissected and collected on PD36 for protein analysis. Using Western Blots, we quantified levels of hippocampal BDNF, its receptor tropomyosin receptor kinase B (TrkB), as well as downstream signaling molecules. Developmental EtOH exposure significantly reduced the levels of mature BDNF without affecting the total amount of BDNF protein present. Importantly, this reduction in BDNF was recovered with choline supplementation. In fact, BDNF levels did not differ between EtOH-exposed subjects treated with choline and controls. Additionally, there were no group effects on TrkB receptors. Thus, these data suggest that choline supplementation acts to restore deficits in hippocampal BDNF signaling in FASD, which may support neuroplasticity and behavioral function.

Disclosures: **S. Liang:** None. **C. Rodriguez:** None. **Z. Kelso:** None. **J.D. Thomas:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.26

Topic: A.07. Developmental Disorders

Title: Rescue of a neurodevelopmental disorder by induction of the heat shock response

Authors: V. BORISOV, N. LEVY, E. LAURY, S. HALPERT, *A. P. LEVY;
Technion Israel Inst. of Technol., Technion Israel Inst. of Technol., Haifa, Israel

Abstract: Purposeful induction of fever for healing, including the treatment of epilepsy, was proposed over two thousand years ago by Hippocrates. More recently, fever has been associated with the abatement of many of the social behavioral abnormalities seen in autism spectrum disorder. However, the lack of mechanistic studies exploring the mechanism for the protection

by fever, due to in large part to the lack of appropriate human disease models, has limited enthusiasm for this mode of therapy. We have focused on a human neurodevelopmental disorder, caused by a missense mutation in the IQSEC2 gene (A350V), resulting in drug resistant epilepsy, autism spectrum disorder and intellectual disability, in which fever in a child with the mutation has been associated with a cessation in seizures and improved social interactions. We have recently demonstrated that a continuous 5d exposure of A350V IQSEC2 mice to an ambient temperature of 37°C dramatically reduced lethal seizures and improved their behavioral interactions. In this study, we sought to determine if a more practical and potentially translatable means of simulating fever could provide a similar benefit and to determine the mechanism for this benefit. Body core temperature in A350V mice was increased to 39°C (assessed telemetrically with IPTT-3000) by increasing the ambient temperature to 39°C for 15 minutes a day. These treatments significantly reduced lethal seizures in A350V mice (66/146, 45% vs. 6/36, 16%, $p<0.002$). Pharmacological induction of the heat shock response with celastrol (1mg/kg/d) significantly reduced lethal seizures in A350V mice (66/146, 45% vs. 1/15, 6.7%, $p<0.004$). Our working hypothesis has been that fever rescues via induction of specific heat shock proteins which serve as chaperones that can help with misfolded mutant proteins. The A350V mutation occurs in the apocalmodulin (ApoCM) binding region of IQSEC2 and thereby prevents ApoCM from serving as an allosteric modulator of the GEF activity of IQSEC2. We therefore assessed the ability of heat to restore the binding of A350V IQSEC2 to ApoCM. We found that *in vitro* brief heat shock resulted in the partial rescue of the impaired binding of A350V IQSEC2 to ApoCM. We have recently identified multiple children with different IQSEC2 mutations that also appear to benefit from fever with a reduction of seizures. Therefore these studies not only provide mechanistic insight into how fever may provide benefit in IQSEC2 disease by induction of the heat shock response but also may provide a practical means to decrease seizures in many children with IQSEC2 mutations.

Disclosures: V. Borisov: None. **N. Levy:** None. **E. Laury:** None. **S. Halpert:** None. **A.P. Levy:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.27

Topic: A.07. Developmental Disorders

Support: T32 ES007026
R01 NS114480
RO1 AA02711
GR530622

Title: Developmental perfluorohexanoic acid (PFHxA) exposure affects brain development

Authors: *E. C. PLUNK[1], A. K. MAJEWSKA[2];
[1]Dept. of Envrn. Med., [2]Dept. of Neurosci., Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Since the phase out of legacy per- and polyfluorakyl substances (PFAS) due to the long-term health effects such as hypothyroidism, decreased immune response to vaccines, attention deficit/hyperactivity disorder, and autism spectrum disorder (ASD) following gestational exposures, industries have effectively replaced them with next generation, short-chain PFAS. Perfluorohexanoic acid (PFHxA) is one of the replacements. PFHxA is a large chemical component in aqueous film forming foam (AFFF) which migrates into water and soil systems contaminating drinking water and food. Epidemiology studies have reported that PFHxA has an efficient blood to breast milk translation showing that infants are exposed via breastmilk. PFHxA has also been reported in serum of pregnant women and passes through the placenta. This suggests that exposure to PFHxA could affect brain development, although this has not yet been studied in depth.
Because long-chain PFAS have been shown to disrupt immune responses, we are interested in how PFHxA exposure could affect microglia, the immune cells of the brain, in the context of the developing nervous system. Additionally, long-chain PFAS disrupt developmental thyroid levels which could impact both neurons and microglia. Therefore, our work investigates the hypothesis that gestational and lactational exposure to PFHxA in a mouse model disrupts the colonization of microglia in the developing brain as well as disrupts neuron growth indirectly through decreased thyroid hormone signaling.
To test this hypothesis, we exposed pregnant C57Bl/6 mice daily from gestational day 0 through postnatal day (P) 21 to ddH2O, propylthiouracil (PTU) which is known to disrupt thyroid signaling, and a low or high dose of PFHxA. On P21 offspring of both sexes were euthanized, and we used immunohistochemistry to immunolabel microglia and mature neurons. We analyzed microglia density in the cerebellum, hippocampus, and corpus callosum, and also assessed cortical thickness. Our preliminary results suggest that cortical thickness is unchanged by PTU or PFHxA and that microglial colonization is not altered in the hippocampus or corpus callosum. The cerebellum, however, showed changes in microglial density that were layer-specific. Additionally, we found elevated PFHxA levels in the brain and serum in both low dose and high dosed exposed animals at P90, long after exposure had ceased. These results suggest that PFHxA might affect brain development in an area-specific manner and might persist in the body. Understanding PFHxA exposure during brain development will aid policy makers in protecting the consumer by regulating PFHxA industrial use.

Disclosures: E.C. Plunk: None. **A.K. Majewska:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.28

Topic: A.07. Developmental Disorders

Support: Department of Defense W81XWH-17-1-0455

Title: Effects of microglial depletion on lysophosphatidic acid (LPA)-induced post-hemorrhagic hydrocephalus (PHH)

Authors: *P. SANCHEZ PAVON[1], C. PALMER[2], C. S. LIU[3], V. TAN[4], R. RIVERA[5], V. BLAHO[4], J. CHUN[4];
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Abstract: Post-hemorrhagic hydrocephalus (PHH) is a neurological disease that primarily affects premature infants, whose brain vasculature is still poorly developed, and is thought to result from the infiltration of blood into the brain's ventricles. Blood components are the primary cause of PHH; however, the exact mechanisms driving PHH development and propagation remain unclear. As PHH progresses, cerebrospinal fluid (CSF) continues to be produced, but it is poorly reabsorbed, driving abnormal CSF accumulation that leads to ventricular enlargement. Current treatment involves surgical introduction of a shunt into the brain ventricles to redistribute the excess CSF. Our group has modeled PHH in postnatal mice by stereotactic injection of lysophosphatidic acid (LPA) into the brain's ventricles of C57/BL6 mice at postnatal day 7 (P7). LPA is a bioactive lipid present in blood that signals through 6 known G protein-coupled receptors (GPCRs), LPA_{1-6}. CSF hypersecretion has been shown to result from an inflammation-dependent response that correlates with increased immune cell numbers, revealed by single-nucleus RNA-seq (snRNA-seq) performed on LPA-injected brains. Our bioinformatic analysis also showed activated microglia to be the most significantly altered cell type. To assess whether microglia or peripheral immune cells influence PHH, as well as to identify the cytokines and chemokines involved in this process, depletion of microglia is being pursued by pharmacological intervention. Using Pexidartinib (PLX3397), a CSF-1R inhibitor that effectively depletes microglia and peripheral macrophages following daily subcutaneous injections, microglia can be vastly reduced in the early postnatal mouse brain. This depletion, in combination with our PHH model, will allow us to identify immune mechanisms of PHH initiation and progression.

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Poster

186. Developmental Disorders: Non-Genetic Models

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Program #/Poster #: 186.29

Topic: A.07. Developmental Disorders

Support: NIH Grant 8P20GM103346

Title: Helminth treatment timing may alter hippocampal dependent context pre-exposure facilitation effect fear conditioning memory

Authors: *K. WINKLER[1], L. L. WILLIAMSON[2];
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Abstract: Autoimmune, allergic, and chronic inflammatory diseases have been on the rise[1]. Commensalist parasites may provide anti-inflammatory signals that attenuate disease-causing inflammation. Early life inflammation has a significant effect on behavior, especially when combined with inflammation in adulthood[2]. This study assesses the interactions between helminth (commensalist parasite) treatment, early-life and adult inflammation, and sex on hippocampal dependent memory using context pre-exposure facilitation effect (CPFE) fear conditioning. Maternal helminths combined with helminth treatment in their offspring at weaning eliminated memory deficits on a fear conditioning task[1]. This study separates the effects of maternal and offspring treatments. In the maternal study, dams received *Hymenolepis diminuta* (rat tapeworms) or saline orally, prior to mating. Their pups received PBS or E. coli on postnatal day 4. In adulthood, the offspring received saline or lipopolysaccharide (LPS) during the CPFE consolidation period on Day 1 of training. Maternal helminths did not specifically alter fear conditioning behavior or prevent impairments. In the offspring study, dams were not treated. Their pups were then treated with PBS or E. coli on P4 as in the previous study, and then at weaning offspring were treated orally with *H. diminuta* or saline. We predict that helminth treatment at weaning will have a more robust effect on fear conditioning behavior. This study will allow us to further understand immune stressors and possible interventions, which later could help aid medicine and human health.

Disclosures: K. Winkler: None. **L.L. Williamson:** None.

Poster

186. Developmental Disorders: Non-Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 186.30

Topic: A.07. Developmental Disorders

Support: Conacyt Postdoctoral scholarship to MOV 660096

Title: The maternal consumption of a high-fat diet could generate long-term mental illness in the progeny: a systematic review

Authors: *M. ORTIZ-VALLADARES[1], R. PEDRAZA-MEDINA[3], N. A. MOY-LOPEZ[4], O. GONZALEZ-PEREZ[5], J. GUZMÁN-MUÑIZ[2];
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Abstract: The first stages of development: gestation, lactation, and early childhood are especially sensitive to changes in the environment due to the concurrence of the cytogenetic processes typical of these stages, which are necessary to guarantee the correct development of the central nervous system. Nutrition is a fundamental element for meeting the demands of the early periods of maturation and brain development, it has been observed that the consumption of inadequate diets could contribute to structural and functional alterations of the brain that have been linked to the development of mental disorders in adulthood. Specifically, studies on the consumption of a high-fat diet during pregnancy have reported involvement in mood regulation, increased stress response, behaviors related to anxiety, and depression. Other studies have shown that HFD during pregnancy contributes to neuroendocrine, metabolic, and immune/inflammatory alterations, affecting maternal and child health due to exposure to hormones and nutrients, and altering the development of critical neural pathways in behavior regulation. This systematic review aims to analyze the previously described mechanisms involved in the development of mood disorders during adulthood due to maternal intake of HFDs during pregnancy and lactation, both in humans and in animal models. The last five years of research were considered for this study to obtain the most recent information on the subject and its contribution to clarifying the mechanisms involved in lipid diets and their influence on early development and contribution to vulnerability to mental illness. The initial search was made in PubMed where 472 articles were found, in addition to 13 titles identified from other sources. No duplicate documents were found, giving a total of 485 documents. Next, 64 articles were selected that met the relevance criteria based on the title and 38 after reading the abstracts. Finally, after a full-text review, 10 were excluded because they met at least one of the exclusion criteria. Alterations due to maternal consumption of HFDs during pregnancy and lactation seem to have multifactorial effects that disturb the quality of life of individuals. The alterations seem to be related to neuroinflammation processes, HPA axis alterations, and epigenetic changes. Animal models are a viable alternative to investigating and understanding the underlying mechanisms of mental illness. The information obtained from animal models can be used as a preamble for more specialized treatments and interventions to prevent mental health deterioration in humans.

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Poster

187. Glutamate and Acetylcholine

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 187.01

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: Conacyt Grant 487731
Conacyt Grant 255087

Title: Co-exposition to fluoride and inorganic arsenic modifies glutamate uptake and translational control in glial cells

Authors: *A. RODRIGUEZ-CAMPUZANO, A. L. GARCIA-LOPEZ, L. HERNADEZ-KELLY, A. ORTEGA;
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Abstract: Glutamate (Glu) is the main excitatory neurotransmitter in the Central Nervous System (CNS), regulation of glutamatergic synaptic transmission requires the uptake of Glu by sodium-dependent high-affinity transporters (EAATs). The gross of Glu uptake is through glial transporters GLAST (EAAT1) and Glt-1 (EAAT2). Within the cerebellum, GLAST highest expression takes places in Bergmann glia cells (BGC), whose processes extend radially through the molecular layer, ensheathing the excitatory synapses between parallel fibers and Purkinje cells. Given their key disposition and their role in Glu uptake, glial cells are important modulators of synaptic activity.Inorganic Arsenic (iAs) and Fluoride (F^-) are two of the most commonly found toxic contaminants in groundwater, co-existing in major hot spots regions due to geogenic and anthropogenic causes, being a worldwide problem affecting millions of people. Chronic co-exposure to iAs and F^- through drinking water has been recognized as a global public health concern. The individual neurotoxicity of iAs and F^- has been largely documented, finding relevant neurological impact that includes neurodevelopmental deficits such as reduced IQ level, lowered memory capacity, retarded attention and slower learning ability, depression, encephalopathy, peripheral neuropathies, and also neurodegeneration. Among the molecular mechanisms associated with these anormalities are oxidative stress, mitochondrial disfunction, inflammation, alterations in synaptic plasticity, increased extracellular Glu levels and excitotoxicity. Given the role of GLAST in controlling the extracellular Glu levels to prevent excitotoxicity, we evaluated the effects of the co-exposure to 3 µM iAs and 500 µM F^- on BGC viability, ^{3}[H]-D-Asp uptake, ribosome profiles and translational control check points proteins phosphorylation (p-eIF2-α and p-eEF2) levels. Co-exposure to iAs and F^- decreases BGC viability in a time and dose dependent manner, a 3 µM iAs/500 µM F^- for 10 min increased significatively the uptake of the non-metabolizable analog of Glu ^{3}[H]-D-Asp, mediated by an increase in the affinity of the transporter. As oxidative stress might be involved in these effects, it was not surprising that antioxidant Trolox reverted the increase in ^{3}[H]-D-Asp uptake. Interestingly the eIF2-α and eEF2 phosphorylation levels were augmented without a clear effect of polysome-to-monosome ratio as judged by ribosomal profiling. The present results shed light into the molecular mechanisms that lie on an important health problem such as Arsenic and Fluoride neurotoxicity, and further support the involvement of glial cells in these processes.

Disclosures: A. Rodriguez-campuzano: None. **A.L. Garcia-Lopez:** None. **L. Hernadez-Kelly:** None. **A. Ortega:** None.

Poster

187. Glutamate and Acetylcholine

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 187.02

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: Fund Scientific Research Flanders (FWO)
SRP-VUB

Title: Deficits in corticostriatal neurotransmission and social interaction in mice with a genetic deletion of system x_c^- disappear with aging

Authors: *A. MASSIE[1], A. VILLERS[2], O. LARA[1], L. VERBRUGGEN[1], H. SATO[3], E. BENTEA[1], L. ARCKENS[4], L. RIS[2], L. DE PAUW[1];
[1]Vrije Univ. Brussel, Brussels, Belgium; [2]Univ. of Mons, Mons, Belgium; [3]Niigata Univ., Niigata, Japan; [4]KU Leuven, Leuven, Belgium

Abstract: In the brain, the cystine/glutamate antiporter system x_c^- is mainly located on astrocytes and imports cystine in exchange for glutamate. This glutamate can modulate glutamatergic neurotransmission by activating extrasynaptic glutamate receptors. Our previous findings show that young-adult mice with a genetic deletion of xCT ($xCT^{-/-}$ mice), the specific subunit of system x_c^-, display deficits in corticostriatal neurotransmission -evaluated by generating input/ouput curves using slice electrophysiology- which can be rescued by adding glutamate to the slice. These deficits are accompanied by behavioral changes, suggestive of autism-spectrum disorder-like (ASD) behavior (Bentea *et al.*, Molecular Psychiatry 2021). Since we recently observed that hippocampal neurotransmission in $xCT^{-/-}$ mice was unaffected by the aging process, contrary to wildtype ($xCT^{+/+}$) littermates (Verbruggen *et al*., Molecular Psychiatry 2022), we hypothesized that also corticostriatal neurotransmission would be maintained with aging in $xCT^{-/-}$ mice and possibly become as efficient as in aged $xCT^{+/+}$ littermates, thereby resulting in similar behavior in both aged genotypes. Repetitive behavior was studied in aged $xCT^{+/+}$ and $xCT^{-/-}$ mice by evaluating grooming and marble burying. Social behavior was analyzed with the reciprocal social interaction and the three-chamber assay test. The corticostriatal neurotransmission was investigated using slice electrophysiology, and Golgi Cox stainings were performed to compare the morphology of the striatal medium spiny neurons (MSN) of $xCT^{+/+}$ and $xCT^{-/-}$ mice. No major differences in behavior were seen between the aged $xCT^{-/-}$ and $xCT^{+/+}$ mice. In accordance with young-adult mice, we could not detect significant effects of xCT deletion on grooming behavior in aged mice. However, contrary to young-adult mice, aged $xCT^{-/-}$ mice did not show increased digging behavior or reduced time spent in social interaction. Furthermore, no deficits in corticostriatal neurotransmission could be detected in aged $xCT^{-/-}$ mice, compared to the aged $xCT^{+/+}$ mice. Finally, no morphological differences were seen between MSNs of aged $xCT^{+/+}$ and $xCT^{-/-}$ mice. We conclude that the differences in corticostriatal neurotransmission and behavior that we previously observed between young-adult $xCT^{-/-}$ and $xCT^{+/+}$ mice, were absent in aged mice. This study will be completed by an in-depth analysis of the corticostriatal synapses at the ultrastructural level to investigate whether the changes in intracellular glutamate that have been observed in the pre- and postsynaptic terminals of young-adult mice, disappear as well with aging, and could explain the observed effects.

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Poster

187. Glutamate and Acetylcholine

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Program #/Poster #: 187.03

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH U19-NS123717
NIH F31NS118949

Title: Multimodal Imaging of Acetylcholine Release and Hemodynamics in the Awake Mouse Brain

Authors: *P. DORAN[1], M. THUNEMANN[1], K. KıLıÇ[1], B. FU[2], N. FOMIN-THUNEMANN[1], J. D. HERBERT[1], A. I. CHEN[1], J. STOCKMANN[2], Y. JIANG[2], B. ZHANG[2], C. T. FARRAR[2], Y. LI[3], B. R. ROSEN[2], X. YU[2], J. B. MANDEVILLE[2], S. SAKADZIć[2], A. DEVOR[1,2];
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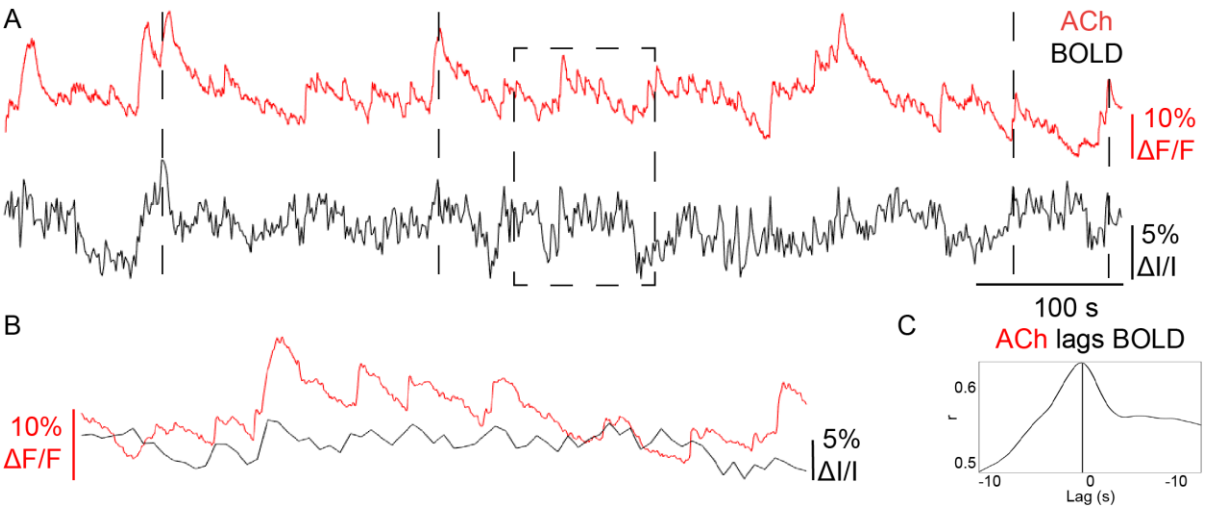
Abstract: Cholinergic neurons in the basal forebrain project across the cerebral cortex releasing acetylcholine (ACh). ACh is known to modulate "brain states" characterized by different efficiency of information processing. ACh is also a known vasodilator and thus is expected to modulate not only activity of neuronal circuits but also hemodynamics reflected by blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI). The cholinergic system is among the earliest neuronal circuits affected in neurodegeneration including Alzheimer's disease and other dementias. Therefore, the ability to infer cholinergic activity from BOLD-fMRI in humans would be useful for basic science and clinical applications alike. In mice, ACh release can be measured directly using genetically encoded optical biosensors. In this study, we combined optical imaging of ACh with BOLD-fMRI to study the relationship between cholinergic activity and BOLD-fMRI signals. We expressed genetically encoded red $GRAB_{ACh}$ sensor (rACh1.4) in the left barrel cortex was induced through intracortical injections of an adeno associated virus. Mice were implanted with large cranial windows to allow optical access to the left hemisphere of the dorsal cortex and PEEK headbars for head fixation within a 9.4T MRI scanner. Mice underwent an extensive training regimen to acclimate them to both head fixation and the sound of the MRI scanner. We engineered an MRI-compatible optical imager to perform fluorescent imaging of ACh inside the scanner. We observed both a BOLD response and ACh release following an airpuff stimulus to the whisker pad. However, the BOLD response preceded ACh release inconsistent with the hypothesis of ACh driving the BOLD signal. Furthermore, we observed intermittent departures between the BOLD signal fluctuations and ACh dynamics during spontaneous ("resting-state") activity. These results suggest that cholinergic control of the resting-state BOLD-fMRI dynamics may vary over time dependent on the local neuronal activity, presence of other neuromodulators, and behavioral context.



Disclosures: **P. Doran:** None. **M. Thunemann:** None. **K. Kılıç:** None. **B. Fu:** None. **N. Fomin-Thunemann:** None. **J.D. Herbert:** None. **A.I. Chen:** None. **J. Stockmann:** None. **Y. Jiang:** None. **B. Zhang:** None. **C.T. Farrar:** None. **Y. Li:** None. **B.R. Rosen:** None. **X. Yu:** None. **J.B. Mandeville:** None. **S. Sakadzić:** None. **A. Devor:** None.

Poster

187. Glutamate and Acetylcholine

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 187.04

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIMH Intramural Research Program

Title: Redox-dependent membrane trafficking of neurotransmitter transporters

Authors: ***S. RADHAKRISHNAN**, R. SCHELLING, S. M. UNDERHILL, S. G. AMARA; Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Plasma membrane neurotransmitter transporters clear neurotransmitters from the extracellular space and play a key role in regulating neuronal signaling. The neuronal excitatory amino acid transporter 3, EAAT3, is essential for glutamate clearance and modulation of glutamatergic tone. However, EAAT3 also transports cysteine into the cell where it serves as a rate-limiting substrate for the synthesis of glutathione, a key neuronal antioxidant. EAAT3 mutations are associated with several neuropsychiatric disorders such as schizophrenia and obsessive-compulsive disorders that may be due to its role in glutamate or cysteine transport. The activity of plasma membrane transporters can be altered by changes in transport kinetics, expression levels and trafficking to and from the plasma membrane. Using radiolabeled neurotransmitter uptake assays and surface biotinylation assays in Neuro2A cells we showed that activation of protein kinase C (PKC) can increase cell surface localization of EAAT3, as

previously reported in other cell lines. There is evidence that redox-triggered mechanisms can influence signaling through PKC by altering the catalytic properties or the subcellular compartmentalization of various PKC isoforms. This suggests a mechanism whereby EAAT3 trafficking could be modulated by cellular redox stress and PKC activation to increase cysteine import and stimulate glutathione biosynthesis. To study this, we examined whether oxidizing agents such as hydrogen peroxide can enhance PKC activation and increase EAAT3 plasma membrane localization. Using a genetically encoded PKC activity sensor, CKAR, we found that application of oxidizing agent, hydrogen peroxide increased PKC activation and enhanced surface expression of EAAT3 in both EAAT3-transfected Neuro2A cells and in primary cultures of mouse cortical neurons that express EAAT3 endogenously. Additional studies have addressed another potential mechanism for redox regulation of neurotransmitter transporters—the direct redox modification of cysteine residues. The dopamine transporter (DAT) contains highly conserved internal lysine and cysteine residues that could trigger a redox-dependent internalization mechanism where the electrophilic modification of a cytosolic cysteine facilitates a conformation change and ubiquitylation of an adjacent lysine residue. We have examined this possibility by assessing DAT trafficking with surface biotinylation, TIRF microscopy and dopamine uptake assays. Taken together our observations suggest multiple mechanisms though which changes in cellular redox states can regulate the activity of neurotransmitter transport systems.

Disclosures: S. Radhakrishnan: None. **R. Schelling:** None. **S.M. Underhill:** None. **S.G. Amara:** None.

Poster

187. Glutamate and Acetylcholine

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 187.05

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Title: Electrophysiological assessment of the pharmacological and functional interactions of the GPCRs mGlu2 and mGlu7

Authors: K. KAMBARA[1], S. A. NEALE[2], *S. BERTRAND[1], S. R. J. HOARE[3], T. E. SALT[2], D. BERTRAND[1];
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Abstract: The metabotropic glutamate receptors represent a family of 8 receptors divided into 3 groups: Group I - mGlu1 and 5; Group II -mGlu2 and 3; Group III - mGlu4, 6, 7 and 8. These receptors have been the focus of attention for their role in normal network function and as possible treatments for neurological and psychiatric disease. Recently there has been interest in the receptors forming functional heterodimers. Here we confirm mGlu2 and mGlu7 receptors can be functionally expressed in the Xenopus oocyte system and demonstrate a shift in

pharmacology and function when mGlu2 and 7 are coexpressed, consistent with formation of heterodimers.
Expression of the mGlu receptors and the inward rectifier potassium (KiR3.1/3.2) reporter was obtained by injection of 15 nL solution containing the desired mRNA mix. Recordings were conducted 2-5 days later using two electrode voltage clamp). To reveal the potassium inward currents, cells were exposed to a high potassium solution (48 mM) and a holding voltage of -80 mV. The time course of the inward current was analyzed by curve fitting to quantify the rate of activation, using GraphPad Prism, employing the Pharmechanics plug-in of custom time course equations.
In oocytes expressing the mGlu7 receptor, application of increasing concentrations of either glutamate, or the Group III selective agonist L-AP4, resulted in a concentration dependent activation of the mGlu7 receptors with EC50 of 226 μM ± 30 and 121 μM ± 5, respectively. The effect of glutamate was attenuated by the mGlu7 receptor antagonist XAP044. Application of the Group II selective agonist LY354740 did not activate the mGlu7 receptor at concentrations of up to 1 μM.
In oocytes expressing the mGlu2 receptor, application of glutamate or LY354740 resulted in activation of the receptor with EC50s of 0.41 μM and 1.8 nM respectively. Although L-AP4 is regarded as selective at Group III receptors it did activate the mGlu2 receptor, albeit at high concentrations (EC50 317 μM ± 6).
When mGlu 2 and 7 were coexpressed the potency of glutamate and LY354740 were similar to expression of mGlu2 alone; however, L-AP4 showed increased potency to an EC50 of 49 μM ± 10, compared to 121 μM ± 5 on mGlu7 alone. In addition, kinetic analysis of the current waveform indicated coexpression decreased the rate of activation by LY354740.
These studies confirm GPCR pharmacology and function can be assessed in *Xenopus* oocytes. Coexpression of the mGlu2 and 7 receptors resulted in a pharmacology that differed from expression of either receptor alone, suggesting an interaction consistent with formation of heterodimers; thereby providing a useful tool to assess the functional consequences of heterodimerisation.

Disclosures: **K. Kambara:** None. **S.A. Neale:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurexpert Limited. **S. Bertrand:** None. **S.R.J. Hoare:** A. Employment/Salary (full or part-time):; Pharmechanics LLC. **T.E. Salt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurexpert.com. **D. Bertrand:** None.

Poster

187. Glutamate and Acetylcholine

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 187.06

Title: WITHDRAWN

Poster

187. Glutamate and Acetylcholine

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 187.07

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIA/NIH Grant R01AG070255

Title: Microtransplantation of native synaptic mGluR5 receptors into Xenopus oocytes.

Authors: *B. MILLER, N. MORENO, B. A. GUTIERREZ, A. LIMON;
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Abstract: Through the study of recombinant synaptic proteins much has been discovered about their structural, biophysical and pharmacological properties; however, there is less knowledge on the properties of the native synaptic protein complexes. Because recombinant expression of synaptic receptors is the interpretation of the cell wherein they are transcribed or translated, they may differ from receptors in native membranes. In this study, we aimed to determine whether microtransplantation of synaptic membranes (MSM) allows the study of native glutamate metabotropic receptors (mGluRs). We microinjected synaptic membranes isolated from frozen rat cortex samples into *Xenopus* oocytes and recorded ion currents elicited by 1 mM glutamate using Two Electrode Voltage Clamp. These trials resulted in a fast ionotropic response of 5.61 ± 0.38 nA (n = 177 oocytes) followed by a delayed oscillatory metabotropic response of 48.59 ± 6.79 nA (n = 177 oocytes). We confirmed the presence of Group 1 mGluRs after observing metabotropic oscillations during the administration of 100 μM of (±)-1-Aminocyclopentane-trans-1,3-dicarboxylic acid (ACPD), a Group 1 specific mGluR agonist, and the loss of glutamate metabotropic currents by 10 μM NPS 2390, a Group 1 specific mGluR antagonist. Further investigation revealed that mGluR1 antagonism (LY 456236) showed little effect on metabotropic oscillations when compared to either prior or following application of glutamate or ACPD. However, antagonism of mGluR5 with 100 μM AZD 9272 showed a reduction of metabotropic currents elicited by ACPD and glutamate. Finally, we confirmed expression of both mGluR1 and mGluR5 in native synaptosomes by immunoblots, where we found protein enhancement in the isolated synaptosomes when compared to rat cortex whole tissue lysate. These results demonstrate the merit of native synaptosomes and their microtransplantation for the study of mGluR5's contribution to metabotropic glutamate signaling, which has been linked with several neurological disorders such as Alzheimer's disease, schizophrenia, and addiction.

Disclosures: B. Miller: None. **N. Moreno:** None. **B.A. Gutierrez:** None. **A. Limon:** None.

Poster

187. Glutamate and Acetylcholine

Location: SDCC Halls B-H

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Program #/Poster #: 187.08

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NRF-2020R1A5A1019023
NRF-2022R1A2C1004913
KHIDI-HU21C0071
NRF-2021R1F1A1049169
BK21 Four Biomedical Science Program

Title: Regulate of local translation in response to stress stimuli in neurons

Authors: *D.-H. PARK[1,2,3], Y. SUH[1,2,3];
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Abstract: Local translation is the rapid protein production from pre-existing synaptic mRNAs independent of the cell body, providing a means to supply the synaptic proteome. Local translation enables key features of neuronal process including development, synaptic plasticity, and information processing. Many neuronal mRNAs are believed to be transported to synapses in a translationally repressed state, however the molecular details regarding the specific stimuli or stress conditions that organize local translation remain uncertain. Since ribonucleoprotein particles (RNPs) such as stress granules and p-bodies, and their associated RNA-binding proteins (RBPs) function to regulate local translation, we traced puromycin-labeled nascent peptides in global or synaptosomal fractions following various neuronal stimuli and stress to explore the signals governing local translation in rat primary cortical neurons. We found that under stress conditions such as CCCP, an inhibitor of mitochondrial oxidative phosphorylation, and sodium arsenite, global translation rate does not fully match the target gene-specific translation rates regulated by RNPs. In addition, oxidative stress suppressed local translation via transitioning RBPs to the detergent-insoluble fraction. These findings provide insight into the stress-responsive role of RNPs in the regulation of neuronal local protein homeostasis.

Disclosures: D. Park: None. **Y. Suh:** None.

Poster

187. Glutamate and Acetylcholine

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 187.09

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH Grant MH104638
UofSC VP for Research

Title: Phasic acetylcholine release induces sharp wave ripples in the basolateral amygdala via nicotinic receptors

Authors: J. X. BRATSCH-PRINCE[1], J. W. WARREN, III[2], G. C. JONES[2], ***D. D. MOTT**[2]; [1]Dept. of Psychiatry, Univ. of California, San Francisco, San Francisco, CA; [2]Dept. of Pharmacology, Physiol. & Neurosci., Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Memory formation and recall requires the proper storage of a past experience in a manner that is available for reactivation. In the hippocampus, these processes are closely associated with network events termed sharp wave ripples (SWR), consisting of a large amplitude, slow frequency (1-20 Hz) envelope (sharp wave, SW) with a brief high-frequency oscillation (150-200 Hz ripple). While the exact circuit and synaptic mechanisms by which SWRs are produced in circuits is not fully understood, SWRs in the hippocampus rely on coordinated bursts of excitatory inputs, largely originating from CA3, and local inhibition from specific classes of inhibitory interneurons (INs). SWRs are also modulated by neuromodulatory inputs and acetylcholine (ACh) muscarinic receptors have been shown to reduce the frequency of hippocampal SWRs. In the basolateral amygdala (BLA), SWRs have been observed both *in vivo* and in brain slice preparations. While these network events in the BLA also rely on coordinated burst activity of excitatory and inhibitory inputs, it is still not entirely clear how they are produced or if they are similarly modulated by cholinergic inputs, which densely project to the BLA. To explore the effects of endogenous ACh release on BLA SWRs, we performed LFP, multi-unit, and cell-type specific whole-cell patch recordings in BLA brain slices in mice. Surprisingly, in response to periodic single pulse optogenetic stimulation of cholinergic fibers in the BLA, robust SWR activity was observed in LFP recordings. Application of ACh muscarinic or nicotinic receptor antagonists, as well as AMPA and $GABA_A$ receptor antagonists, revealed that SWR activity was mediated by nicotinic receptors and relied on both local BLA glutamatergic and GABAergic signaling. At the level of single excitatory pyramidal neurons (PNs), single pulse cholinergic stimulation induced a rapid onset (<10 ms) barrage of both excitatory and inhibitory synaptic events that induced ripple-frequency oscillations of PN membrane potential. These events were sensitive to a nicotinic receptor antagonist. Interestingly, PNs in the BLA were not directly activated by nicotinic receptors, indicating that nicotinic-induced excitation driving SWR activity at the single cell level was produced by glutamate release from long-range axons innervating the BLA. Whole-cell recordings from parvalbumin (PV), cholecystokinin (CCK), and somatostatin (SOM) INs revealed differential nicotinic activation of these IN classes and distinct involvement in SWR network activity. Together, these results indicate a novel mechanism by which phasic ACh signaling can induce SWRs in the BLA.

Disclosures: **J.X. Bratsch-Prince:** None. **J.W. Warren:** None. **G.C. Jones:** None. **D.D. Mott:** None.

Poster

187. Glutamate and Acetylcholine

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 187.10

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: CIHR
NSERC
Alzheimer Society of Canada

Title: A novel transgenic mouse model expressing primate-specific nuclear choline acetyltransferase: insights into potential cholinergic vulnerability

Authors: *H. E. ALQOT, R. J. RYLETT;
Univ. of Western Ontario, London, ON, Canada

Abstract: Choline acetyltransferase (ChAT), the acetylcholine synthesizing enzyme, is a fundamental component of cholinergic neurons whose level and activity is reduced in normal and pathological aging. In humans, the M-transcript of ChAT can translate to both 69-kDa ChAT and 82-kDa ChAT, the former found in the cytoplasm while the latter resides primarily within the nucleus. Interestingly, this unique nuclear localization of 82-kDa ChAT is altered in aging, mild cognitive impairment and Alzheimer's disease, shifting to a more diffuse cytoplasmic distribution. While the function of nuclear ChAT is unknown, a few studies indicate a role in altering the expression of genes related to stress and inflammation, key features associated with aging, suggesting a potential role for nuclear ChAT in neuronal vulnerability. In this regard, and since 82-kDa ChAT is not expressed endogenously in mice, our laboratory has successfully generated transgenic mice expressing human 82-kDa ChAT. As this is a new mouse model, behavioral and biochemical characterization of 82-kDa ChAT expressing mice is necessary to provide a baseline for future studies. The aim of this study is to characterize the 82-kDa ChAT mouse to elucidate the physiological implications of expression of this nuclear protein. Using qPCR, RNAscope and immunohistochemical analysis, we were able to demonstrate 82-kDa ChAT mRNA and protein expression in basal forebrain neurons of our mouse model. Moreover, there was a similar spatial/temporal distribution to studies performed using human necropsy brain. Longitudinal behavioral characterization did not reveal significant differences between control and the 82-kDa ChAT mice. Unexpectedly, there was a reduction in age-related dystrophies in mircoglia and cholinergic nerve fibers in older 82-kDa ChAT mice compared to age-matched controls. This was complimented by increased levels of M2 microglial markers (Arginase, Cd206) and reduced levels of M1 markers (iNos, Cd86). Additionally, an Aging PCR array revealed downregulation of genes involved in inflammation (C3 and C1q) and upregulation of genes related to neuron-glia interaction (CxCl16) in 82-kDa ChAT mice. In conclusion, we have generated a mouse model that expresses the human 82-kDa ChAT that recapitulates many of the features observed in cholinergic neurons in human necropsy brain. Moving forward, these mice will provide a valuable tool to study the impact of this nuclear ChAT isoform on disease pathologies involving cholinergic neurons.

Disclosures: H.E. Alqot: None. **R.J. Rylett:** None.

Poster

187. Glutamate and Acetylcholine

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 187.11

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH R01 NS111767
NIDA R01 DA051205

Title: Positive allosteric modulation of glutamate transporter EAAT2 as a novel therapeutic approach for neurological disorders

Authors: *K. L. REEB, O. V. MORTENSEN, A. C. K. FONTANA;
Pharmacol. and Physiol., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Excitatory amino acid transporters (EAATs) are key proteins that regulate glutamate levels in the synaptic clef and are therefore crucial to prevent excitotoxicity. Aberrant glutamatergic signaling occurs in many neurological disorders, such as stroke, epilepsy, and addiction, and others. Consequently, positive allosteric modulation (PAM) of EAATs is an attractive, novel therapeutic approach for these conditions. EAAT PAMs, previously identified in our lab, are hypothesized to act by altering the interactions between the scaffold and the transport domains, which move in an elevator-like motion. To test this hypothesis, we used computational modeling, followed by site-directed mutagenesis and radioligand uptake assays. We identified mutations in specific amino acid residues located between the transport and scaffold domains on astrocytic transporters EAAT1 and EAAT2 that result in increased activity without changes in expression, suggesting that interactions between the domains are responsible for enhanced transport efficiency. These gain-of-function mutated EAAT1 and EAAT2, and PAMs of these transporters, are currently being studied in single molecule Förster Resonance Energy Transfer (smFRET) approaches to explore effects on the kinetics and dynamics of EAATs. We also evaluated other mutant EAATs in dose-response radioligand uptakes with EAAT PAMs to identify critical amino acids that mediate the positive allosteric action of these compounds. Additionally, we explored the potential clinical utility of our EAAT2-specific PAMs, which are of clinical interest since EAAT2 is responsible for most of the glutamate clearance in the CNS. Previously, we demonstrated neuroprotective properties of EAAT2 PAMs in an *in vitro* stroke model. We have now expanded our studies to an *in vitro* epilepsy model, using live calcium signaling measured in primary neuron-glia cultures. Additionally, we validated the action of these EAAT2 PAMs by measuring the concentration of extracellular glutamate from these cultures using HPLC. We also examined the effect of our lead EAAT2 PAM, NA-014 in *in vivo* models of drugs of abuse, as glutamatergic projections have been shown to influence cue-induced drug seeking behavior and relapse. Using the conditioned place preference (CPP) model, we showed that NA-014 decreased cocaine-seeking behavior in both males and females, without rewarding effects. Locomotion was not altered by NA-014 either, suggesting that this compound is not sedative or stimulating. Collectively, these studies expand our understanding of the mechanisms of these PAMs and broaden their potential therapeutic indications.

Disclosures: K.L. Reeb: None. **O.V. Mortensen:** None. **A.C.K. Fontana:** None.

Poster

187. Glutamate and Acetylcholine

Location: SDCC Halls B-H

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Program #/Poster #: 187.12

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: KBRI Grant 22-BR-03-01

Title: Impaired muscarinic acetylcholine-dependent persistent firing and short-term memory function in a chronic ketamine-induced mouse model of schizophrenia.

Authors: *J. YU[1,2], J. CHOI[2], J.-C. RAH[1,2];
[1]Brain Sci., Daegu Gyeongbuk Inst. of Sci. and Technol., Daegu, Korea, Republic of; [2]Korea Brain Res. Inst., Deagu, Korea, Republic of

Abstract: Short-term memory (STM) is the ability to maintain immediately valid information for a short period of time. Although the underlying circuit mechanism of STM remains unknown, it has been demonstrated that persistent neural activity in high-order cortical areas, including the prefrontal cortex, is indispensable for the function. Previously, studies have shown that reverberatory neural activity can be induced by the activation of muscarinic acetylcholine receptor (mACh), coupled with depolarizing current influx in the medial prefrontal cortex (mPFC) and suggested the phenomenon as a physiological substrate of the STM. The glutamate hypothesis of schizophrenia (SZ) proposes that dysfunction of the N-methyl-D-Aspartate receptor (NMDAR) and excessive glutamate may alter the balance between excitation and inhibition, leading to cognitive impairment of SZ, including STM. The hypothesis is developed based on human imaging and post-mortem study and corroborates well with animal models. In the present study, we found that the chronic administration of ketamine, a partial NMDAR antagonist, significantly impaired the performance of an STM-dependent behavioral task. In corroboration, we found that the fraction of mPFC neurons that show mAChR-dependent persistent activity was significantly reduced in the mouse. Furthermore, we found that the mPFC neurons of the mouse have reduced connectivity and decreased synaptic transmission. we assessed impaired short-term memory performances in SZ mouse model, using the delayed-alternation task in Y maze. By using whole-cell patch-clamp, we found that the muscarinic acetylcholine receptor-dependent persistent activity is reduced in the mPFC L2/3 neurons of the SZ mouse model. We also found that the reduced synaptic transmission in mPFC L2/3 neurons of SZ mice, implicating an altered neuronal connectivity in mPFC. Together, our results provide evidences that reduced acetylcholine-evoked persistent activity in mPFC is caused by altered synaptic connectivity, which may explain the mechanism of working memory impairment in SZ patients.

Disclosures: **J. Yu:** None. **J. Choi:** None. **J. Rah:** None.

Poster

187. Glutamate and Acetylcholine

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 187.13

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: HHMI Hanna Gray

Title: Dynamics of dopamine and acetylcholine in decision making

Authors: *L. CHANTRANUPONG[1], J. A. ZIMMER[1], C. C. BERON[1], M. J. WEN[1], W. WANG[1], B. L. SABATINI[2];
[1]Harvard Med. Sch., Boston, MA; [2]Neurobio., Harvard Med. Sch. Dept. of Neurobio., Boston, MA

Abstract: The striatum is a brain region that is critical for the selection and reinforcement of motor actions and the execution of decisions, and dopamine (DA) and acetylcholine (Ach) are essential for these functions. In vitro studies have revealed a circuit local to the striatum in which these two neurotransmitters regulate one another. Ach is released by a unique population of cholinergic interneurons (CINs) and drives DA release. In turn, DA inhibits CIN activity via dopamine D2 receptors (D2R). Whether and how this circuit contributes to striatal function in vivo remains a longstanding question. In mice performing a reward-based decision-making task, we monitored the release of DA and Ach in the ventrolateral striatum. We establish that DA and Ach exhibit multiphasic and anticorrelated release that is modulated by choice history and reward outcome. CIN perturbations reveal that DA release does not require Ach. However, CIN-specific deletion of D2Rs show that DA inhibits Ach release in a D2R-dependent manner, and loss of this regulation impairs task performance. To determine how other CIN inputs in addition to DA may shape Ach release, we assessed the contribution of the cortex and thalamus and found that glutamate release from both these sources are necessary to evoke Ach release. Altogether, the interaction of DA, Ach and glutamate contribute to striatal function during decision making.

Disclosures: L. Chantranupong: None. **J.A. Zimmer:** None. **C.C. Beron:** None. **M.J. Wen:** None. **B.L. Sabatini:** None.

Poster

187. Glutamate and Acetylcholine

Location: SDCC Halls B-H

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Program #/Poster #: 187.14

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: This work was supported by funds from the NINDS IRP

Title: Cholinergic signaling in ventral hippocampus contributes to cue-conditioned fear learning

Authors: *C. ZHONG, N. DESAI, G. WATKINS, D. A. TALMAGE, L. W. ROLE;
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Abstract: The cholinergic fibers of the medial septal/diagonal band of Broca (MS/DB) are implicated in learning and memory by modulating properties of the hippocampal network. The ventral hippocampal region (vHipp) might be involved in cue-conditioned fear memory. However, the precise spatial and temporal profile of acetylcholine release in vHipp remains unclear making it difficult to define specific roles for cholinergic signaling in vHipp mediated cue-conditioned fear learning.Here, we have combined imaging of genetically encoded calcium indicator (GCaMP, axonGCaMP) and acetylcholine ($GRAB_{ACh}$) sensors, with transcriptional activity markers to investigate whether and how cholinergic signaling in vHipp controls cue-conditioned fear behaviors. We put mice through an auditory cue conditioned fear training and recall paradigm and then imaged acute vHipp or MS/DB slices. In vHipp slices, we found: 1) increased neuronal immediate early gene (IEG) expression after cue-induced recall; and 2) after recall, neurons that had been activated during training had more active Ca^{2+} dynamics (changes in frequency and amplitude of GCaMP6f fluorescent signals) than neurons that had not been activated during training. In acute slices from the MS/DB following the same cued fear training and recall, we also found increased IEG expression in cholinergic neurons. With GCaMP expressed specifically in cholinergic neurons in Chat-Cre mice, we observed transiently (up to 5 hours) increased Ca^{2+} dynamics of MS/DB cholinergic neurons after training. The increase in Ca^{2+} dynamics was no longer present 24 hours after training. We next used a tTA-dependent activity labeling system (Robust Activity Marker, or RAM) to label neurons activated during the training session. 24 hours after training, when Ca^{2+} dynamics were compared in RAM^{+} cholinergic neurons (i.e. cholinergic neurons activated during training) in mice left in home cage to mice subjected to tone recall, Ca^{2+} dynamics were significantly enhanced following tone presentation. Ongoing studies are focused on imaging the activity of cholinergic axons within the vHipp using axonal GCaMP and $GRAB_{ACh}$ sensors after fear training / recall. Taken together, these data are consistent with an important role for septal-ventral hippocampal cholinergic signaling participating in both the acquisition and retrieval processes in this auditory cued fear conditioning paradigm.

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Poster

187. Glutamate and Acetylcholine

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Program #/Poster #: 187.15

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: This work was supported by funds from the NINDS IRP

Title: Appetitive and aversive stimuli activate two distinct populations of ventral pallidal cholinergic neurons

Authors: *R. KIM, L. W. ROLE, D. A. TALMAGE;
NIH/NINDS, Bethesda, MD

Abstract: The ventral pallidum (VP) is a brain region involved in encoding hedonic value of external stimuli and mediating motivated behaviors There are multiple subtypes of neurons in the VP, including a population of cholinergic neurons. However, the functional role of VP cholinergic neurons remains unclear. A previous study found exposure to innately threatening stimuli elicits defensive behaviors and increases the number of activated cholinergic neurons in the VP/SI (Rajebhosale et al, 2021). Whether this increase is specific to aversive stimuli, or if the same cholinergic neurons are also activated in response to appetitive stimuli remains unknown. Accordingly, the behavioral responses of mice to an appetitive (2-phenylethanol) or aversive (predator urine) odor was assessed in a Y-maze. The subpopulation of VP cholinergic neurons activated in response to each odor was then examined. Both odors elicited the predicted innate behavioral responses (approach vs avoidance) and activated a subset of VP cholinergic neurons. To address whether distinct or overlapping populations of VP cholinergic neurons were engaged by aversive vs appetitive odors, a combination of genetically modified mice (Chat-cre x fos tTa/eGFP) and a viral vector that tags activity- and cre-dependent neurons (ADCD) was used. ADCD positive neurons are cholinergic neurons that are activated and permanently labeled with mCherry using a Tet-Off system (in the absence of a doxycycline (DOX) diet) to isolate specific time windows. In addition, cFos IHC reveals the total population of activated neurons in distinct contexts. Utilizing ADCD, we found that there are two distinct subpopulations of VP cholinergic neurons: one activated in response to appetitive odor and a second, non-overlapping population activated upon exposure to an aversive odor. In sum, the present studies reveal that the VP contains two distinct and non-overlapping subpopulations of cholinergic neurons, which are uniquely engaged by either appetitive or aversive stimuli. In ongoing studies, we are exploring how these subpopulations of VP cholinergic neurons differ and are directly assessing their contribution to innate behaviors with in vivo activity measures.

Disclosures: R. Kim: None. **L.W. Role:** None. **D.A. Talmage:** None.

Poster

187. Glutamate and Acetylcholine

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 187.16

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: This work was supported by funds from the NINDS IRP

Title: Heterogenous cholinergic circuits: defining factors that confer vulnerability with age

Authors: *M. ANANTH, D. A. TALMAGE, L. W. ROLE;
NINDS, Bethesda, MD

Abstract: Alterations to basal forebrain cholinergic circuits are a hallmark of cognitive impairment, however the time-course and relevance of these changes are not well understood. Close examination of these changes reveals that not all cholinergic projections are created equal. Specifically, some cholinergic projections are affected far sooner and to a much greater degree than others. Here we sought to investigate what confers resilience or vulnerability of cholinergic circuits with age. To assess vulnerability of cholinergic neurons and projections in aging we focused on two cholinergic terminal fields that are vulnerable with age: the entorhinal cortex (EC) and the basolateral amygdala (BLA). These regions receive cholinergic input from distinct cholinergic nuclei and have different timescales of deterioration with age. We used two behavioral assays that uniquely engage each circuit as a readout of function: displaced object recognition (DOR task, EC-function) and cued fear conditioning (cFC task, BLA-function). We asked whether functionally engaged cholinergic neurons were affected in aged animals. We found that young animals consistently spend more time exploring the displaced object during the DOR task, however this was no longer seen with age. We found that DOR performance in young animals activated cholinergic neurons of the medial septum/diagonal band, while the number of activated cholinergic neurons was lower with age and lower in animals with poor performance. Using a cFC task, we previously found that normal performance (i.e., elevated freezing in response to the tone) requires activation of cholinergic neurons within the nucleus basalis/substantia innominata (NBM/SI) and proper cholinergic signaling in the BLA. With age, we find less freezing behavior across the recall session coupled with fewer activated cholinergic neurons within the NBM/SI. Using the RNAscope assay, we identified subsets of cholinergic neurons across the basal forebrain that express mRNA for glutamatergic or GABAergic markers. Ongoing studies investigate whether these activated cholinergic neurons preferentially subscribe to a cell-identity and whether the proportion of these co-expressing subtypes changes with age. These studies provide valuable insight into the involvement of cholinergic neurons in cognitive behaviors and the consequence of age on cholinergic circuits. Importantly, they underscore a growing body of literature that shows incredible diversity and functional organization within the cholinergic system. This diversity may be key in understanding which factors lead to vulnerability of cholinergic circuits with age.

Disclosures: M. Ananth: None. **D.A. Talmage:** None. **L.W. Role:** None.

Poster

188. Trans-Synaptic Nanoscale Organization

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 188.01

Topic: B.04. Synaptic Transmission

Support: NIH R00 MH118425

Title: Characterizing the expression and localization of MDGA1 at hippocampal synapses

Authors: *M. SANDOVAL[1], M. A. BEMBEN[2], V. N. CHAU[1], R. A. NICOLL[2], J. DÍAZ-ALONSO[1];
[1]Anat. and Neurobio., UC Irvine, Irvine, CA; [2]Cell and Mol. Pharmacol., UC San Francisco, San Francisco, CA

Abstract: MAM domain-containing glycosylphosphatidylinositol anchor 1 (MDGA1) is an entirely extracellular putative synaptic adhesion molecule, attached to the plasma membrane through a GPI anchor. In humans, the gene encoding MDGA1 is associated with schizophrenia. In mice, while the MDGA1 KO is viable, lacking gross anatomical defects, it shows impaired excitation/inhibition balance, as well as defects in long-term potentiation and cognitive function. Based on overexpression studies, MDGA1 has been proposed to act as one of the few known synaptic repressors. It is believed to bind preferably to NLGN2 to block interaction with presynaptic neurexins, repressing inhibitory synapse formation. However, proteomic data suggests that MDGA1 localizes to excitatory, not inhibitory synapses. Given these apparently contradictory findings, a reliable analysis of MDGA1 expression is essential to gain understanding about its physiological role, but analyses of the localization of MDGA1 at the protein level have been hampered by the lack of a suitable antibody against MDGA1. Thus, for our current investigation of the specific expression pattern and localization of MDGA1 we generated a knock-in mouse line expressing HA-tagged MDGA1. Utilizing this model, we initially performed Western blot semi-quantification to compare the age-specific expression of MDGA1 in the mouse brain at six ages from postnatal day 3 (P3) to P130 (n = 3-4 mice of either sex/age). MDGA1 was found to follow a developmentally regulated expression pattern, peaking between the second and third postnatal week. Then, we examined the expression of MDGA1 across different brain regions taken at P15 through Western blot analysis (n = 3 mice of either sex/region). Our data indicated high expression of MDGA1 in the striatum, thalamus, cortex, hippocampus, pons and cerebellum, largely consistent with previous *ISH* data. Lastly, we completed a series of qualitative immunofluorescence imaging to elucidate the localization of MDGA1 within the hippocampus at P15. In accordance with previous proteomics data, immunofluorescence analyses showed a high degree of co-distribution of MDGA1 with the excitatory synaptic proteins VGluT1 and Homer1 compared to their inhibitory counterparts, VGAT and NLGN2, within area CA1. Future steps will include the analysis of the subcellular localization of MDGA1 in the hippocampus and other brain regions, and an assessment of MDGA1's synaptic function, including the exploration of potential sex differences. Overall, our data expands our understanding of the expression and function of MDGA1, shedding light on a relatively unexplored protein relevant to some psychiatric disorders.

Disclosures: **M. Sandoval:** None. **M.A. Bemben:** None. **V.N. Chau:** None. **R.A. Nicoll:** None. **J. Díaz-Alonso:** None.

Poster

188. Trans-Synaptic Nanoscale Organization

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 188.02

Topic: B.04. Synaptic Transmission

Title: A comprehensive proteomic analysis of the Neurexin interactome in the mammalian brain

Authors: J. SCHWENK[1], S. THIVAIOS[1], F. H. STERKY[2], W. BILDL[1], *B. FAKLER[1]; [1]Univ. of Freiburg, Inst. of Physiol., Freiburg, Germany; [2]Clin. chemistry and transfusion medicine, Univ. of Gothenburg, Gothenburg, Sweden

Abstract: Neurons are polarized cells, which build functional networks of communication, through synapses. These are specialized junctions between neurons and their formation is mediated by cell adhesion proteins. Among those, Neurexins are widely recognized as key proteins for synapse organization and loss-off-function mutations are associated with neurodevelopmental disorders as autism or schizophrenia. Three coding genes and extensive alternative splicing generate Neurexin proteins with various binding motifs, a flexibility which may determine synapse specification. A comprehensive understanding of the underlying molecular mechanisms is however largely missing. Our aim is to unravel the molecular composition of Neurexin networks in the brain by using unbiased quantitative proteomic approaches. Here we show results from targeted proteomics, combining affinity isolation of endogenous Neurexin complexes from mouse brain with high-resolution quantitative mass spectrometry. The identified interactome of Neurexin 1-3 contains members of different classes of secreted, adhesion or integral membrane proteins. This includes known binding partners (such as Cerebellin, Neuroligin and LRRTM proteins), but also quite a number of proteins that lack any annotation of primary function(s) and relation to synaptic physiology. We present in detail the common protein building blocks of Neurexins at the pre-synapse, the repertoire of ligands and trans-synaptic bridges and the differences among the isoforms. Furthermore, the impact of heparan sulfate modifications on the interactome will be presented. Together, we provide insights into the complex molecular environment of Neurexins in the brain and therefore open a possibility for future targeted analysis of molecular mechanisms involved in synapse formation, maintenance and function.

Disclosures: J. Schwenk: None. **S. Thivaios:** None. **F.H. Sterky:** None. **W. Bildl:** None. **B. Fakler:** None.

Poster

188. Trans-Synaptic Nanoscale Organization

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 188.03

Topic: B.04. Synaptic Transmission

Support: NINDS INTRAMURAL FUNDS

Title: Complex structural entanglement of transsynaptic complexes create association domains within rat hippocampal synapses in culture shown by EM tomography

Authors: *A. COLE, T. S. REESE;
NINDS, Bethesda, MD

Abstract: Even the simplest animal behavior hinges on the efficient yet imperfect transmission of the chemical synapse. While much is known of the chemical synapse's molecular composition, understanding the organization of these molecules may be key to understanding how the chemical synapse strikes a balance between efficiency and stochasticity. Recently, advanced light and cryoelectron microscopy revealed the alignment of elements across the synaptic cleft. The collection of aligned elements is called a *transsynaptic assembly*. Here, transsynaptic assemblies are visualized by tomographic electron microscopy of dissociated rat hippocampal neuronal cultures prepared by high-pressure freezing and freeze-substitution. In our renderings, nearly all cleft spanning structures connect to intracellular material to form transsynaptic assemblies. These transsynaptic assemblies can even form a complex connection between synaptic vesicles in the presynaptic compartment with postsynaptic density scaffolding. Moreover, assemblies can possess multiple intracellular components of varying morphologies and share them with other assemblies. This leads to complex entangled associations across the synapse. Thus, many presynaptic and postsynaptic structures are linked in association domains, which we think constitute the recently discovered nanocolumns. As such, we conclude that the structural basis for nanocolumns is the complex connectivity of transsynaptic assemblies. While our renderings show transsynaptic assemblies directly linking synaptic vesicles with bulky structures resembling glutamate receptors, we suggest instead that the link between domains is more functionally significant when considering the efficiency and stochasticity of the chemical synapse.

Disclosures: A. Cole: None. **T.S. Reese:** None.

Poster

188. Trans-Synaptic Nanoscale Organization

Location: SDCC Halls B-H

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Program #/Poster #: 188.04

Topic: B.04. Synaptic Transmission

Support: NIH R01MH116901
NIH R21MH129620-01
NIH 3T32GM007635-41S1

Title: Neurexin-3 controls excitatory synapse nano-organization in hippocampus

Authors: *B. A. LLOYD, R. ROTH, J. N. AOTO;
Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: Synaptic adhesion molecules are a diverse class of proteins that participate in synaptic formation, maintenance, and function which are critical for efficient synaptic transmission and plasticity. Recent evidence suggests that synaptic adhesion molecules allow for effective synaptic transmission via clustering and alignment of presynaptic active zones, where neurotransmitter is released, and postsynaptic receptors. Neurexins (Nrxns) are a class of essential, disease relevant presynaptic adhesion molecules which have been proposed to modulate synaptic nano-organization due to their known roles in regulating pre and postsynaptic structure via intracellular and transsynaptic signaling respectively. Nrxns are encoded by three evolutionarily conserved Nrxn genes (Nrxn1, 2, and 3) which were initially proposed to be redundant at all synapses; however, it is becoming increasingly apparent that individual Nrxns govern distinct aspects of synapse function. For example, Nrxn3 has been shown to control AMPA receptor strength but how Nrxn3 dependent nano-organization may contribute to this effect remain unknown. Here, I am investigating the role of Nrxn3 in synapse nano-organization using our Nrxn3 conditional knockout mouse and double helix 3D STORM to examine the clustering and alignment of proteins critical for synaptic transmission and have shown that Nrxn3 is required for the organization and alignment of excitatory synapses. These data represent, to the best of our knowledge, the first evidence that neurexins, and more widely presynaptic adhesion molecules, are required for the nano-organization of excitatory synapses and provides further insight into the mechanism by which Nrxn3 controls AMPA receptor strength. Future studies utilizing our novel epitope tagged Nrxn3 mouse will focus on defining the nanoscale endogenous localization of Nrxn3 at excitatory synapses and to study the relative distribution of multiple Nrxns in the same synapse.

Disclosures: B.A. Lloyd: None. **R. Roth:** None. **J.N. Aoto:** None.

Poster

188. Trans-Synaptic Nanoscale Organization

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 188.05

Topic: B.04. Synaptic Transmission

Support: NIH R00 MH118425

Title: Exploring novel extracellular signaling mechanisms regulating AMPAR trafficking and trans-synaptic localization

Authors: *G. SANDOVAL, J. DIAZ-ALONSO;
Anat. and Neurobio., Univ. of California, Irvine, Irvine, CA

Abstract: AMPA receptors (AMPARs) mediate fast excitatory synaptic transmission. Several synaptic plasticity mechanisms thought to be critical for learning and memory processes converge in the regulation of synaptic AMPAR abundance. Intracellular mechanisms have been shown to play a role orchestrating AMPAR trafficking. In addition, recent studies suggest that

subsynaptic localization of AMPAR is also crucial for their activation. Nanocolumns have been identified connecting postsynaptic AMPAR-rich clusters with presynaptic active zones, enriched in glutamate release machinery, across the synaptic cleft. Thus, the dynamic modulation of transsynaptic AMPAR alignment may influence synaptic plasticity, but the molecular mechanisms underlying transsynaptic AMPAR anchoring have yet to be identified. We and other laboratories recently discovered that the AMPAR amino terminal domain (ATD), which accounts for almost half of the protein size and protrudes substantially into the synaptic cleft, is essential for AMPAR synaptic trafficking and LTP. It is conceivable that the AMPAR ATD contributes to AMPAR transsynaptic positioning via interactions with extracellular proteins, constituting an "extracellular AMPAR slot." The present study aims at probing this hypothesis. In order to identify novel AMPAR ATD-interacting proteins, we initially conducted proteomic analyses. Preliminary AMPAR ATD interactors include the astrocyte-secreted glycoprotein thrombospondin-1 (TSP1) and its receptor, the voltage-gated calcium channel subunit α2δ1. Combining whole-cell patch-clamp electrophysiology using hippocampal acute slices from 3-6 week old mice of either sex with pharmacological and genetic approaches, we aim to explore this putative tripartite protein interaction and its potential contribution to AMPAR trafficking, subsynaptic localization, and function. Our co-immunoprecipitation studies in a heterologous system confirmed that TSP1 can directly interact with α2δ1 and suggest that it can also bind to GluA1. However, our preliminary observations indicate that TSP1 KO CA1 pyramidal neurons have unaltered basal synaptic transmission ($n = 8\text{-}15$, $p = 0.795$), and pairing induced LTP at CA3-CA1 synapses is comparable to WT counterparts ($n = 9\text{-}17$, $p = 0.973$). Ongoing experiments will determine whether specific presynaptic deletion of α2δ1 affects basal AMPAR synaptic transmission and plasticity. Future research will test other putative AMPAR ATD interactors. Our results suggest that extracellular signaling mechanisms involving the AMPAR ATD may play a modulatory role in AMPAR synaptic localization and function.

Disclosures: **G. Sandoval:** None. **J. Diaz-Alonso:** None.

Poster

188. Trans-Synaptic Nanoscale Organization

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Topic: B.04. Synaptic Transmission

Support: NIH Grant R01MH116901
NIH Grant 3T32GM007635-43S1

Title: Neurexin3a mutations distinctly modulate synaptic organization and function

Authors: *E. G. STOKES, J. N. AOTO;
Pharmacol., Univ. of Colorado, Anschutz Med. Campus, Aurora, CO

Abstract: Neurexins are essential but poorly understood presynaptic cell-adhesion molecules commonly thought to organize synaptic structure and transmission. Nrxns are encoded from three evolutionarily conserved genes (Nrxn1-3), each of which producing long alpha (α-Nrxn) and short beta (β-Nrxn) isoform. Although β-Nrxns have received a disproportionate amount of attention, α-Nrxns are more abundantly expressed than β-Nrxns in almost all neurons studied and more commonly associated with neuropsychiatric and neurodevelopmental disorders. Moreover, the elaborate extracellular sequences specific to α-Nrxns contain interaction domains for cognate post-synaptic ligands, potentially encoding for distinct and nonoverlapping functional synaptic properties. However, due to the complexity of α-Nrxn extracellular structure, a functional understanding of sequences essential for synapse function has remained largely enigmatic. As a first step toward understanding extracellular sequences of α-Nrxns, we have utilized a Nrxn3α mutation (A687T) identified in a patient with epilepsy and profound intellectual disability to investigate changes in synaptic properties. This mutation in the extracellular region of Nrxn3α modulates binding with LRRTM2 and produces a robust gain-of-function phenotype at excitatory synapses. To determine the properties of this amino acid residue that affects synapse integrity, I developed pseudo-mimetic Nrxn3α mutations (A687V, A687S). Utilizing a multidisciplinary approach, I expressed the Nrxn3α mutants in heterologous cells to investigate hemi-synapse formation and cellular aggregation. I also systematically interrogated these mutations in neuronal cultures using a knockdown-replacement approach, and employed immunocytochemistry and electrophysiology. I discovered that residue A687 plays an important role in modulating Nrxn3α interactions with post-synaptic ligands leading to distinct modifications in synapse organization and function. These findings further highlight the importance of studying α-Nrxn sequences. Revealing novel and non-redundant functionality of Nrxn3α in the synapse potentially illustrates the importance of how mutations in α-Nrxn specific sequences impact synaptic function and mental health.

Disclosures: E.G. Stokes: None. **J.N. Aoto:** None.

Poster

188. Trans-Synaptic Nanoscale Organization

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 188.07

Topic: B.04. Synaptic Transmission

Support: NIH Grant HL146833-02
AHA 915397

Title: Cholinergic collateral projections between sympathetic neurons in the murine stellate ganglia

Authors: *C. CLYBURN[1], M.-H. LI[1], M. C. ANDRESEN[1], S. L. INGRAM[2], B. A. HABECKER[1];
[1]Chem. Physiol. and Biochem., [2]Neurolog. Surgery, Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Postganglionic stellate ganglia (SG) neurons receive cholinergic inputs from the spinal cord (T2 inputs) and send noradrenergic projections to the heart via the inferior cardiac nerve (iCN). Several lines of evidence suggest that cholinergic collateral projections may be present within sympathetic ganglia. We tested the hypothesis that cholinergic collateral projections occur between sympathetic neurons of the intact SG. $ChAT^{TH+CreERT2/lox}$ mice were treated with tamoxifen to induce the deletion of choline acetyltransferase (ChAT) in tyrosine hydroxylase (TH) positive cells ($ChAT^{TH+}$ KO). This should prevent cholinergic collateral transmission while leaving preganglionic inputs intact. Whole-cell patch clamp recordings were made of intact SG neurons from male and female $ChAT^{TH+}$ KO mice (N=7 males and 9 females) or littermate controls without tamoxifen (N=14 males and 9 females). Collateral synapses were assessed by stimulating the preganglionic nerve trunk, T2, to evoke excitatory postsynaptic currents (eEPSCs; orthograde), or by stimulating the postganglionic nerve trunk (iCN) to evoke retrograde EPSCs and action currents (rACs). We measured the amplitude, jitter (i.e. SD of latency), and success rate of the orthograde and retrograde EPSCs and tested the effects of the nicotinic acetylcholine receptor antagonist, hexamethonium (300μM; Hex). Retrograde EPSCs were completely blocked by Hex in 7/7 control neurons and were detected in the majority of neurons from control mice, but not in $ChAT^{TH+}$ KO mice (17/20 cells vs. 0/16 cells, $P<0.05$ from χ^2 test). Spontaneous EPSCs were completely blocked by Hex in 5/5 control neurons and the frequency of sEPSCs was significantly reduced in ChAT KO mice (0.31±0.339 events/s vs. 0.057±0.061 events/s, $P<0.05$ from Student's unpaired t-test, N=20-28 cells). The jitter of orthograde eEPSCs was significantly reduced in ChAT KO mice (285.7±167.12μs vs. 75.2±54.5μs, $P<0.05$ from Student's unpaired t-test, N=9-11 cells). The presence of retrograde eEPSCs and high jitter orthograde eEPSCs are consistent with added synaptic connections to the conventional monosynaptic sympathetic transmission in the SG. The selective loss of retrograde eEPSCs after deletion of ChAT from TH^+ cells suggest that these collaterals are cholinergic. These data also suggest that cholinergic collaterals contribute to increased sEPSC frequency and network activity in the SG. Understanding SG neurocircuitry is critical in deciphering changes in sympathetic activity in many pathophysiological conditions such as myocardial infarction and heart failure and may identify novel therapeutic targets for the treatment of autonomic imbalance.

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Poster

188. Trans-Synaptic Nanoscale Organization

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Program #/Poster #: 188.08

Topic: B.04. Synaptic Transmission

Support: NIH Grant R01DC011099
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Title: The molecular components of an Electrical Synapse

Authors: *S. CÁRDENAS-GARCÍA, S. IJAZ, A. PEREDA;
ALBERT EINSTEIN COLLEGE OF MEDICINE, Bronx, NY

Abstract: Chemically-mediated synapses can be identified by the presence of various pre-and postsynaptic structural components. On the other hand, electrical synapses are identified and defined by the presence of a neuronal gap junction. While gap junctions serve as the communicating mechanism, it is unclear if electrical transmission relies on additional synaptic structures. We investigated this at identifiable single auditory synaptic contacts on the teleost Mauthner cells, at which neuronal gap junctions coexist with structural specializations for neurotransmitter release. Using expansion microscopy we were able to increase 5X the resolution and resolve with higher accuracy spatial distribution of the synaptic components. We identified at each terminal multiple gap junctions of variable size by the presence of their molecular component, including Zonula occludens-1 (ZO1), a scaffolding protein that forms part of the gap junctions plaques. Expansion microscopy confirmed the postsynaptic location of ZO1, making it possible to detect the lack of colocalization between the fluorescence for the presynaptic gap junction hemichannel and ZO1. Strikingly, while chemical synapses occupy a small area in the periphery of the contact, most of the surface is occupied by interleaving gap junctions the adherens junction proteins N-Cadherin and beta-catenin, suggesting a functional relationship between these structures. In summary, the observations indicate that an electrical synapse can operate using multiple gap junctions. Moreover, gap junctions might require the functional contribution of additional structural components such as adherens junctions. The association between gap junctions and adherens junctions could thus help identifying the boundaries of an electrical synapse.

Disclosures: S. Cárdenas-García: None. **S. Ijaz:** None. **A. Pereda:** None.

Poster

188. Trans-Synaptic Nanoscale Organization

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Program #/Poster #: 188.09

Topic: B.04. Synaptic Transmission

Support: NIH Grant R37 MH080046
NIH Grant F31 MH117920

Title: The Impact of Ionotropic Glutamate Receptors on Trans-Synaptic Nanostructure

Authors: *P. A. DHARMASRI, M. C. ANDERSON, A. D. LEVY, T. A. BLANPIED;
Physiol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Information processing in the brain relies on the dynamic and precise organization of molecular ensembles at the excitatory synapse. Proteins subserving the mechanism of synaptic

transmission are heterogeneously organized on the nanometer scale. Presynaptic proteins such as Munc13 are found in ~80 nm diameter subsynaptic regions of high local density. These presynaptic *nanoclusters* (NCs) are aligned across the synapse with NCs of proteins such as PSD-95 and ionotropic glutamate receptors (iGluRs), forming a *trans-synaptic nanocolumn*. Our lab established that this nanocolumn is the preferential site of action potential evoked release and that dispersal of AMPARs from the nanocolumn to the remainder of the synapse specifically reduces the strength of the response to evoked release. This highlights how the nanocolumn is critical for synaptic transmission and underscores the importance of knowing what generates and regulates this alignment. We reasoned that a mechanism underlying trans-synaptic alignment would involve a protein that could act as either a structural or functional marker of successful transmission. Intriguingly, the iGluRs themselves embody both aspects. First, either AMPARs or NMDARs could trigger downstream mechanisms that sample receptor activation and direct receptor positioning. Second, often underappreciated, both AMPARs and NMDARs have an extended extracellular structure with distal N-terminal domains that could nucleate direct or indirect interaction with presynaptic proteins. iGluRs could also organize scaffold NCs through multivalent interactions, either through NMDAR c-tail or AMPAR TARP binding. Previous studies show that receptor structure, particularly that of the AMPAR subunit GluA2 and its N-terminal domain, influences synaptogenesis and presynaptic maturation. Thus, we hypothesized that the iGluRs align the trans-synaptic nanocolumn. To test this, we generated both GluA2 and GluN1 CRISPR knockouts in dissociated rat primary hippocampal neuronal culture. Here we report the effects that loss of iGluRs have on nanostructure of scaffold proteins within the synapse. On-going experiments seek to elucidate the structural or functional influence of iGluRs. The organization of iGluRs at the synapse is typically thought to be downstream of pre-existing scaffold structure. This study instead tests an unorthodox view of AMPA and NMDA receptors: that they actively shape the structure of synapses and thus may dictate their own activation.

Disclosures: P.A. Dharmasri: None. **M.C. Anderson:** None. **A.D. Levy:** None. **T.A. Blanpied:** None.

Poster

188. Trans-Synaptic Nanoscale Organization

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Program #/Poster #: 188.10

Topic: B.04. Synaptic Transmission

Support: F32-MH119687
F31-MH117920
R37-MH080046
R01-MH119826

Title: Differential nanoscale organization of excitatory synapses onto excitatory vs inhibitory neurons

Authors: *A. D. LEVY, P. A. DHARMASRI, T. A. BLANPIED;
Physiol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The strength of excitatory synaptic transmission relies on the precise, nanometer-scale organization of synaptic proteins. Within single synapses, presynaptic release proteins and postsynaptic receptors and scaffolds concentrate in ~80 nm sub-regions of higher protein density, termed nanoclusters (NCs). Pre and postsynaptic NC alignment across the synaptic cleft results in stronger action potential-mediated excitatory postsynaptic currents, demonstrating a role for nanostructure in regulating synaptic strength. One context in which this may be particularly important is dictating excitatory drive onto diverse cell types in a circuit. For example, in the hippocampus, excitatory synapses are made onto both dendritic spines of excitatory principal neurons as well as dendrites of parvalbumin (PV)-expressing fast-spiking interneurons. These cellular and circuit contexts require different excitatory drive - compared to principal neurons, excitatory drive onto PV neurons must be stronger to overcome the electrotonic limitations of a non-compartmentalized synapse and sustain fast spiking, enabling PV neurons to deliver rapid and precise inhibition. Despite these different requirements, excitatory synapses onto both cell types consist largely of the same widely conserved group of proteins. Therefore, we reasoned synapses on these two cell types might differentially utilize nanoscale organization to tune the strength of excitation. We used immunocytochemistry and a combination of confocal and super-resolution microscopy to compare organization of common scaffold proteins at these synapses. We find that PV neurons have larger presynaptic Munc13 and postsynaptic PSD-95 puncta, consistent with stronger synapses. However, while the larger Munc13 puncta contained more Munc13 per area, PSD-95 did not, suggesting a possible nanostructural difference at these two cell types. Indeed, Munc13 and PSD-95 at PV cells were organized into more NCs (Munc13: 1.85x, PSD-95: 1.35x) that were larger (Munc13: 1.7x, PSD-95: 1.3x) than their excitatory cell counterparts. Analysis and modeling of protein localization autocorrelation, which measures nanoscale heterogeneity of protein distribution, indicates both Munc13 and PSD-95 are more concentrated into nanoclusters at PV cells. These results suggest that while excitatory synapses onto PV neurons are both larger and have more protein, the nanostructure is also optimized in such a way that predicts larger responses to action potential-evoked release. More broadly, we conclude that specialized aspects of nanostructural organization may contribute to functional diversity of excitatory synapses.

Disclosures: **A.D. Levy:** None. **P.A. Dharmasri:** None. **T.A. Blanpied:** None.

Poster

188. Trans-Synaptic Nanoscale Organization

Location: SDCC Halls B-H

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Program #/Poster #: 188.11

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: Skidmore College Faculty Student Summer Research Award
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NINDS Grant R01 NS123439 01

Title: Identification of modifiers of DIP-α-mediated neuromuscular connectivity in Drosophila melanogaster

Authors: B. WU[1], T. J. GATTON[1], Y. WANG[2], R. A. CARRILLO[2], ***C. G. VECSEY**[1]; [1]Neurosci., Skidmore Col., Saratoga Springs, NY; [2]Mol. Genet. and Cell Biol., Univ. of Chicago, Chicago, IL

Abstract: Synaptic connectivity during development is highly specific and stereotyped, but much is still unknown about why these processes occasionally fail. In *Drosophila*, emerging evidence suggests that motor neurons achieve target recognition through a network of interacting cell surface proteins (CSPs), the defective proboscis extension response proteins (Dprs) and their partners, Dpr-interacting proteins (DIPs). Here, we aimed to elucidate mechanisms regulating Dpr/DIP-mediated target specificity during development of neuromuscular innervation. The X-linked gene *DIP-α* in neurons and its binding partner Dpr10 in muscles have been shown previously to be required for RP2 motor neurons to innervate body wall muscle 4 (m4). To identify genes that regulate *DIP-α* and RP2 innervation, we performed a dominant modifier screen using the Bloomington Deficiency Kit. Flies that were either mutant for *DIP-α* or both *DIP-α* and *Dpr10* were crossed with a series of genomic deficiency lines, and innervation of m4 by RP2 neurons was assayed by immunostaining. Several deficiency lines modified the mutant phenotypes, some that further worsened and others that enhanced connectivity. One particular deficiency (DF7634) improved connectivity deficits seen in hemizygous *DIP-α* mutant males as well as in *DIP-α*/*dpr10* double-heterozygous female mutants. As there are about 20 identified genes within this deficiency region, a future aim is to identify the individual gene(s) within this region responsible for these changes. Overall, our findings suggest that proteins expressed from genes present within the DF7634 genomic region may be part of the Dpr/DIP signaling pathway that regulates neuromuscular junction wiring patterns. These data shed new light on processes that shape synaptic connectivity during development.

Disclosures: B. Wu: None. **T.J. Gatton:** None. **Y. Wang:** None. **R.A. Carrillo:** None. **C.G. Vecsey:** None.

Poster

189. Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 189.01

Topic: B.03. Ion Channels

Support: NRF-2018M3C7A1056897
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IBS-R001-D2

Title: Distinct mechanisms determine the differential activation of TTYH-mediated volume-sensitive organic osmolyte/anion channel (VSOAC) and LRRC8A-mediated volume regulated anion channel (VRAC)

Authors: *Y.-E. HAN[1], H. KANG[2,3], T.-Y. KIM[2], P. PRASHANT[1], E.-S. YOON[1], J. C. LEE[2,3], S.-J. OH[1];
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Abstract: In the brain, a reduction in extracellular osmolality causes changes in intracellular ionic strength (μ) and swelling during water influx which subsequently triggers Cl^-- and osmolyte-efflux via volume-regulated anion channel (VRAC) and/or volume sensitive organic osmolyte/anion channel (VSOAC). LRRC8 family, an essential component of the VRAC channel, was firstly identified as pore-forming subunits of low intracellular ionic strength-dependent VRAC ($VRAC_\mu$) in response to either hypo-osmotic challenges or low ionic strength internal solution rather than cell swelling itself. In a recent study, we also demonstrated that (Ttyh) family encodes the pore-forming subunits of swelling-dependent anion channel in astrocytes with hypo-osmotic solution. However, the experimental conditions for treatment of hypo-osmotic-solution cannot distinguish between low cytoplasmic ionic strength (μ)-dependent activation of LRRC8 family and swelling-dependent activation of TTYH family. To discriminate between these two different channels, we recorded the stretch-activated Cl^- conductance in astrocytes by clamping positive-pressure and identified that Ttyh family encodes the pore-forming subunits of VSOAC activated by not only hypo-osmotic solution, but also positive-pressure induced membrane stretch without any osmotic perturbation. The stretch-activated Cl^- conductance in astrocytes was completely impaired by simultaneous gene-silencing of all homologs of *Ttyh*, but not by gene-silencing of *Lrrc8a*. Interestingly, we performed co-immunoprecipitation and Duo-link assay and found that TTYH1 and AQP4 or LRRC8A physically interact with each other, indicating that two kinds of volume channels, TTYH family-mediated VSOAC and LRRC8 family-mediated $VRAC_\mu$, could cooperate as volume channel complexes in astrocytes under hypo-osmotic conditions. Our results demonstrate that Ttyh family confers a bona fide stretch-activated VSOAC in the brain.

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Poster

189. Other Ion Channels

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Program #/Poster #: 189.02

Topic: B.03. Ion Channels

Support: R37NS045876

Title: The ion channel calhm2 as a translocator of the alpha subunit of mitochondrial trifunctional protein

Authors: *S. BATTER[1], A. ROBSON[2], N. MNATSAKANYAN[3], M. KHOKHA[2], E. JONAS[4]; [1]Cornell Univ., Ithaca, NY; [2]Pediatrics, Yale school of medicine, New Haven, CT; [3]Cell. and molecular physiology, Penn State Col. of Med., Hershey, PA; [4]Intrnl. Med., Yale Sch. of Med., New Haven, CT

Abstract: Mitochondria play a pivotal role in cell survival and cellular metabolic function. Alterations in mitochondrial metabolic function or structure can cause neurological disease. One of the many metabolic reactions inside the mitochondrial matrix is fatty acid beta oxidation, which generates acetyl-CoA leading to ATP synthesis in later metabolic steps. A protein called mitochondrial trifunctional protein (mTFP) localizes to the matrix and catalyzes the last 3 steps of fatty acid beta oxidation. mTFP is an octamer with 4 units of ECHA (alpha subunit of mTFP) and 4 subunits of ECHB (beta subunit of mTFP). How these enzyme subunits translocate from the cytosol to the matrix where they carry out their function is not known. CALHM2 has recently been identified as a calcium-inhibited ion channel. Mutations in the gene that encodes CALHM2 have been associated with heart and neuropsychiatric disease. Our group had found previously that CALHM2 is localized to mitochondria and that knockdown of CALHM2 in cells causes severe changes in mitochondrial morphology and function including abnormal cristae, calcium imbalance, and respiratory dysfunction. Immunoprecipitation of CALHM2 demonstrated that it is bound to ECHA and ECHB, but in cells depleted of CALHM2, ECHA is prevented from reaching the mitochondrial matrix as assayed by immunofluorescence and immunoblotting. ECHB matrix amount remains normal in CALHM2 knockdown cells. Based on the large pore diameter of CALHM2, it is possible that this channel acts as a protein translocator for ECHA. Single channel patch clamp recordings of the purified CALHM2 protein reconstituted into liposomes revealed that channel activity was inhibited by calcium as has been reported previously for whole cell recordings. We found that the channel opens when peptides of ECHB are added to the solution. Addition of ECHA peptides produces rapid gating (opening and closing) of the channel, followed by complete channel activity inhibition, suggesting ECHA's transit into and through the pore. Thus, we hypothesize that ECHB acts as an activator of the CALHM2 channel, which then subsequently translocates ECHA.

Disclosures: S. Batter: None. **A. Robson:** None. **N. Mnatsakanyan:** None. **M. Khokha:** None. **E. Jonas:** None.

Poster

189. Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 189.03

Topic: B.03. Ion Channels

Support: Startup fund from the medical college of Georgia at Augusta University

Title: Hcn1 channelopathy in the mouse model of social avoidance

Authors: J. KIM, ***C. KIM**;
Neurosci. and Regenerative Med., Augusta Univ., Augusta, GA

Abstract: The CA1 pyramidal neurons have a non-uniform distribution of voltage-gated ion channels. They control how incoming information is processed and how synaptic plasticity is induced. Hyperpolarization-activated cyclic nucleotide gated nonselective cation 1 (HCN1) channels are highly expressed in the hippocampus. A gradient of increasing HCN1 channel density along the somatodendritic region of CA1 is an essential feature. An increasing amount of evidence suggests that HCN1 channel mislocalization in CA1 neurons is linked to the abnormal cellular and behavioral properties. While chronic stress increases hyperpolarization-activated current (I_h) in dorsal hippocampal CA1 neurons, the underlying molecular mechanisms are entirely unknown. Following chronic social defeat stress (CSDS), susceptible mice displayed social avoidance and impaired spatial working memory, which were linked to decreased neuronal excitability, increased perisomatic HCN1 protein expression, and elevated I_h in dorsal but not ventral CA1 neurons. In control mice, bath application of corticosterone (CORT) reduced neuronal excitability, increased tetratricopeptide repeat-containing Rab8b-interacting protein and HCN1 protein expression, and elevated I_h in dorsal but not ventral CA1 region/neurons. CORT-induced upregulation of functional I_h was mediated by the glucocorticoid receptor, HCN channels, and the protein kinase A but not the calcium/calmodulin-dependent protein kinase II pathway. Three months after the end of CSDS, susceptible mice displayed persistent social avoidance when exposed to a novel aggressor (i.e., a trigger). The sustained behavioral deficit was associated with lower neuronal excitability and higher functional I_h in dorsal CA1 neurons, both of which were unaffected by CORT treatment. Our findings show that CORT treatment mimics the pathophysiological effects of dorsal CA1 neurons/region found in susceptible mice. The aberrant expression of HCN1 protein along the somatodendritic axis of the dorsal hippocampal CA1 region might be the molecular mechanism driving susceptibility to social avoidance.

Disclosures: **J. Kim:** None. **C. Kim:** None.

Poster

189. Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 189.04

Title: WITHDRAWN

Poster

189. Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 189.05

Topic: B.03. Ion Channels

Title: Glycine alpha1 receptors in GlyT2 positive cells are central to the control of motor function

Authors: Z. JARRETT[1], D. M. LOVINGER[2], ***L. ZHANG**[1];
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Abstract: Glycine receptor alpha1 subunits (GlyRα1s) are implicated in regulating motor activity in both animals and humans. However, the cell-type specific mechanisms underlying GlyRα1 regulation of motor function have not been well defined. The current study explores the in vivo consequences of GlyRα1 deficiency in glycine transporter type 2 (GlyT2) positive cells. We generated a GlyT2 GlyRα1 deficient mouse line by crossing GlyT2-Cre mice with GlyRα1-flox mice. In line with previous studies, GlyT2 and GlyRα1 signals were primarily colocalized in the intermediate gray matter of mouse spinal cord, as revealed by RNAscope analysis. However, such colocalization nearly disappeared in spinal slices from homozygous GlyT2 specific GlyRα1 deficient mice. Western blot assay revealed a significant reduction in the expression levels of spinal GlyRα1 protein from homozygous mutant mice. In contrast, depletion of GlyRα1 from GlyT2 cells did not significantly affect the colocalization of spinal GlyRα1 with vGluT2, a biomarker specific for glutamatergic neurons. Both male and female homozygous mutant mice displayed hind limb paralysis from 14 days after birth. As a result, these mice showed near complete disruption of locomotor activity and motor coordination. On the other hand, male and female heterozygous mutant mice did not show impaired locomotor activity, whereas these mutant mice performed worse on the rotarod than their wild type littermates. No group, including the homozygous mutant mice, showed differences on the tail-flick test, suggesting that GlyRα1 in GlyT2 cells is not involved in spinal nociception. These data demonstrate that GlyRα1 subunits expressed in GlyT2 positive cells of the spinal cord are essential for motor function. Heterozygous GlyT2-GlyRα1 deficient mice, with selective reduction in coordination and balance skills, may provide a particularly valuable animal model to study the in vivo consequences of GlyRα1 subunits specifically expressed in GlyT2 cells.

Disclosures: Z. Jarrett: None. **D.M. Lovinger:** None. **L. Zhang:** None.

Poster

189. Other Ion Channels

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Program #/Poster #: 189.06

Topic: B.03. Ion Channels

Support: NIH Grant NS116327
GSU MBD
GSU B&B fellowship

Title: C-terminal post-translational modification of ion channels by the small ubiquitin like modifier (SUMO) promotes rab11a mediated slow recycling of endocytosed channels

Authors: *L. A. FORSTER[1], D. J. BARO[2];
[1]Neurosci. Inst., Georgia State Univ., Atlanta, GA; [2]Biol., Georgia State Universsity, Atlanta, GA

Abstract: HCN2 channels mediate the hyperpolarization activated current, Ih. Their SUMOylation at K669 increases HCN2 surface expression and Ih. The molecular mechanism by which HCN2 SUMOylation enhances channel surface expression is unknown. Surface expression can be increased by promoting trafficking from the trans-Golgi to the plasma membrane, by reducing channel endocytosis and/or by increasing recycling of the endocytosed channel. The effect of SUMOylation was investigated by stably expressing HCN2 in Human Embryonic Kidney (HEK) 293 cells. SUMOylation was or was not experimentally increased either by overexpressing the SUMO2 peptide and the SUMO-conjugating enzyme, ubc9, or by including SUMO2 and SUMO3 peptides in the patch pipette. Whole cell patch clamp experiments with the endocytosis inhibitor, Pitstop2, mimicked and occluded the effect of K669 SUMOylation, suggesting that SUMOylation reduces endocytosis and/or enhances recycling of the endocytosed channel (one way ANOVA, $F(3, 31) = 4.672$, $p=0.008$). This was confirmed by measuring the fraction of biotinylated HCN2 channels that were internalized over a 2.5hr period. The fraction of internalized channels was greatly reduced by enhanced SUMOylation (t-test, $p=0.005$). This effect was not observed when expressing the HCN2 mutant, K669R (t-test, $p=0.98$). During endocytosis, the AP2 complex recruits HCN2 into clathrin coated vesicles. Co-IP experiments indicated that enhanced SUMOylation did not alter the association of HCN2 with the AP2 subunit, α-adaptin (t-test, $p=0.67$), suggesting that SUMOylation is not altering clathrin mediated endocytosis and is perhaps enhancing recycling of the endocytosed channels. Rab11a is necessary for slow recycling and is used as a marker for the endosomal recycling compartment. Immunohistochemistry experiments followed by confocal microscopy showed that enhanced SUMOylation significantly increased colocalization of HCN2 with Rab11a (Pearson's coefficient: 0.1391 vs 0.1829, $p=0.04$). Furthermore, over-expressing a dominant negative Rab11a mutant blocked the effect of enhanced K669 SUMOylation (t-test, $p=0.88$). These data suggest that HCN2 SUMOylation at K669 increases Ih by promoting recycling of endocytosed channels. Similar studies on HEK cells over-expressing a Kv4.2 ternary complex (Kv4.2, KChIP2a, DPP10) suggested that Kv4.2 SUMOylation at K579 increased surface expression of the ternary complex in a rab11a-dependent manner. C-terminal SUMOylation of ion channels may represent a general mechanism for regulating the extent of ion channel recycling.

Disclosures: L.A. Forster: None. **D.J. Baro:** None.

Poster

189. Other Ion Channels

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Program #/Poster #: 189.07

Topic: B.03. Ion Channels

Support: Supported by Janssen Research & Development, L.L.C.

Title: Structural basis of small-molecule inhibition of acid-sensing ion channel 1

Authors: J. MA[1], J. SCHOELLERMAN[2], R. HAGAN[2], R. L. DESJARLAIS[1], J. RECH[3], D. LIN[3], C. LIU[2], R. MILLER[1], J. LUO[4], M. LETAVIC[3], B. GRASBERGER[1], M. MAHER[2], ***Y. LIU**[2];
[1]Discovery Sci., Janssen Res. and Develop., Spring House, PA; [2]Neurosci. Discovery, [3]Discovery Sci., Janssen Res. and Develop., San Diego, CA; [4]Lead Engin., Janssen Res. and Develop., Spring House, PA

Abstract: Acid-sensing ion channels (ASICs) are proton-gated cation channels expressed in the nervous system. Studies of ASIC1a, a major ASIC isoform and primary sensor of acidosis, have identified an extracellular region enriched in acidic residues, termed the acidic pocket, as a key participant in proton sensing and channel gating. Recently, we reported that a small molecule modulator of ASIC1 (JNJ-799760) binds at the acidic pocket and stabilizes the closed state[1]. Here, we describe patch clamp studies of human and rat ASICs and crystal structures (at 2.5-3.2 Å resolutions) of chicken ASIC1 in complex with four structurally distinct small molecule ASIC1 modulators. Despite the chemical diversity, all four molecules bind to the same site in the acidic pocket as JNJ-799760 in the closed state of the channel, and potently inhibit pH-induced ASIC1a homomeric and ASIC1a/ASIC2a heteromeric channel currents (pIC50 = 7.2±0.1 - 8.9±0.1; n=3-15). Our findings unveil a novel and significant drug-binding site for small molecule modulation of ASIC1a and provide molecular and structural insight into chemical modulation of ASIC1. 1. Liu et al., Commun. Biol., 2021. doi: 10.1038/s42003-021-01678-1.

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Poster

189. Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 189.08

Topic: B.03. Ion Channels

Support: NIH Grant 5R01MH120637-02

Title: Inhibitory, antidepressant like effects of a novel compound series derived from cardiac bradine drugs on VTA dopamine neurons

Authors: *E. M. TEICHMAN[1], J. HU[1], X. HU[1], S. E. MONTGOMERY[1], S. J. RUSSO[1], C. MOREL[1], J. JIN[1], M.-H. HAN[2];
[1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Shenzhen Inst. of Advanced Technol., Shenzhen, China

Abstract: Depression is a devastating disease, associated with profound neurophysiological alterations. Upregulation of the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in ventral tegmental area (VTA) dopamine neurons is associated with depressive-like symptomology in mice. Inhibition of these channels by the HCN inhibitor Cilobradine alleviates those symptoms. Cilobradine is part of the "bradine" family of HCN-inhibiting cardiac drugs which includes Ivabradine, an FDA-approved drug to treat heart disease and heart failure. Here, we aim to augment and refine the HCN-inhibiting, minimally blood brain barrier (BBB)-penetrant features of Cilobradine so as to improve rapid-acting and long-lasting therapeutic effects. 11 analogs of HCN inhibitor Cilobradine and 1 analog of Zatebradine were designed and synthesized. We investigated their effects on VTA dopamine neuron I_h current and firing rate utilizing electrophysiology of brain slices from adult C57Bl6 mice. We also determined the pharmacokinetic profile and brain plasma ratios of the potent analogs. We demonstrated that these different analogs have a variety of inhibitory effects on I_h currents in VTA dopamine neurons. Compounds 10 and 12 were chosen for further study based on their strong inhibition of not only the I_h current but also firing rate; Cilobradine reduced the firing rate of VTA dopamine neurons by 66%, while compounds 10 and 12 led to 91.5% and 92.4% reductions, respectively. Pharmacokinetic analysis determined that the brain plasma ratios of compounds 10 and 12 are 0.28 and 0.57, respectively, greatly improved from the 0.076 brain plasma ratio of parent compound Cilobradine. We demonstrate that minimal changes to the Cilobradine scaffold can alter, and even improve, its inhibitory effect on VTA dopamine neurons. Furthermore, these minimal changes can drastically improve its BBB permeability. Future studies will assess the antidepressant behavioral effects of compounds 10 and 12 *in vivo*. Our results provide a new avenue of research for the development of novel therapeutics to alleviate psychiatric disorders associated with dopamine dysfunctions.

Disclosures: E.M. Teichman: None. **J. Hu:** None. **X. Hu:** None. **S.E. Montgomery:** None. **S.J. Russo:** None. **C. Morel:** None. **J. Jin:** None. **M. Han:** None.

Poster

189. Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 189.09

Topic: B.03. Ion Channels

Title: Electrophysiological characterization of functional and pharmacological effects of β subunit expression on BK channel activity

Authors: *L. HUTCHISON, C. BROWN, E. PARKER, D. DALRYMPLE, I. MCPHEE, D. PAU;
SB Drug Discovery, Glasgow, United Kingdom

Abstract: BK channels are large conductance potassium channels which are expressed in a broad range of excitable as well as non-excitable cell types and are activated by elevations in cytosolic Ca2+ concentration and membrane depolarization. It is thought that these channels have discrete physiological roles that are adapted to the specific cell type requirements and depend on cell-specific membrane potentials and cytosolic Ca2+ concentrations. To accomplish such a broad diversity in physiological roles, BK channels consist of a pore forming α subunit and employ auxiliary subunits (β1-4 or γ1-4) to enable the variety of functional roles. Modulation of BK channels has been suggested as a therapeutic strategy for a number of disorders including epilepsy, Alzheimer's disease and schizophrenia. However, the widespread expression of BK channels and their participation in a variety of essential physiological processes mean that any therapeutic strategy aimed at one particular cell type, tissue, or organ system risks impacting other cells and tissues unrelated to the pathology, perhaps with highly undesirable consequences. To reduce this risk, specific targeting of particular BK / auxiliary subunit combinations may prove beneficial, with the aim to selectively modulate specific BK subsets and reduce unwanted side-effects.
To investigate the functional and pharmacological effect of different β subunits on BK activity, recombinant HEK cell lines expressing BK α, αβ1, αβ2 and αβ4 combinations were developed and characterized using a high throughput automated patch clamp platform. Simultaneous assessment of multiple BK subtypes allowed for rapid optimization and characterization. Firstly, the current-voltage (IV) relationship was assessed in the presence of various free internal Ca2+ concentrations (0, 1, 3, and 10 μM), with the β containing BK channels displaying increased sensitivity to intracellular Ca2+ in line with literature values. The general BK activator NS1619 was assessed with calculated EC50 values in the range of 10-20 μM, while the activator Arachidonic Acid was selective for αβ1 over α only expressing cells.
In inhibitor mode, the calculated IC50 of the BK antagonist Paxilline was in the range of 30-130 nM depending on the specific BK αβ subunit complex, while Iberiotoxin was selective for BKα alone, in line with previous studies.
The characterization performed here enables the identification of pharmacological compounds targeting particular BK αβ subunit combinations and provides opportunities to selectively target BK channels involved in particular physiological disorders.

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Poster

190. Somatic and Dendritic Integration

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 190.01

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Title: Dendritic stimulus processing deficits in human cortical microcircuits in depression

Authors: *H. YAO, E. HAY;
Krembil Ctr. for Neuroinformatics, Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: Reduced inhibition from somatostatin (SST) interneurons and dendritic atrophy of pyramidal (Pyr) neurons are implicated in underlying cognitive deficits in treatment-resistant major depressive disorder (depression). Cortical SST interneurons primarily inhibit the apical dendrites of Pyr neurons to facilitate feedforward inhibition and modulate input integration. To study the effects of reduced inhibition from SST interneurons and dendritic atrophy on dendritic stimulus processing, we expanded our previous data-driven models of human cortical microcircuits in health and depression to include active dendritic properties in Pyr neurons such as backpropagating action potentials, and dendritic atrophy in terms of synapse and spine loss, and reduced dendrite length. We then characterized the functional implications of reduced SST interneuron inhibition and dendritic atrophy in depression on dendritic processing of stimuli, and showed decreased signal-to-noise ratio and increased stimulus detection errors in depression microcircuits. Our study thus mechanistically links reduced inhibition and dendritic atrophy in depression to dendritic stimulus processing deficits in human cortical microcircuits.

Disclosures: H. Yao: None. **E. Hay:** None.

Poster

190. Somatic and Dendritic Integration

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 190.02

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

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Scottish Rite Charitable Foundation of Canada (SRCFC) research grant

Title: Dendritic channelopathies alter the synaptic integration of excitatory inputs in the basal dendrites of layer 5 pyramidal neurons in a mouse model of Fragile X Syndrome

Authors: D. E. MITCHELL[1], S. MIRANDA-ROTTMANN[2], M. G. BLANCHARD[3], ***R. ARAYA**[4];
[1]Neurosciences, Univ. of Montreal/ The CHU Sainte-Justine Res. Center, Montreal, QC, Canada, Montreal, QC, Canada; [2]Neurosciences, Univ. of Montreal/ The CHU sainte-justine Res. Ctr., Montreal, QC, Canada; [3]Univ. of Montreal, Montreal, QC, Canada; [4]Neurosciences, Univ. of Montreal/ The CHU Sainte-Justine Res. Ctr., Montreal, QC, Canada

Abstract: Fragile X syndrome (FXS) is the most frequent form of inherited intellectual disability and common known cause of autism. Defects in the processing and integration of excitatory inputs in cortical neurons likely contributes to the behavioral phenotype associated with FXS. A key function of the neocortex is to associate external sensory information with an internal representation of the world to make predictions about the future. Layer 5 (L5) pyramidal neurons integrate sensory inputs onto their basal dendrites with information from other cortical areas at their distal dendrites. Here, we aimed to uncover how L5 pyramidal neurons from Fmr1KO mice integrate synaptic inputs at the level of single spines in the basal dendrites. We used two-photon uncaging of caged glutamate to activate nearly simultaneously two clustered spines in L5 pyramidal neurons. While excitatory inputs onto spines integrate linearly before the generation of a dendritic spike in wild-type animals, surprisingly those of Fmr1KO mice summate sublinearly. Since FXS is characterized by several channelopathies in pyramidal cells, we are currently investigating the role of calcium-activated potassium channels in explaining the observed integration defects using genetic manipulations and numerical simulations. Taken together, the results from these experiments will help uncover the role of ion channels in excitatory input integration and identify novel targets for the design of specific drugs to successfully treat FXS. This work was funded by the CIHR, as well as FRQS and QART postdoctoral fellowships to DEM.

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Poster

190. Somatic and Dendritic Integration

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 190.03

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Title: The function of I_h in layer 2/3 pyramidal cells

Authors: *V. J. OLAH, M. J. M. ROWAN;
Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Neocortical layer 2/3 pyramidal cells are a major component of the canonical cortical circuit, yet little is known about their subcellular excitability. These cells differ in several crucial aspects from their more extensively studied (e.g., L5) pyramidal counterparts. Specifically, their

morphology is less polarized, they often lack a distinctive apical tuft, and their ion channel expression is also assumed to differ. However, due to the absence of thick dendritic protrusions, the expression and distribution of dendritic conductances in L2/3 cells are mostly unknown. Hyperpolarization-activated nonselective cation (HCN) channels are essential for regulating resting membrane potential, the temporal normalization of synaptic events arriving at spatially mismatched locations, and establishing oscillation frequency-selectivity. Although it is well known that pyramidal cells in deeper cortical layers and the hippocampus express this conductance in their dendrites, layer 2/3 pyramidal cells have been widely regarded to lack it, due to the absence of the characteristic "sag" potential in current clamp recordings. Here we report that layer 2/3 pyramidal cells express functionally relevant HCN channels throughout the cortex. These channels induce steady-state membrane response rectification and their currents (Ih) are kinetically and pharmacologically similar to previous reports in other pyramidal cell types. We found that HCN channel activation influences the amplitude and time-course of synaptic events arriving onto various parts of the dendritic tree. Importantly, HCN channels have previously been found to be enriched within the distal apical tufts of cortical L5, and hippocampal CA1 pyramidal cells. Here, using direct dendritic patch-clamp recordings and spatially restricted synaptic stimulation, we found that HCN channels also exhibit a unique expression pattern in layer 2/3 cells, thereby influencing synaptic integration distinctively depending on input location. Our results demonstrate that layer 2/3 pyramidal cells not only express dendritic HCN channels but also employ these conductances in a previously unobserved manner.

Disclosures: V.J. Olah: None. **M.J.M. Rowan:** None.

Poster

190. Somatic and Dendritic Integration

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 190.04

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NINDS Grant 5F31NS113353-03
NINDS Grant R37NS046579
NIH Grant 1RF1MH117042-01
Vannevar Bush Faculty Fellowship
Brain Research Foundation Scientific Innovation Award
Harvard Brain Initiative Grant
Life Sciences Research Foundation Grant

Title: Dendritic branch-specific voltage and calcium imaging in layer 2/3 pyramidal neurons

Authors: *P. PARK[1], J. WONG-CAMPOS[1], H. TIAN[1], A. T. LANDAU[3], B. L. SABATINI[3], A. E. COHEN[1,2];

[1]Dept. of Chem. and Chem. Biol., [2]Dept. of Physics, Harvard Univ., Cambridge, MA; [3]Howard Hughes Med. Institute, Dept. of Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Neurons convert synaptic inputs arriving onto dendrites into action potentials that propagate outward along axons. Back-propagating action potentials (bAPs) also go from soma into dendrites and interact with synaptic inputs to modulate synaptic strengths at individual spines. Understanding how bAPs propagate within neurons is critical, but we have a limited technical tools to map voltage and calcium signals throughout dendritic arbors. We developed tools for simultaneous voltage and calcium imaging by using a new genetically encoded voltage indicator (GEVI) called QuasAr6a, which has a higher brightness, sensitivity, and speed than any existing GEVI. We introduced QuasAr6a and membrane-targeted GCaMP6f into a sparse subset of L2/3 neurons through in utero electroporation (IUE). On the optics front, we used a new instrument that combines holographically patterned red (635 nm) illumination for voltage imaging, micromirror-patterned blue (488 nm) illumination for wide-field Ca^{2+} imaging, and two-photon (2P) illumination for structural imaging. Under whole-cell patch clamp configuration in mouse brain slices (3-4 weeks of age), we studied dendritic Ca^{2+} heterogeneity in response to bAPs triggered by a current injection through the patch pipette. Our data suggests that some branches show a failure of bAP-dependent Ca^{2+} influx, and this branch-specific Ca^{2+} variability is dependent on the sister branch structures and voltage-gated calcium channels (VGCCs).

Disclosures: P. Park: None. **J. Wong-Campos:** None. **H. Tian:** None. **A.T. Landau:** None. **B.L. Sabatini:** None. **A.E. Cohen:** None.

Poster

190. Somatic and Dendritic Integration

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 190.05

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant R01NS113079

Title: Backpropagation-like error signals in apical dendrites of cortical neurons during a brain-computer interface task.

Authors: *V. FRANCIONI, N. J. BROWN, V. D. TANG, M. T. HARNETT;
MIT, Cambridge, MA

Abstract: The deep learning algorithm ‘backpropagation’ has led to significant advances in artificial intelligence. Recent theoretical models suggested how biological neurons could potentially implement backpropagation-like learning by semi-independently processing feedforward and feedback information streams at separate dendritic compartments. This represents a compelling but untested hypothesis for how cortical circuits may solve the credit assignment problem, a longstanding and crucial unknown in our understanding of how brains learn. We designed a neurofeedback Brain-Computer Interface (BCI) task with an experimenter-

defined cost function to test the hypothesis that individual neurons receive tailored error signals onto their apical dendrites, according to their causal contribution to task performance. We trained mice to modulate activity in two arbitrarily-selected neural populations (~5 neurons each) to rotate a visual grating to a target orientation while recording GCaMP7 activity from somas and corresponding apical dendrites. Our data revealed systematic amplitude mismatches of coincident GCaMP signals in somas and dendrites of individual layer 5 neurons, which could be predicted from local network activity. Somato-dendritic amplitude mismatches contained information about task-related variables including reward and error, defined as the distance between target and output activity. Our data show that the sign of error signals depends on the causal role of individual neurons in the BCI task. These neuron-specific error signals further correlate with changes in firing rates during the course of learning. Our results demonstrate that individual neurons receive backpropagation-like error signals onto their apical dendrites during learning, providing a biological mechanism for solving credit assignment in the mammalian neocortex.

Disclosures: **V. Francioni:** None. **N.J. Brown:** None. **V.D. Tang:** None. **M.T. Harnett:** None.

Poster

190. Somatic and Dendritic Integration

Location: SDCC Halls B-H

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Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: European Union (B.A.B., MSCA-IF 845956)
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Wellcome Trust (M.H., PRF 201225/Z/16/Z)

Title: Computational specialization of cortical dendrites

Authors: *K. SHENG, B. A. BICKNELL, M. HAUSSER;
Univ. Col. London, London, United Kingdom

Abstract: The transformation of synaptic input into action potential output is governed by dendritic physiology. Synaptic potentials are subject to severe attenuation on their way to the soma due to passive cable filtering, yet dendritic active conductances and local spatial interactions may compensate for, or even enhance the processing of particular input features. Dendritic integration thus presents both fundamental constraints on signal transmission and opportunities for computation. It has been known for decades that dendritic morphology and biophysics vary widely across cortical cell types, suggesting that different cortical neurons may be specialized for different computations. However, how the properties of dendrites determine the computational repertoire of neurons remains unclear. Here, we introduce a biophysical modelling and machine learning framework to address this question. We develop a general learning rule with which detailed models of neurons, comprising 3D reconstructed morphologies

and an array of active conductances, can be 'trained' to perform sophisticated computational tasks. Applying our learning rule in experimentally validated models of excitatory and inhibitory neurons from the Allen Cell Types Database, we translate the biology of each model neuron into a measure of its computational abilities. Training the database of models to implement nonlinear feature-binding computations, Boolean logic operations, and a regression task designed to test the limits of stimulus selectivity, we find performance varies both across and within cell types. Regression analysis identifies a diverse set of morphological and biophysical features that together explain ~85% of the variance in task performance. Moreover, we find that different features, such as dendritic branching parameters and input resistance, contribute differentially across tasks. In summary, we establish a systematic approach for investigating the computational specialization of single neurons, predicting tight relationships between the functional roles of different cell types, their structure, and biophysical properties.

Disclosures: K. Sheng: None. **B.A. Bicknell:** None. **M. Hausser:** None.

Poster

190. Somatic and Dendritic Integration

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 190.07

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Life Sciences Research Foundation

Title: All-optical electrophysiology reveals mechanisms of dendritic integration in vivo

Authors: *D. WONG-CAMPOS, P. PARK, H. TIAN, H. DAVIS, Y. QI, D. KIM, A. E. COHEN;
Chem. and Chem. Biol., Harvard Univ., Cambridge, MA

Abstract: Neurons convert synaptic inputs to spiking outputs, yet we lack a coherent picture of how dendrites integrate synaptic inputs to produce spikes, and how back-propagating action potentials (bAPs) interact with synaptic inputs to drive plasticity. The ability to map voltage dynamics throughout a dendritic tree would be a transformative capability for studying dendritic physiology. Here, we combine patterned channelrhodopsin activation and high-resolution structured illumination one-photon voltage imaging, to control and measure electrical activity throughout a dendritic tree *in vivo*. We recorded the voltage dynamics of the apical dendrites of 2/3 pyramidal neurons in the visual cortex of mice at different brain states and mapped the propagation of synaptic inputs and bAPs. We use localized optogenetic perturbations to estimate electrical cross-couplings among dendritic branches and to probe how the excitation, inhibition, and bAPs travel through the dendritic tree. The combination of advanced optical control with improved molecular reagents for all-optical electrophysiology has the potential to unravel mechanisms of dendritic integration and activity-dependent plasticity.

Disclosures: **D. Wong-Campos:** None. **P. Park:** None. **H. Tian:** None. **H. Davis:** None. **Y. Qi:** None. **D. Kim:** None. **A.E. Cohen:** None.

Poster

190. Somatic and Dendritic Integration

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 190.08

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: WhiteHall2018-12-09

Title: Modelling how the spatial relationship of dendritic excitation and inhibition affects synaptic integration in the posterior parietal cortex neurons

Authors: *A. PRODDUTUR[1], D. RINDNER[1], G. LUR[2];
[1]Univ. of California Irvine, Irvine, CA; [2]Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Functional interaction and distribution of excitatory and inhibitory synapses on dendritic trees shape multimodal integration properties of neurons. We have previously shown that distinct subclasses of pyramidal cells in the posterior parietal cortex (PPC) characterized by either regular spiking (RS) or intrinsic burst spiking (IB) perform nonlinear integration of inputs from auditory (A1, bottom-up) and anterior cingulate cortex (ACC, top-down). In preliminary studies using multicompartmental models and electrophysiological recordings, we found that coincident but not delayed (50 ms) activation of A1-ACC synapses resulted in multimodal enhancement in IB cells, whereas delayed (50 ms) but not coincident activation of A1-ACC synapse resulted in supralinear integration in RS cells. Based on model predictions, we found that, unlike IB cells, RS cells received more potent feedforward inhibition, which was instrumental for distinct temporal integration profiles in these cells. However, it is unclear what role morphological parameters and the spatial organization of synapses on the dendrites play in these distinct temporal integration dynamics. To test whether clustered or distributed synapses recapitulate our electrophysiological findings, here we used multicompartmental model cells, one with apical dendrite alone and the second model with basal and apical dendrites with proximal and distal segments in each dendrite. We simulated models with either synapses arriving on the same or different segments (distal or proximal) of the dendrites. From the simulations, we find that clustered excitatory synapses, that is, both A1 and ACC inputs arriving on the same segment, especially in the distal dendritic segment of both the models, resulted in multimodal enhancement in IB cells that recapitulated our experimental observations. Similarly, we recapitulated RS cell experimental observations when the excitatory and inhibitory synapses clustered on the distal segment of the tested models. Additionally, in RS cells, we observed that inhibitory input arriving on any segment of the dendrite with respect to excitatory synapses is efficient in suppressing supralinear integration when A1-ACC inputs were activated simultaneously. Together, based on our simulations, we speculate that in the distinct subclasses

of PPC principal neurons, regardless of apical or basal dendrite morphology, the multimodal synapses are likely to arrive or cluster on the distal dendritic segments, which seem to drive supralinear synaptic integration.

Disclosures: **A. Proddutur:** None. **D. Rindner:** None. **G. Lur:** None.

Poster

190. Somatic and Dendritic Integration

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Program #/Poster #: 190.09

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIMH R01MH123686
NIDCD T32DC010775-12
Whitehall Foundation grant 2018-12-9

Title: Distinct ionic mechanisms of feedforward and feedback synaptic integration in parietal cortex layer 5 neuron subclasses

Authors: *D. J. RINDNER, A. PRODDUTUR, G. LUR;
Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Combining feedforward (sensory) and feedback (contextual) signals is a key function of the neocortex. Yet, synaptic-level insight to how these pathways interact remains limited. Using a dual-color optogenetic approach, we show that intracortical feedforward and feedback afferents have monosynaptic convergence on cells in layer 5 of the mouse posterior parietal cortex. Temporal dynamics of integration differed between the two major subclasses of layer 5 neurons. Intrinsically bursting (IB) cells boosted coincident synaptic events, while regular spiking (RS) cells preferentially enhanced delayed inputs. Pharmacological manipulations coupled with computational modelling identified sodium channel, calcium channel, and NMDA receptor conductances necessary for coincident and delayed integration. Notably, a difference in NMDA decay kinetics between IB and RS cells was crucial for driving cell type-specific delayed interactions. Retrograde labelling then revealed unique long-range axonal targets for IB and RS cells. Parietal cortex projections to the pons primarily comprised of IB cells, while outputs to the dorsal striatum were predominantly RS. Thus, IB and RS subclasses are likely to represent distinct channels of information flow in the neocortex with specific roles in integrating feedforward and feedback signals.

Disclosures: **D.J. Rindner:** None. **A. Proddutur:** None. **G. Lur:** None.

Poster

190. Somatic and Dendritic Integration

Location: SDCC Halls B-H

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Program #/Poster #: 190.10

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: ARC (DP160103047)
NHMRC (APP1085708)
Sylvia and Charles Viertel Charitable Foundation

Title: Dendritic encoding of multimodal behaviour in the posterior parietal cortex

Authors: *R. MASUDA[1,2], K. ZHOU[1,2], L. CHEUNG[1,2], L. PALMER[1,2];
[1]Florey Neurosci. Inst., Melbourne, Australia; [2]Univ. of Melbourne, Melbourne, Australia

Abstract: The ability to integrate sensory information from multiple modalities allow us to respond appropriately and dynamically to our environment. While we are beginning to unravel where in the brain this may occur, we are yet to fully understand the cellular mechanisms which drive multimodal integration. Specifically, we do not know how dendritic processes integrate information from different senses, and how cellular mechanisms change to match behavioral needs. To this end, we performed two-photon calcium imaging from the apical tuft dendrites of layer 2/3 pyramidal neurons in the posterior parietal cortex (PPC) - a brain region heavily implicated in multisensory integration. Compared to unimodal stimuli (tactile or auditory), dendritic calcium activity was greater in response to passive presentations of a multimodal stimulus (tactile + auditory). To probe whether this enhanced dendritic encoding of multisensory information also occurs during behavior, we designed a Go/NoGo task where mice had to discriminate between unimodal and multimodal stimuli, and respond appropriately to receive a water reward. In expert mice performing the task, sensory encoding was increased compared to the naïve state, with a greater frequency and decreased latency of dendritic signals. Specifically, the multimodal Go stimulus had a greater decrease in the dendritic response latency compared to unimodal NoGo stimuli, which was also reflected in the behavioral response speed of mice. Taken together, PPC dendrites shift from enhanced encoding of multimodal stimuli in a passive context, to quicker encoding during behavior. The present study uncovers a novel cellular mechanism underlying the ability to adapt the efficiency of multimodal processing when the behavioral requirements necessitate it, highlighting the importance of PPC dendrites in this process.

Disclosures: **R. Masuda:** None. **K. Zhou:** None. **L. Cheung:** None. **L. Palmer:** None.

Poster

190. Somatic and Dendritic Integration

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 190.11

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Foundation for Polish Science, TEAMNET, POIR.04.04.00-00-14DE/18-00

Title: Morphologically realistic neurons are alternatives to artificial neural networks

Authors: *Z. SLAWINSKI[1,2], I. PODOLAK[2], D. WÓJCIK[1,2];
[1]Lab. of Neuroinformatics, Nencki Inst. of Exptl. Biology, Polish Acad. of Sci., Warsaw, Poland; [2]Jagiellonian Univ., Kraków, Poland

Abstract: From the perspective of synaptic input and spiking output, a morphologically complex neuron is (almost) equivalent to a single output multilayered artificial neural network. However, it is not known if they can perform equivalent computations. Recently Bicknell and Hausser (2021) showed that a single neuron of complex morphology can be trained to distinguish different synaptic patterns which previously were considered to require multilayer neural networks. Here, using the method of Bicknell and Hausser, we study minimal computational models of a dendritic tree trained on a classification task that requires complex, nonlinear computations. We show which aspects of dendritic morphology and synaptic locations influence feature differentiation and how to select them optimally.
Bicknell, B. A., & Häusser, M. (2021). A synaptic learning rule for exploiting nonlinear dendritic computation. Neuron, 109(24), 4001-4017.

Disclosures: Z. Slawinski: None. **I. Podolak:** None. **D. Wójcik:** None.

Poster

190. Somatic and Dendritic Integration

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 190.12

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Title: Refining a table-based approach to prediction of active dendritic responses

Authors: *C. R. JEWELL[1], T. RAMDAS[2], B. W. MEL[1];
[1]Biomed. Engin., USC, Los Angeles, CA; [2]Program in Neurosci., Harvard Univ., Cambridge, MA

Abstract: Refining a table-based approach to prediction of active dendritic responses
Christopher R. Jewell, Biomedical Engineering Department, USC
Tejas Ramdas, Neuroscience Program, Harvard University
Bartlett W. Mel, Biomedical Engineering Department, USC.
Understanding how neurons convert synaptic inputs into trains of action potentials is a central problem of systems neuroscience. The question is complicated by the fact that neurons contain many types of active channels (Major et al. 2008), and support nonlinear synaptic interactions on spatial scales ranging from microns to millimeters (Jarsky et al. 2005; Larkum et al. 2009; Branco and Hausser 2010; Gidon and Segev 2012; Jadi et al. 2014). Various modeling approaches have been developed to try to predict neural responses to spatiotemporal patterns of

excitatory and inhibitory stimulation (Mel 1992; Poirazi et al. 2003; Jadi et al. 2014; Ufjalussy et al. 2018; Moldwin et al. 2021; Beniaguev et al. 2021). An important factor in determining neural responses is the degree of spatial clustering of excitatory input to dendrites (Mel, 1993; Polsky et al. 2004; Katona et al. 2011; Kastellakis et al. 2015; Ujfalussy et al. 2020), which is significant in light of accumulating evidence for spatial clustering of like-activated synaptic contacts in the brain (Fu et al. 2012; Kleindienst et al. 2011; McBride & DeBello 2015; Lee et al. 2016; Wilson et al 2016; Adoff et al. 2021). Many questions remain, however: in numerous pilot simulations for this project, we have found that nonlinear synaptic interactions in dendrites, and therefore dendritic outflows to the soma, are influenced not only by the degree of spatial clustering of activated synapses, but also by the number of activated synapses, the biasing of inputs towards or away from the soma, the locations of coactivated inhibitory synapses, the NMDA-AMPA ratio, NMDA peak conductance, short term synaptic dynamics, spine neck resistance, the spine density profile, dendritic resting potential, and the state of somatic excitation (up-state vs. down-state). We are currently extending a previously developed table-based approach to dendritic response prediction (Jin and Mel, 2019 SFN abstract), to accommodate a wider range of stimulus variables (see above), and to allow prediction of a neuron's output firing rates under multi-branch stimulus conditions.

Disclosures: C.R. Jewell: None. **T. Ramdas:** None. **B.W. Mel:** None.

Poster

190. Somatic and Dendritic Integration

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Program #/Poster #: 190.13

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant R01MH115832

Title: Cholinergic modulation of firing rate adaptation in hippocampal CA1 pyramidal neurons via TRPM4 channel activation: implications for place cell firing

Authors: C. L. COMBE[1], C. M. UPCHURCH[2], C. C. CANAVIER[2], *S. GASPARINI[1]; [1]Neurosci. Ctr., [2]Cell Biol. and Anat., LSU Hlth. Sci. Ctr., New Orleans, LA

Abstract: Many CA1 pyramidal cells function as place cells, firing at higher rates within a preferred place field. Upon repeated traversals of the place field in the same direction, the center of the place field shifts in the opposite direction, as novel environments become more familiar. Higher levels of the neuromodulator acetylcholine (ACh) are associated with novelty, and thus may decrease with experience. Such a decrease in ACh may partially account for the backward shift in the center of mass of the place field, by modulating intrinsic neuronal properties. Using *in vitro* electrophysiology in slices from male rats and *in silico* simulations in a multicompartmental model of a CA1 pyramidal neuron, we investigated cholinergic modulation of the firing rate adaptation that occurs in response to symmetric ramps of depolarizing current

input. This symmetric input approximates the spatially-tuned, temporally-diffuse depolarizing synaptic input received by these neurons while traversing a place field. In control, fewer spikes are elicited on the down-ramp than on the up-ramp; moreover, at equal levels of injected current, the frequencies are lower on the down ramp. The cholinergic agonist carbachol (CCh) reverses this spike rate adaptation and causes more spikes to be elicited on the down-ramp than the up-ramp. This reversal is equivalent to a shift in the place field center in the same direction as the place field is traversed. The non-specific TRP antagonist flufenamic acid reverses the effect of CCh, suggesting that the CCh-induced shift is due to activation of the Ca^{2+}-activated nonspecific cation current, I_{CAN}, carried by TRP channels. The TRPC-specific antagonist SKF 96365 does not significantly affect the CCh-associated shift, but CBA, a blocker specific for TRPM4 channels, does. The model suggests that the IP_3 receptor is the locus that requires both CCh and sustained depolarization to produce the rightward shift in firing rate along the ramp. In the model, a step change in IP_3, representing the activation of muscarinic receptors by CCh, coupled with an increase in bulk $[Ca^{2+}]_i$ due to voltage-gated Ca^{2+} influx during the ramp, activates Ca^{2+}-induced Ca^{2+} release. This Ca^{2+}-induced Ca^{2+} release in turn activates TRPM4 channels, via a nanodomain required by the micromolar half-activation for TRPM4. In summary, our synergistic experimental/computational approach led to an improved mechanistic understanding of the intrinsic mechanisms involved in the cholinergic modulation of firing rate in response to symmetric ramp current injections, which has implications for the dependence of place cell firing on position within the place field.

Disclosures: C.L. Combe: None. **C.M. Upchurch:** None. **C.C. Canavier:** None. **S. Gasparini:** None.

Poster

190. Somatic and Dendritic Integration

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 190.14

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: R01NS104911

Title: Synaptic integration by space-specific neurons in the owl auditory system

Authors: *P. HARRAST[1], N. GORGAS[1], N. HAMED[2], C. RESNGIT[1], E. A. BUSHONG[3], K. KIM[4], M. MADANY[4], D. FIORAVANTE[1], M. H. ELLISMAN[5], W. M. DEBELLO[1];
[1]UC Davis, Davis, CA; [2]Harvard Univ., Cambridge, MA; [3]Ctr. for Res. in Biol. Systems, [4]Univ. California - San Diego, La Jolla, CA; [5]Dept Neurosci, Univ. California - San Diego Sch. of Med., La Jolla, CA

Abstract: Space-specific neurons (SSNs) in the barn owl inferior colliculus are computational hubs for synaptic integration, plasticity and learning. We discovered an SSN subtype with unusually large dendritic spines, each of which integrate multiple axonal inputs. These ‘toric

spines,' named for their topological holes, also exhibit low connection fractions suggesting a role in experience-dependent rewiring. The current work combines patch-clamp recordings and volume electron microscopy to determine the rules of subthreshold integration in SSNs and their microanatomical substrates. (1) Patch-clamp recordings are being used to characterize glutamatergic EPSPs and EPSCs elicited by electrical stimulation of the input nucleus, the lateral shell of the inferior colliculus central nucleus (ICCls). Data indicate that the *in vivo* topography of synaptic inputs is preserved in ex vivo slices; EPSCs elicited by synchronous stimulation at two distinct input locations summate, which provides a tool to probe rules of integration across interaural time differences or sound frequencies, and; the ICCls-SSN synapse exhibits paired-pulse facilitation and depression. These observations establish a baseline for investigating learning-driven changes in SSN computation. (2) Serial block-face scanning electron microscopy (SBEM) is being used to reconstruct SSN network architecture. Horizontal and feed-forward connections were labeled in vivo for retrospective identification in SBEM. Reconstruction of a 2.4 x 106 mm^3 image volume is proceeding using an AI-assisted pipeline. To investigate learning-driven changes in connectivity, owls were adapted to prismatic spectacles (prisms) which cause reorganization of the ICCls-SSN connection. SBEM volumes corresponding to the normal and learned circuits of a prism-adapted owl are in process. One goal of this reconstruction is to test predictions of the dendritic input clustering hypothesis. In total, this project uses an integrative approach to study principles of synaptic integration in a circuit that mediates behavioral learning.

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Poster

190. Somatic and Dendritic Integration

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Program #/Poster #: 190.15

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Australian Research Council Grant DP160103047
Sylvia and Charles Viertel Charitable Foundation

Title: Learning dependent modulation of dendritic activity during auditory discrimination

Authors: *L. GODENZINI, L. M. PALMER;
Florey Inst. of Neurosci. and Mental Hlth., Melbourne, Australia

Abstract: Neural activity in the auditory cortex is strongly modulated by both learning and the behavioural state. Such modulation is believed to occur via top down projections, which target the apical tuft dendrites of cortical pyramidal neurons. Due to their non linear properties, tuft dendrites can actively integrate different streams of information and are a great candidate for

driving the dynamic changes required during learning. Here, we used two photon calcium imaging to investigate dendritic activity of layer 2/3 pyramidal neurons within the auditory cortex during learning of an auditory discrimination task. Comparing passive listening (naïve) and behaving (novice and expert) mice, we found task-dependent activity in tuft dendrites following learning. Specifically, dendritic activity was overall similar in naïve and novice mice, whereas auditory-evoked responses were selectively increased during correct (HIT) performance in expert mice. Additionally, task engagement increased the proportion of dendrites with dampened activity during the auditory stimuli, suggesting that the balance of excitation and inhibition is important for learning. Overall, our findings illustrate that apical tuft dendrites of cortical pyramidal neurons can flexibly encode task-relevant information, suggesting that dendrites can be primary drivers in modulating the cortical activity that is required during learning.

Disclosures: L. Godenzini: None. **L.M. Palmer:** None.

Poster

190. Somatic and Dendritic Integration

Location: SDCC Halls B-H

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Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: MEXT/JSPS Kakenhi Grant Numbers 17H06310
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Title: Learning to discriminate stimulus sequences by dendritic computation of a single cerebellar Purkinje cell model

Authors: *K. TAMURA[1], Y. YAMAMOTO[2], T. KOBAYASHI[3], T. YAMAZAKI[1];
[1]The Univ. of Electro-Communications, The Univ. of Electro-communications, Chofu, Japan;
[2]Dept. of Psychiatry and Behavioral Sci., Tokyo Med. and Dent. Univ., Bunkyo-ku, Japan;
[3]Yamaguchi Univ., Yamaguchi, Japan

Abstract: Our daily lives such as movements and thoughts are realized by advanced motor and cognitive functions of the brain. The brain is a large and complex network of neurons, and the dynamics of this network seems essential for such functions. In other words, such functions are assumed to emerge from interactions of a number of neurons by exchanging spikes over the network. This hypothesis also implies that individual neurons are rather simple elements that only accumulate incoming spikes and emit another spikes. On the other hand, several studies have shown that even single neurons can perform complex computations by taking advantage of the characteristic spatial shape of neurons and the nonlinearity of ion channels present on their surfaces. For example, cerebellar Purkinje cells have rich spatially organized dendrites and various types of ion channels on them, which makes the cells capable of complex computations. The cerebellum is known to play essential roles in motor control and learning, which allows a

number of muscles in our body to contract with appropriate timings to achieve fast and smooth body movements. By these observations, we assumed that cerebellar Purkinje cells could process sequential information on stimuli through parallel fiber inputs. In this study, by conducting computer simulation of a multi-compartment model of cerebellar Purkinje cells, we investigated whether a single cerebellar Purkinje cell could discriminate sequences of parallel fiber input stimuli. Specifically, we chose about ten synapses randomly aligned sequentially on a dendrite, and stimulated them in two directions, from proximal to distal and from distal to proximal, with a relatively long time interval (~ 100 ms). We observed that the cell fired when stimulated in one order, but not when stimulated in the reverse order. We also observed that by setting weights of those parallel fiber synapses appropriately, the order of the stimuli for the cell to fire could be reversed. These results suggest that if appropriate weights were acquired through learning, the cell could learn sequential information on parallel fiber stimuli. To address this learning issue, we implemented a spike timing-dependent Hebbian plasticity rule on the synapses, and fed a short pulse that activated the entire dendrites strongly immediately after the sequential stimulation. We confirmed that by this learning mechanism, sequential information was acquired correctly. These results suggest that Purkinje cells can discriminate and learn input sequences from parallel fibers.

Disclosures: **K. Tamura:** None. **Y. Yamamoto:** None. **T. Kobayashi:** None. **T. Yamazaki:** None.

Poster

191. Networks and Signal Propagation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 191.01

Topic: B.07. Network Interactions

Support: KAKENHI 16H06532
KAKENHI 19H01142
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KAKENHI 21H03532
KAKENHI 20H04341

Title: Heterogeneous ipsilateral cortical excitation spread and contralateral activation of the mouse prefrontal cortex imaged with voltage-sensitive dye

Authors: *T. TOMINAGA[1,2], P. GUSAIN[1,3], M. TAKETOSHI[1], Y. TOMINAGA[1];
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Abstract: The prefrontal cortex (PFC) is essential in integrating higher brain activities, and disruption can cause schizophrenic and other neuropsychiatric phenotypes. However, the neuronal circuit activities of PFC are not well known. We have developed a stable and reliable voltage-sensitive dye (VSD) imaging system for large-scale network activity. Here we report the functional dissection of mouse PFC with the VSD imaging method with high speed (1 ms/frame), high resolution (250×256 pixels), and a large field of view (ca. 10 mm in diameter). From a slice, about 1 mm from the bregma, electrical stimulation to layer II/III of cg1 of the anterior cingulate cortex (ACC) resulted in the neural propagation to the most medial side of the ACC (subfield 33). It subsequently induced interhemispheric transmission to the other side of subfield 33. To evaluate the functional pathway of the propagation and elucidate functional connectivity among the areas in the PFC, we made five acute serial slices 350 μm thick from the bregma. We assigned slices 1 to 5 based on the mouse brain atlas from 1.70 mm to 0.26 mm from the bregma. We collected the neural response to electrical stimulation at nine sites on them. We developed a method to produce the average neural activities of slices to nine stimulation sites and made a functional map of the propagation patterns from 6 to 8 slices.The results indicate that the intracortical propagation occurred in slices 3, 4, and 5, which are the slices with the ACC and the corpus callosum (CC). The activity seemed to spread at area 33 of the ACC. The direct stimulation to the white matter induced the activation of area 33 in both hemispheres, suggesting that the CC has reciprocal termination at area 33 of the ACC. The observation was consistent with the Di-I staining of the CC. It was also evident that the propagation within the ACC was heterogeneous. The spread from cg1 to cg2 was weaker than from cg2 to cg1. Thus, intrahemispheric propagation preferentially occurs when directly stimulating cg2. The heterogeneous activity spread should shape the information at the ACC.

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Poster

191. Networks and Signal Propagation

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Program #/Poster #: 191.02

Topic: B.07. Network Interactions

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ISF #1871/17
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CRCNS-NSF-BSF #2015577
Zimin-Foundation

Title: Deciphering the optical timescale of cortical networks required for information transmission

Authors: *A. LEVI, L. SPIVAK, H. E. SLOIN, S. SOMECK, E. STARK;
Sagol Sch. of Neurosci. and Dept. of Physiol. and Pharmacol., Tel Aviv Univ., Tel Aviv, Israel

Abstract: During sensory processing, cognitive thought, and action generation, spiking signals are propagated accurately and rapidly across multiple brain regions. However, spike transmission is also accompanied by increased variability. Understanding the mechanisms underlying the precise and reliable propagation of spiking signals between neurons is an open question in neuroscience. Here, we focus on information transmission at the interface between pyramidal cells (PYR) and inhibitory interneurons (INT) in neocortex and hippocampal region CA1 of freely-moving mice. Initially, we used an optogenetic Gaussian white-noise (WN) signal with a timescale of 3 ms as input to PYR. We found that spike generation of directly-activated (DA) PYR exhibited precision of 2-4 ms. Instead of being distorted by transmission, precision of postsynaptic INT was higher than the precision of DA PYR. INT precision was higher when a larger presynaptic PYR pool was recruited. Data driven modeling showed that coincidence detection of the convergent inputs enables spiking patterns to be precisely propagated between cortical PYR and INT. Next, we asked whether the improved precision of the postsynaptic INT depends on the input timescale, and what input timescale yields the highest precision. We therefore applied multi-scaled optogenetic WN signals to PYR in neocortex and CA1 of freely-moving mice. The same seed WN signal was scaled using alpha functions with different time constants, ranging from 1 to 20 ms. We found that compared to DA PYR, postsynaptic INT showed improved precision at every tested WN timescale. Furthermore, precision improved at shorter timescales. However, DA PYR reached a precision asymptote at short timescales, suggesting a lower bound for directly-activated units. In contrast, postsynaptic INT precision continued to improve at short timescales. Thus, although PYR precision is limited, the convergence of information across the PYR-to-INT interface allows high precision transmission of input signals, regardless of the signal timescale.

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Poster

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Topic: B.07. Network Interactions

Support: NSF 2024364

Title: Spontaneous neural fluctuations are coordinated topographically across cortical and subcortical areas

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Abstract: Traveling waves are often observed in large scale population activity in different brain areas and in various brain states. However, the detailed spatiotemporal patterns of wave propagation have not been well characterized, especially in awake mammals. Critically, it is still largely unknown if spatiotemporal patterns are shared and coordinated across different brain structures. Here we show that propagating activity patterns are shared across multiple cortical maps and thalamus.
Using transcranial widefield imaging of the dorsal cortex in awake GCaMP7-expressing transgenic mice, we robustly observed spiral waves in the 2-8Hz frequency range. Spiral centers were detected using custom detection algorithms and optical flow methods applied to phase data after Hilbert transformation. Spirals tended not to occur during overt movements of the mouse, and were more prevalent during periods of quiet wakefulness, which are also characterized by high 2-8Hz power. Spirals were also present in epochs during which mice actively consumed rewards, though primarily in between bouts of licking. The presence of spirals in wild-type mice was separately validated with 4-shank Neuropixels 2.0 probe recordings in the cortex.
These spirals were nearly always mirrored on the left and right hemispheres with opposing rotation directions, reflecting the mirrored topographic anatomical connectivity between hemispheres. Similarly, we frequently observed simultaneous mirrored spirals split along the S1/M1 or M1/M2 borders within the same hemisphere. The spatially coordinated distribution of spiral patterns in the cortex suggests that spontaneous neural activity is shared across interconnected topographic maps. Indeed, with kernel regression analysis, we are able to accurately predict spatiotemporal neural activity and recapitulate spiral patterns in the posterior cortex from simultaneously recorded activity in the frontal cortex.
To determine whether spatiotemporal dynamics are globally coordinated beyond cortex, we made simultaneous widefield imaging in the cortex and extracellular recordings in subcortical areas including the thalamus with 4-shank Neuropixels 2.0 probe. As above, spiral patterns in cortical activity were recapitulated when predicting that activity from simultaneously recorded thalamic neurons. Overall, these results demonstrate that spontaneous fluctuations in neural activity in the mouse are coordinated more broadly than previously known, across multiple topographic maps cortically and subcortically.

Disclosures: Z. Ye: None. **N.A. Steinmetz:** None.

Poster

191. Networks and Signal Propagation

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Topic: B.07. Network Interactions

Support: NIH Grant R01NS121084
NIH Grant R01NS124592

Title: Subthreshold oscillating waves in neural tissue propagate by volume conduction and generate interference

Authors: *C.-C. CHIANG, D. M. DURAND;
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Abstract: Subthreshold neural oscillations at different frequency bands have been observed in several brain regions and can influence the timing of neural spikes. However, the spatial extent and function of these spontaneous oscillations remain unclear. To study the mechanisms underlying these waves, we generated oscillating subthreshold activity at different frequencies in the longitudinal hippocampal slice expressing optopatch proteins by optical stimulation with blue laser pulse trains. We found that optogenetic stimulation can induce low-amplitude oscillating waves in addition to high-amplitude neural spikes. Neural oscillating waves can propagate bidirectionally across the hippocampal slice and go through a complete transection of the tissue. The propagation speed of these neural waves is independent of the oscillating frequency. The endogenous electric fields generated by these waves are 0.5 to 0.7 mV/mm perpendicular to the direction of propagation and about 0.3 mV/mm parallel to the propagating direction. We also observed that these oscillating waves could interfere with each other in the middle of the slice when two waves were initiated simultaneously at both ends of the slice. Interference was maximum when two waves were in phase (constructive interference). When two waves were slightly out of phase with a 90° or 270° phase shift, interference decreased and reached a minimum value when the phase shift was 180° (destructive interference). Finally, our pharmacological experiments show that the optically-induced oscillating waves are not affected by the NMDA blocker (APV) and still propagate in the presence of tetrodotoxin (TTX) but at a significantly lower amplitude. These results suggest that the neural tissue is wired to generate waves producing interference patterns without or in combination with synaptic transmission. Although the function of the subthreshold waves in the neural system and their interaction with suprathreshold activity is unknown, the results described above open the possibility that signal processing of very low amplitude signals could produce interfering patterns below the threshold of firing, leading to another level of neural computation not previously described.

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Poster

191. Networks and Signal Propagation

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Topic: B.07. Network Interactions

Support: Arnold O. Beckman Postdoctoral Fellowship Award
Larry L. Hillblom Foundation

Title: Internally generated sequential firing patterns in human brain organoids

Authors: *T. SHARF[1,2], T. VAN DER MOLEN[1], L. R. PETZOLD[1], P. K. HANSMA[1], K. S. KOSIK[1];
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Abstract: Human brain organoids represent a self-organized neuronal system that replicates key facets of cellular diversity, developmental anatomy and functional connectivity found in *in vivo* brain networks (Sharf *et al*. 2022). An ongoing conjecture in neuroscience is that cognition depends on self-generated sequential activation of neuronal assemblies within the brain (Pastalkova *et al*. 2008). Brain organoids provide an experimental framework to investigate intrinsic temporal dynamics within hierarchically organized neuronal assemblies, composed of both excitatory and inhibitory networked neurons, that emerge devoid of an external input. Utilizing high-density CMOS microelectrode arrays containing 26,400 recording sites, we measured extracellular action potentials generated by spontaneous spiking activity across the surface of a human brain organoid. From tracking the transient dynamics of single-unit firing rates we identified ensembles of neurons that initiate, align and disperse within a time course of a few hundred milliseconds. Utilizing these readouts of neuronal activity, we found neuronal ensembles with reliable, sequentially activated spike patterns that form temporally aligned sequences. A subset of these neurons exhibit a sharp increase in temporal alignment with respect to peak activity within neuronal avalanches and form attractors with stable phase-space trajectories. Surprisingly, temporal dynamics generated by our brain organoids resemble temporal signatures of firing patterns observed in neuronal assemblies hypothesized to function as computational units observed *in vivo*, and are retained and activated during sleep when largely disengaged from external inputs (Peyrache *et al*. 2015). These results suggest that perturbation of intrinsically self-organized neuronal patterns with electrical and/or optogenetic stimulation may open a new paradigm for encoding and reading information delivered from an external input to a human brain organoid.

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Poster

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Program #/Poster #: 191.06

Topic: B.07. Network Interactions

Support: NIMH Grant F31MH126465-01

Title: The mouse claustrum synaptically connects cortical network motifs

Authors: *H. QADIR[1], B. W. STEWART[3], J. W. VANRYZIN[1], Q. WU[4], S. CHEN[2], D. A. SEMINOWICZ[3], B. N. MATHUR[1];
[1]Pharmacol., [2]Biostatistics and Bioinformatics, Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Univ. of Maryland Sch. of Dent., Baltimore, MD; [4]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Spatially distant areas of cerebral cortex coordinate their activity into networks that are integral to cognitive processing. A common structural motif of cortical networks is co-activated frontal and posterior cortical regions. The neural circuit mechanisms underlying such widespread inter-areal cortical coordination are unclear. Using a discovery based functional magnetic resonance imaging (fMRI) approach in mouse we observed frontal and posterior cortical regions that demonstrate significant functional connectivity with the subcortical nucleus the claustrum. Examining whether the claustrum synaptically supports such fronto-posterior cortical network architecture, we observed cortico-claustro-cortical circuits reflecting the fMRI data: significant trans-claustral synaptic connectivity from frontal cortices to posteriorly lying sensory and sensory association cortices contralaterally. These data reveal discrete cortical pathways through the claustrum that are positioned to support cortical network motifs central to cognitive control functions and add to the cannon of major extended cortico-subcortico-cortical systems in the mammalian brain.

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Poster

191. Networks and Signal Propagation

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Program #/Poster #: 191.07

Topic: B.07. Network Interactions

Support: Tiny Blue Dot Foundation

Title: Neural microcircuits underlying EEG responses evoked by electrical stimulation in mice

Authors: *S. RUSSO[1,2], I. REMBADO[2], L. CLAAR[2], L. MARKS[2], C. KOCH[2];
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Abstract: Electroencephalography (EEG) is one of the most widely used methods for the investigation of the electric activity of the brain. Embedded within the EEG are the neural responses associated with specific perturbations, so-called event-related potentials (ERPs). Previous studies directly stimulating the brain showed that some ERPs' features, such as spectral content and waveform, depend on the cortical area that is perturbed (Rosanova M., 2009; Parmigiani, S., 2022), whereas other information-related metrics depend on the brain state of the subject, such as the presence of consciousness (Massimini M., 2005; Casali A.G., 2013). Despite

its widespread use, we know little about the microcircuits that give rise to the ERPs. To uncover the underpinnings of these ERPs features, we developed an electrophysiological setup in head-fixed mice that combines the simultaneous recording of the LFP and 102-103 individual units via Neuropixels probes (Jun J.J., 2017) with 30 channel EEG electrodes, while applying electrical stimulation in the secondary motor, primary somatosensory, and primary visual cortical areas. We compare the ERPs elicited by direct stimulation of each area in terms of whole-brain peak latency and waveform. Leveraging our multi-scale setup, we investigate the local bases of these macroscopic differences in terms of current source density and single unit firing activity, disentangling the contribution of the stimulated area from that of the regions indirectly engaged by the stimulation. Furthermore, to assess whether each area-specific feature depends on the intrinsic structure of the network or on the functional properties of the network, we evaluate how these metrics are affected during anesthesia induced via isoflurane. Preliminary results show that the latency of the ERPs peak after the stimulation onset and the waveform depend on the perturbed area. Intriguingly, the area-specific differences in the latency of the first component are preserved under anesthesia, while the differences between the ERPs' waveform are partially abolished. We infer that the response features preserved during anesthesia may reflect intrinsic properties of the stimulated area. Conversely, the response features affected by anesthesia are likely to reflect operational properties of the stimulated area, that fade with loss of consciousness during anesthesia. We will relate these macroscale differences captured by EEG to the underlying microscale dynamics. This study will lead to a better understanding of ERPs by interrogating the contributions of specific brain regions and ultimately inform their application in a clinical setting.

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Poster

191. Networks and Signal Propagation

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Program #/Poster #: 191.08

Topic: B.07. Network Interactions

Title: Neural architecture of the midbrain periaqueductal gray

Authors: *H. H. SUBRAMANIAN[1], G. HOLSTEGE[2];
[1]Boston Scientific, Valencia, CA; [2]Emeritus Professor, Groningen Academic Hosp., Haren, Netherlands

Abstract: The midbrain PAG is a critical relay center of the emotional motor system and controls neural circuits that regulate breathing, blood pressure, bladder function, vocalization, coughing, sneezing, vomiting and maintenance of abdominal and intrathoracic pressure. Dysfunction of PAG circuits lead to dysreflexia such as dyspnea, ataxic breathing, hyper & hypotension and micturition disruption (urinary incontinence). Circuit specific neuromodulation

of the PAG could reverse specific types or combined dysfunctions paving way for development of neurologic clinical therapy. However, PAG circuit dynamics are unkown. For this reason, we stereotaxically mapped the PAG in the rat in vivo to investigate its neural architecture, circuit physiology and anatomical topography. The PAG was found to be predominantly quiescent in the resting state and could be activated by iontophoresis of excitatory amino acid glutamate agonists. Cells activated ceased function when glutamate ejection was terminated or by co-iontophoresis of muscimol (GABA agonist). Very few spontaneously active cells were found in the PAG, mainly in its dorsal region and these cells, typically fired in a slow and irregular pattern. Activation of either behavioral and/or emotional motor interventions caused immediate activation of PAG neurons mainly in the lateral and ventrolateral PAG. showing two distinct types of activity patterns; 1) single spike firing and 2) burst firing, The cells fired both tonically and phasically when correlated with specific emotional motor output such as the diaphragm EMG. Predominantly the non-bursting PAG neurons had a near normal distribution around 200 to 250 msec, while burst-firing cells typically showing a bimodal distribution. The functional implications of PAG neuronal activity are discussed in terms of descending motor and emotional motor control and effective translation for application of neuromodulation clinical therapy for specific emotional motor diseases.**Acknowledgement and Declaration**This work was wholly undertaken in the laboratories of HHS-RJB at The University of Sydney and The University of Queensland with respective institutional ethics approvals. HHS, RJB and GH conceived and designed the projects, performed the experiments, analyzed primary data and made figure illustrations. GH curated and approved the final data/figure representation in this presentation/poster. None of the work described here were undertaken at either of the current work designations of Hari Subramanian or Gert Holstege.

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Poster

191. Networks and Signal Propagation

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Topic: B.07. Network Interactions

Support: NIH Brain Initiative MH117815
NSF CPBF PHY-1734030

Title: Characterizing neuropils in the whole-brain Drosophila connectome

Authors: *A. LIN[1], R. YANG[1], S. DORKENWALD[1], A. MATSLIAH[1], F. CONSORTIUM[2], S. SEUNG[1], M. MURTHY[1];
[1]Princeton Univ., Princeton Univ., Princeton, NJ; [2]Flywire.ai, Princeton, NJ

Abstract: Most brains are compartmentalized organs, with many brain regions having either known or suspected biological functions. The FlyWire project has now completed the

proofreading of a connectome for a *Drosophila* female brain (FAFB) which contains both complete hemispheres of the central brain and includes neurons that receive inputs in the optic lobes. This reconstruction gives us the opportunity to better understand the structure of the *Drosophila* brain at this mesoscale. We characterized 75 anatomically defined brain regions, or neuropils, spanning most of the central brain of the fruit fly, and found that different neuropils are statistically distinct. By identifying the strongest input and output brain regions for each neuron, we constructed a neuropil projectome, a network describing the number of neurons connecting each neuropil pair, and identified neuropils critical to information flow across the brain. These results demonstrate that, despite being strongly interconnected, different brain regions have distinct network properties.

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Poster

191. Networks and Signal Propagation

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Topic: B.07. Network Interactions

Support: NIH/NIGMS

Title: Murine brain networks induced by selective stimulation of TRPA1 vagus nociceptors

Authors: *D. C. LEE[1,2], O. HASHIMOTO[1], S. S. CHAVAN[1,3,4], K. J. TRACEY[5,3,4];
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Abstract: Afferent and efferent vagus nerve fibers in the inflammatory reflex are a physiological interface between the brain and the inflammatory response. We have shown previously that activation of Transient Receptor Potential Ankyrin-1(TRPA1)- expressing vagus nociceptors activates the afferent arc of the inflammatory reflex. Here, we reasoned that TRPA1-signaling in the vagus nerve will activate specific neuronal ensembles in the brain. To label neuronal populations activated in response to TRPA1 signaling, we generated Targeted Recombination-in Active Populations (TRAP2)-tdTomato mice by crossing TRAP2 mice with a Cre-dependent tdTomato reporter line. When exposed to 4-hydroxytamoxifen, active neurons persistently express fluorescent protein tdTomato. Vagus TRPA1 fibers were activated by opto-pharmacological stimulation with optovin + 405 nm light, and the brain was assessed for tdTomato expression. tdTomato+ cells were normalized to cells stained for NeuN+ within the same brain region, and the optovin-stimulated groups (n=9) were compared to sham-stimulated control groups (n=8) by utilizing the QUINT pipeline linked to the 2017 3D Allen Brain Atlas.

The QUINT pipeline is comprised of QuickNii (for 3D brain atlasing), Nutil (compilation and analysis tool), and Ilastik (training-based segmentation tool). The groups were analyzed using three custom brain region filters: higher hierarchical brain regions (n=44), TRPA1-associated brain regions (n=13), and brain regions associated with vagus TRPA1 stimulation (n=8). Within brain regions associated with vagus TRPA1 stimulation, two-way ANOVA revealed significant differences between treatment groups ($p=0.0019$) and activated brain regions ($p<0.0001$). We observed increased brain activity in the following regions: the paraventricular nucleus of the hypothalamus (23%), locus coeruleus (16%), and parabrachial nucleus (9%). These results provide a novel and low-tech methodology of combining TRAP2 and QUINT to screen for activation of brain neurons in response to vagus TRPA1 signals. The expression of tdTomato - expressing neurons was validated by manual cell count of the paraventricular nucleus of the hypothalamus using confocal microscopy (<20% percent error). Together, these findings indicate that specific vagus TRPA1 nociceptors induce specific brain neuron activity that can be identified using this QUINT strategy.

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Poster

191. Networks and Signal Propagation

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Program #/Poster #: 191.11

Topic: B.07. Network Interactions

Support: OSCAR-URSP grant

Title: Shrink to clean: Exploring the glymphatic system and volume change in brain

Authors: *M. AHMADI, J. R. CRESSMAN;
George Mason Univ., Fairfax, VA

Abstract: The glymphatic system is the brain's waste clearance system which is most active during sleep. Astroglia, through their aquaporin-4 water channels, play an important role in the glymphatic system by facilitating the exchange of interstitial fluid (ISF) and cerebrospinal fluid (CSF). Previous studies show that astroglia swell in response to high extracellular K^+ by uptaking it through Sodium Potassium Chloride Cotransporter 1 (NKCC1). Collectively, these studies lend support to glial swelling during high activity (high extracellular potassium). Previous studies also show that the extracellular space expands when transitioning from wakefulness to sleep. The goal of our study was to assess the possibility of activity dependent glia shrinkage during sleep to enable the extracellular expansion, thus resulting in less resistance for the CSF flow. We assessed the volume changes in neuronal, glial, and extracellular space during wakefulness, light sleep (stage N1), and deep sleep (stage N3). We utilized an existing model of neural dynamics, with the additional implementation of NKCC1 for glial dynamics. We drove our neuronal model to generate action potentials at frequencies equivalent to those seen in

natural wake/sleep cycles and looked for the corresponding volume changes, and concentrations of extracellular K^+. Our findings indicate that neurons and glia decrease in volume when transitioning from light to deep sleep. The results also suggest that the concentration of the extracellular K^+ is higher during wakefulness compared to deep sleep and the volume of the extracellular space increases during sleep and decreases during wakefulness. Collectively, our findings suggest that both neurons and glia shrink in response to reduced activity and may compensate for the expansion of extracellular space during sleep.

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Poster

192. Network Interactions

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Program #/Poster #: 192.01

Topic: B.07. Network Interactions

Title: Spontaneous activity in organotypic cultures of human spinal cord slices: potential for use in the drug discovery process

Authors: *C. MATHES, **T. COTTA**, Y. MIRON, N. ABI-GERGES, A. GHETTI, K. CARLIN; AnaBios Corp., San Diego, CA

Abstract: Neurons and neuronal networks in cultured neuronal tissues are often seen to generate spontaneous activity (SA). This activity can consist of both uncoordinated random firing or can be coordinated into bursts or oscillatory-like activity. The underlying nature of this activity in organotypic cultures of human spinal cord (hSC) slices has not been explored. Therefore, using calcium imaging and pharmacological agents, we sought to gain an understanding of the underlying mechanisms of this SA occurring in the superficial dorsal horn of these cultures. We then sought to determine if this preparation could provide insight into CNS-induced side effects of various centrally active drugs by comparing the inhibition of spontaneous activity in slices to free (unbound) therapeutic plasma concentrations (FTPC). hSCs obtained from consenting organ donors were prepared as 400 μm transverse slices and placed in culture. Slices were loaded with Fluo-8 AM and imaged at 2 Hz in a flowing bath chamber. A typical experiment consisted of 10 min of vehicle baseline, followed by 5 min of drug perfusion, followed by 10 min of data acquisition. This is repeated for each drug / concentration. SA in these slices was persistent and the frequency stable for up to 20 days in culture. Bath application of TTX (1 μM) inhibited the vast majority of the SA indicating a voltage-gated sodium channel component to the calcium signal. Co-application of AMPAR antagonist CNQX (10 μM) and the NMDAR antagonist APV (100 μM) also abolished the SA. Strychnine (10 μM) and picrotoxin (100 μM) increased SA. These latter results suggest the SA was driven by glutamatergic inputs and was inhibited by both GABA and glycine releasing synaptic inputs. Next we used a group of sodium channel modulators (carbamazepine, lacosamide, and mexiletine) that are known to have CNS-related side effects (including dizziness, nausea, mood swings and depression) at their FTPC. We also

included vixotrigine - a newer sodium channel modulator with less CNS side effects. SA was reduced by all compounds in a concentration-dependent manner. Carbamazepine, lacosamide and mexiletine had values fall within their respective FTPC ranges. Conversely, the SA IC_{50} for vixotrigine was right-shifted relative to its FTPC range, in agreement with prior studies indicating that it is a well-tolerated drug. In conclusion, these initial data indicate that SA can be reliably measured in adult human CNS tissue up to 3 weeks *in vitro* and that organotypic hSC slice cultures can provide a valuable translational tool for the evaluation of the safety and possible efficacy of novel CNS drug candidates.

Disclosures: **C. Mathes:** A. Employment/Salary (full or part-time):; AnaBios Corporation. **T. Cotta:** A. Employment/Salary (full or part-time):; AnaBios Corporation. **Y. Miron:** A. Employment/Salary (full or part-time):; AnaBios Corporation. **N. Abi-Gerges:** A. Employment/Salary (full or part-time):; AnaBios Corporation. **A. Ghetti:** A. Employment/Salary (full or part-time):; AnaBios Corporation. **K. Carlin:** A. Employment/Salary (full or part-time):; AnaBios Corporation.

Poster

192. Network Interactions

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 192.02

Topic: B.07. Network Interactions

Title: Synapse specific post synaptic density-95 association with actin is perturbed in APP/PS1 mice

Authors: *H. P A[1,2], R. P. KOMMADDI[1], V. RAVINDRANATH[1];
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Abstract: Alzheimer's disease (AD) is a progressive form of dementia associated with loss of dendritic spines and F-actin from synapses, leading to memory deficit and cognitive impairment. Actin has significant functions in establishment and maintenance of synapses including post synaptic density organization, vesicle trafficking, anchoring of post synaptic receptors, and translational machinery. Proteomic analysis of actin interacting proteins in the synaptosomes isolated from 6 month old wildtype and APP/PS1 mice showed that the protein post synaptic density-95 (PSD- 95) interact with actin. PSD-95 is a major scaffolding protein enriched at the glutamatergic synapse where it acts as a potent regulator of synaptic strength and plasticity. However the dynamics of PSD-95-actin interaction at the synapse remains unclear. Therefore, we tried to investigate the critical role of PSD-95 association at the synapse with actin, AMPA and NMDA receptors using APP/PS1 mice. We have isolated synaptosomes from WT and APP/PS1 mice and performed immunoprecipitation studies to determine the association of PSD-95 with actin. PSD-95 association is detected with actin in synaptosomes isolated from adolescent and middle aged WT and APP/PS1 mouse brain cortex. We found that PSD95-actin association is significantly decreased in middle aged APP/PS1 mice compared with WT while it

remains unaffected in the F-actin fraction of synaptosomes in APP/PS1 mice. In order to determine whether F-actin plays a role at the synapse, we injected actin stabilizing agent in mice and found that the intrathecal injection of jasplakinolide rescued the fear memory, restored the reduced association and synaptic F-actin levels in APP/PS1 mice. The association of PSD-95 with both AMPAR and NMDAR subunits were also detected and the association with only GluA1 subunit is affected in middle aged APP/PS1 mice. Interestingly, interaction of NMDAR subunits (GluN1 and GluN2B) with PSD-95 is greatly enhanced at adolescent APP/PS1 mice even though these subunit protein levels are significantly diminished. Thus, our results suggest an essential role of PSD-95 as a crosstalk between glutamatergic receptors and F-actin. We conclude that altered PSD-95 - actin interaction may have critical implications in glutamatergic neurotransmission, synaptic strength and plasticity in AD.

Disclosures: H. P a: None. **R.P. Kommaddi:** None. **V. Ravindranath:** None.

Poster

192. Network Interactions

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 192.03

Topic: B.07. Network Interactions

Title: Facial motion energy is more temporally accurate than pupil size in reflecting the cortical state in mice primary visual cortex

Authors: *E. SABRI[1], T. TOOSI[3], R. BATISTA-BRITO[2];
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[3]Neuroscince, Columbia Univ., New York, NY

Abstract: Neural information processing is highly affected by behavioral states. At the brain level, behavioral states are associated with distinct cortical activity patterns (or cortical states). Over the past decade, it has been established that changes in pupil size can accurately track neuronal spiking throughout the brain. In rodents, the animal's facial motion has also been shown to accurately follow the brain's spontaneous activity. Here, we set out to determine which one of these indicators (facial motion energy or pupil size) more timely reflects the arousal state. We recorded the spiking activity of neurons in mice V1 with multi-site linear probes alongside the pupil size and facial motion (n=14). Both at the single-cell level and with population decoding, we observed that the pupil size is 1 second delayed relative to neural spiking in mice V1, whereas facial motion energy is more synchronously predicted by neural activity. We used linear regression and recurrent neural networks for decoding facial motion energy from population spiking. The performance of these two models was comparable in decoding facial motion energy, suggesting a linear relation between facial motion energy and neural activity at the population level. We then used Partial Least Square (PLS) regression to identify the number of dimensions in spiking activity that contributes to the decoding of facial motion energy. We observed that with two dimensions, PLS has the best performance in predicting facial motion

energy, which suggests that the neural correlate of facial motion energy is two-dimensional. Intriguingly, these two dimensions characterize the predictability of facial motion energy by each neuron's activity, as we found out that the contribution of each neuron to each of these two dimensions reflects, respectively, the magnitude and delay of correlation between spiking of that neuron and the facial motion energy. Finally, in terms of spiking activity at single neuron level, some cells are following the facial motion energy while others are temporally ahead of it; the peak of correlation between spiking activity of single neurons and facial motion energy can happen with a delay up to ±500ms. In summary, our study shows that compared to pupil size, facial motion energy is a temporally-more-accurate predictor of spontaneous cortical activity, it is represented by two dimensions in population spiking activity, and the time difference of spiking activity of single neurons with facial motion can span up to ±500ms.

Disclosures: E. Sabri: None. **T. Toosi:** None. **R. Batista-Brito:** None.

Poster

192. Network Interactions

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 192.04

Topic: B.07. Network Interactions

Support: NIH-National Human Genome Research Institute
Schmidt Futures
Simons Foundation (Autism research)

Title: High-density recordings for long-term electrophysiology in developing brain organoids with automated fluidics platform integration

Authors: *C. PAZ FLORES[1,2], J. SEVETSON[3,2], K. VOITIUK[3], S. T. SEILER[3], S. TORRES MONTOYA[6], D. EHRLICH[4], Y. ROSEN[3], M. A. T. ELLIOTT[3], D. HAUSSLER[2,3,5], M. TEODORESCU[6,2], S. SALAMA[3,2,5];
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Abstract: lt&Human stem cell-derived brain organoids are self-organizing three-dimensional tissue models which offer unprecedented access to developing human neural tissues. Recent work in brain organoid electrophysiology demonstrates the emergence of coordinated neural activity patterns. However, there remain technical challenges in characterizing the activity. Organoids typically develop under metabolic stress due to greatly varying nutrient, dissolved gas, pH, and toxin concentration cycles from conventional, pipette media changes. Consistent nutrient levels are especially critical for neural tissue, which has the highest metabolic requirements in the body. We present an automated fluidic platform for high-density CMOS

multielectrode arrays. The platform sustains the organoids through regular, frequent feeding intervals, over a long period of time with minimal perturbation. The design is compatible with MaxWell Biosystems MaxOne HD-MEA wells but can be adapted to fit other electrophysiology arrays. The device uses off-the-shelf microfluidics equipment and 3D printed components to be reproducible. The user can set feeding intervals and volumes through a user-friendly interface, allowing the system to run longitudinal experiments spanning multiple days. The proposed system better recapitulates the primary, in vivo environment to facilitate greater health and consistency for developing neurons while minimizing the amount of manual manipulation by researchers.&gt

Disclosures: **C. Paz Flores:** None. **J. Sevetson:** None. **K. Voitiuk:** None. **S.T. Seiler:** None. **S. Torres Montoya:** None. **D. Ehrlich:** None. **Y. Rosen:** None. **M.A.T. Elliott:** None. **D. Haussler:** None. **M. Teodorescu:** None. **S. Salama:** None.

Poster

192. Network Interactions

Location: SDCC Halls B-H

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Program #/Poster #: 192.05

Topic: B.07. Network Interactions

Support: NSERC Grant 210977
NSERC Grant 05830
FRQNT Doctoral Scholarship
NSERC Master's scholarship

Title: Inhibitory control of network dynamics revealed through large-scale validated cell-type-specific recordings

Authors: *E. GIRAUD, M. LYNN, J.-C. BÉÏQUE, J.-P. THIVIERGE;
Univ. of Ottawa, Ottawa, ON, Canada

Abstract: The ability of brain networks to process information depends on the connectivity and functional coupling between distinct subpopulations. In the prefrontal cortex (PFC), an elaborate repertoire of computations arises from the interaction of single excitatory (E) and inhibitory (I) neurons. It remains unclear, however, how defined subpopulations modulate activity dynamics in the surrounding network. To examine the contribution of different neuronal subtypes to cortical dynamics, we recorded and stimulated spiking activity in acute slices of PFC using high-density multi-electrode arrays containing 4096 closely spaced electrodes. To parse out the contribution of distinct cell types, we developed a spike sorting technique utilizing waveform kinetics and spline interpolation to distinguish putative regular-spiking excitatory neurons from fast-spiking inhibitory interneurons. We validated this classification using a combination of optogenetic and pharmacological strategies. Using a sequential pharmacological approach, we systematically tested the contribution of connections between distinct subpopulations to the activity patterns of

each cell type. In intact networks, surprisingly, optogenetically activating parvalbumin (PV) neurons had no effect on their firing rate. However, with GABAergic and glutamatergic transmission blocked, activating PV neurons reliably increased their firing rate, indicating counterintuitive dynamics in intact networks. To further investigate how coupling between distinct populations was affected by inhibitory drive, we analyzed the effect of targeted inhibition on pairwise correlations. In intact networks, consistent with predictions from past theoretical work, we found that PV stimulation led to a decrease in E to E correlations. Our framework offers a platform to investigate cell-type-specific neuronal dynamics in high-density circuits. Together, our results provide insights on how complex connectivity motifs can affect respective E and I cell population activity.

Disclosures: **E. Giraud:** None. **M. Lynn:** None. **J. Béïque:** None. **J. Thivierge:** None.

Poster

192. Network Interactions

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 192.06

Topic: B.07. Network Interactions

Title: Novel technique for constructing small neuronal networks in culture

Authors: *N. DIAZ[1], T. D. NGUYEN[2], K. O'KEAN[2];
[2]Physics, [1]The Col. of New Jersey, Ewing, NJ

Abstract: Novel techniquefor constructing small neuronal networks inculture Inoptical interrogations of neuronal networks, e.g. calcium imaging, voltage-sensitivedye imaging, etc., the size of the network is oftensignificantly larger thanthe imagingfield-of-view (FOV), making it difficult toperform precise investigations of global networkproperties. Typically, many datasamples must be included inorder todraw meaningfulconclusions. Toovercome this limitation, we developed anovel technique toeasily andefficiently construct small networks consisting of about 600-1200neurons within1-μm diametercircular area.This technique is based onspatially limiting the applicationof poly-L-lysine hydrobromide(PLL), aproteinneeded for neuronal adhesiontoculturing substrates like glass. This ideahasbeenpursued by other authors inprevious works (Yamamotoet al. 2016, Tibau et al. 2020). Inthis work, we used 0.002in. thick silicone membranes tocreate amask inorder tolimit PLLcoating. A 3x3array of 1-um diameter holes were punched through the membrane using acustom built “cookie cutter”type tool. This cutter consisted of sharpened stainless steel tubesattached toa3D-printed holder. The mask was placed over 12-mm diam. glass coverslip, whichwas thencoated with PLL and subsequently seeded with plating medium. After afew days, themask was removed and neurons were only observed withinthe circular regions throughout thefollowing weeks.Neuronal networks of varying densities were cultured and their network properties andactivities were probed. We used Ca^{2+} fluorescent imaging torecord the activity of networks overan8-day period. We alsoused laser scanning photostimulationtomeasure the correspondingfunctional connectivity (Nguyenet

al. 2017)from which network properties were determined. Wediscuss the conclusions drawnfrom these dataas well as the feasibility of producing smallnetworks in-bulk for investigating the role connectivity plays insynchronized network bursting.**References**Yamamoto, Hideaki, et al. Size-dependent regulationof synchronized activity inliving neuronalnetworks. *Physical Review E***94**, 012407(2016).Tibau, Elisenda, et al. Neuronal spatial arrangement shapes effective connectivity traits of invitrocortical networks. *IEEETransactions onNetwork ScienceandEngineering***7**,435-448(2018).Nguyen, TuanD., et al. Mapping functional connectivity of bursting neuronal networks. *AppliedNetwork Science***2**, 1-10(2017).

Disclosures: N. Diaz: None. **T.D. Nguyen:** None. **K. O'Kean:** None.

Poster

192. Network Interactions

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 192.07

Topic: B.07. Network Interactions

Title: Brain Mapping the Effects of Chronic Aerobic Exercise in the Rat Brain Using FDG PET

Authors: *P. THANOS[1], C. HANNA[2], J. HAMILTON[2], K. BLUM[3];
[1]Pharmacol. and Toxicology, State Univ. of New York, Buffalo, Buffalo, NY; [2]U at Buffalo, Buffalo, NY; [3]Western Univ. Hlth. Sci., Pomona, CA

Abstract: Exercise is a key component to health and wellness and is thought to play an importantrole in brain activity. Changes in brain activity after exercise have been observed through various neuroimaging techniques, such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). The precise impact of exercise on brain glucose metabolism (BGluM) is still unclear; however, results from PET studies seem to indicate an increase in regional metabolism in areas related to cognition and memory, direction, drive, motor functions, perception, and somatosensory areas in humans. Using PET and the glucose analog [18F]-Fluorodeoxyglucose (18F-FDG), we assessed the changes in BGluM between sedentary and chronic exercise in rats. Chronic treadmill exercise treatment demonstrated a significant increase in BGluM activity in the following brain regions: the caudate putamen (striatum), external capsule, internal capsule, deep cerebellar white matter, primary auditory cortex, forceps major of the corpus callosum, postsubiculum, subiculum transition area, and the central nucleus of the inferior colliculus. These brain regions are functionally associated with auditory processing, memory, motor function, and motivated behavior. Therefore, chronic daily treadmill running in rats stimulates BGluM in distinct brain regions. This identified functional circuit provides a map of brain regions for future molecular assessment which will help us understand the biomarkers involved in specific brain regions following exercise training, as this is critical in exploring the therapeutic potential of exercise in the treatment of neurodegenerative disease, traumatic brain injury, and addiction.

Disclosures: **P. Thanos:** None. **C. Hanna:** None. **J. Hamilton:** None. **K. Blum:** None.

Poster

192. Network Interactions

Location: SDCC Halls B-H

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Program #/Poster #: 192.08

Topic: B.07. Network Interactions

Support: DIRP, NIMH, USA, ZIAMH002797
BRAIN initiative Grant U19 NS107464-01

Title: Stable neuronal avalanche dynamics in the prefrontal cortex of awake mice during behavioral state changes

Authors: *P. KELLS, T. LINS RIBEIRO, D. PLENZ;
Section on Critical Brain Dynamics, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: The prefrontal cortex (PFC) is crucial for cognitive functions such as working memory, rule-based decision making, and goal-directed behavior. To achieve these demanding tasks, the PFC communicates with many cortical areas. Theory suggests that such network communication might be optimized when networks establish critical dynamics. Here we studied whether PFC exhibits criticality in the form of neuronal avalanches and whether avalanche dynamics remain stable across behavioral states that are known to elicit large fluctuations in neuronal firing rate. We transfected adult mice (N=3; >P35; C57BL/6) with a nonspecific viral vector to express the red-shifted jRGECO1a in PFC neurons. A dorsal cranial window was combined with a microprism to record across the midline in the contralateral medial PFC and anterior cingulate cortex. After recovery, mice were head-fixed on a running wheel and we used 2-photon imaging (2PI) to record simultaneously from >200 neurons in layer 2/3 of PFC. We recorded continuously behavioral states of quiet resting and self-initiated locomotion for periods of 30 min (N=11) over multiple recording sessions. The arousal state of the animal was tracked using pupillometry. Raw 2PI movies were motion corrected, denoised, cell segmented, and subsequent neuropil subtracted fluorescence traces were deconvolved to obtain spiking probabilities. The rotational speed of the wheel was converted to linear speed and thresholded to obtain epochs of locomotion and quiet resting. The time course of pupil dilation and constriction was extracted from movies, z-scored, and thresholded. We found that pupil diameter correlated with running speed (0.27±0.14 (mean±std)). Pupil sizes correlated with firing rates across states (rest 0.47±0.24Hz, locomotion 0.62±0.40Hz; constriction 0.50±0.20Hz, dilation 0.53±0.24Hz). Neuronal cross-correlations remained unchanged between behavioral states (rest 0.03±0.03, locomotion 0.03±0.03; constriction 0.03±0.03, dilation 0.03±0.02). Despite these changes in the first and second order of neuronal firing, avalanche dynamics was maintained for each behavioral state and level of arousal as indicated by their power laws in avalanche size and duration distributions as well as scaling in mean avalanche size vs. duration with an exponent close to 2 (rest 1.8±0.1, locomotion 1.8±0.1; constriction 1.8±0.1, dilation 1.7±0.1). Our results

demonstrate stable neuronal avalanche dynamics in PFC during diverse behavior and arousal states which may support optimal information transmission in frontal networks.

Disclosures: P. Kells: None. **T. Lins Ribeiro:** None. **D. Plenz:** None.

Poster

192. Network Interactions

Location: SDCC Halls B-H

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Program #/Poster #: 192.09

Topic: B.07. Network Interactions

Support: NIH Grant 5R01HL136710

Title: Orexin facilitates the peripheral chemoreflex via corticotrophin releasing hormone neurons and the nucleus of the solitary tract

Authors: *R. BEN MUSA, J. CORNELIUS-GREEN, D. KLINE, E. HASSER, K. CUMMINGS;
Univ. of Missouri-Columbia, Univ. of Missouri, Columbia, Columbia, MO

Abstract: Orexinergic drive is highest in the active phase of the circadian cycle. Previously we showed that orexin contributes to the peripheral chemoreflex (PCR)-mediated hypoxic ventilatory response (HVR), especially in the active phase. This effect was associated with increased Fos-immunoreactivity (IR) in orexin neurons (indicating activation). Orexin neurons project to the paraventricular nucleus of the hypothalamus (PVN) and nucleus of the solitary tract (nTS), two nuclei integral to the HVR. Many PVN neurons with projections to the nTS are activated by acute hypoxia (Hx), and a majority of these are also immunoreactive (IR) for corticotropin-releasing hormone (CRH). Whether orexin neurons facilitate the HVR through an excitatory action on CRH neurons in the PVN and/or the nTS is unknown. Here we hypothesized that hypoxia activates orexin neurons that project to the PVN, and that orexin facilitates the hypoxia-induced activation of CRH neurons and neurons in the nTS. To test these hypotheses, we performed bilaterally microinjection of fluorescent retrobeads into the PVN to label projecting orexin neurons. Male Sprague Dawley rats (age 3-4 months) were exposed to hypoxia (FI_{O2}=0.11; n=4) or normoxia (FI_{O2}=0.21; n=4) for 2 hrs in the active phase. Immunohistochemistry (IHC) was performed to quantify the number of Fos-IR orexin neurons also labeled with retrobeads. In separate experiments, rats were exposed to hypoxia (n=4), or normoxia (n=4), in the active phase, with or without the dual orexin receptor antagonist, suvorexant (20 mg/kg). IHC was performed to quantify the activation (i.e., Fos-IR) of CRH neurons in the PVN and nTS neurons. Compared to rats exposed to normoxia, hypoxia increased the number of activated PVN-projecting orexin neurons by 35 % (O_2 level: p=0.0015). OxR blockade significantly reduced the number of activated CRH neurons in the PVN (by ~54%; drug: p=0.0041), and nTS neurons (by ~50%; drug x O_2 level: p=0.033). These data suggest that

orexin facilitates the PCR via a neural circuit that includes CRH neurons in the PVN and the nTS neurons.

Disclosures: **R. Ben Musa:** None. **J. Cornelius-Green:** None. **D. Kline:** None. **E. Hasser:** None. **K. Cummings:** None.

Poster

192. Network Interactions

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Program #/Poster #: 192.10

Topic: B.07. Network Interactions

Support: MOE2017-T3-1-002

Title: Inhibitory connectome of the claustrum

Authors: ***M. GRAF**, G. J. AUGUSTINE;
Lee Kong Chian Sch. of Med., Singapore, Singapore

Abstract: Little is known about the local circuitry of the claustrum, the most highly interconnected part of the brain. We have filled this gap by characterizing the claustral inhibitory connectome. Optogenetics and whole-cell patch clamp recordings were combined in brain slices to examine inhibitory synaptic responses to photostimulation of parvalbumin (PV), somatostatin (SST) and vasoactive-intestinal peptide (VIP) interneurons (INs). By using optogenetics-based circuit mapping, we defined the connectivity and 2-dimensional spatial organization of local circuits (Cell. Rep. 7:1601). PV-IN and SST-IN connections onto claustral projection neurons (PNs) had broader input areas, greater convergence and generated larger inhibitory postsynaptic currents (IPSCs) than connections with other claustral INs. The converse was true for VIP-INs: their connections onto INs had larger input areas, convergence numbers and IPSCs compared to their connections with PNs. Thus, PV-INs and SST-INs preferentially target claustrum projection neurons, while VIP-INs preferentially target other INs. By comparing responses to wide-field photostimulation and small laser spots, it was possible to obtain information about the 3-dimensional organization of the inhibitory circuits. With this approach, we found that PV-INs connect only locally to neighboring neurons, while VIP-INs connect to both local and more distant targets. Our results indicate that (1) the claustrum uses inhibitory motifs identical to those found in other brain areas, and (2) the 3-dimensional spatial organization of claustrum inhibitory circuits depends upon the presynaptic IN type. Lastly, based on the spatial architecture and preferential connectivity of VIP-INs and PV-INs, these IN types could play contrasting roles in claustral signal processing. VIP-INs may use their widespread disinhibitory connections to act as a general on/off switch for claustrum inputs, while PV-INs may use distance-dependent inhibition and disinhibition to improve input-specific signal processing and noise filtering. Our study greatly expands knowledge of claustral inhibitory circuitry and suggests novel roles for the claustrum in higher-order brain signalling.

Disclosures: **M. Graf:** None. **G.J. Augustine:** None.

Poster

192. Network Interactions

Location: SDCC Halls B-H

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Program #/Poster #: 192.11

Topic: B.07. Network Interactions

Support: DIRP, NIMH, USA, ZIAMH002797
BRAIN initiative Grant U19 NS107464-01

Title: Using machine learning to study the information encoded in visually evoked neuronal avalanches

Authors: *S. PAJEVIC, T. L. RIBEIRO, K. SRINIVASAN, A. GUPTA, D. PLENZ;
Section on Critical Brain Dynamics, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Deciphering how visual information is encoded in spatiotemporal patterns of neural activity has been a long-standing question in neuroscience. It is well accepted that stimulus information is distributed over many neurons with contributions from selective inputs as well as intrinsic network dynamics. Besides the high dimensionality in the representation of a given stimulus, over many neurons, the response selectivity of single cortical neurons is in addition highly nonlinear, and hence simple models perform poorly in predicting the encoding and stimulus properties. Here, we show that machine-learning using the responses of many neurons classifies neuronal avalanche responses elicited by visual stimuli in the primary visual cortex (V1) of awake mice. We recorded such responses to semi-randomly presented drifting Gabor gradings (1 s, 8 directions, 8 s interstimulus interval) using 2-photon imaging (2PI) of ~150 - 250 pyramidal neurons in the V1 of quietly resting mice (~125 stimuli/session; n = 3 mice). To extract the coding information, we use a prediction framework, utilizing deep neural networks and random forests. We establish a hierarchy among the neuronal cells using the direction and orientation selectivity indices (DSI, and OSI, respectively), as well as using a linear discriminant analysis (LDA) framework that we used to select the most discriminant cells. Using only 10%-30% of the highest selective cells greatly improves the overall predictability, increasing from ~50% (random guessing is at 12.5%), when full responses of all neurons are analyzed, to ~70% when using highly selective neurons based on DSI and OSI, and >80% when the select groups were based on LDA. A time window of ~250 ms carries most information about the stimulus, with information preserved for many seconds, before the presentation of the next stimulus. We present our results in the context of long-range spatial and temporal correlations present in critical neural networks that display avalanche dynamics.

Disclosures: **S. Pajevic:** None. **T.L. Ribeiro:** None. **K. Srinivasan:** None. **A. Gupta:** None. **D. Plenz:** None.

Poster

192. Network Interactions

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Program #/Poster #: 192.12

Topic: B.07. Network Interactions

Support: NIH/NINDS ZIA NS003144

Title: Stable long-term functional connectivity metrics capture different aspects of cortico-cortical evoked potentials

Authors: *N. FATUROS, J. CHAPETON, S. INATI, K. A. ZAGHLOUL;
NIH, Natl. Inst. of Neurolog. Disorders & Stroke (NINDS), Bethesda, MD

Abstract: Direct cortical stimulation holds the potential to treat a range of neuropsychiatric disorders in which the connectivity of distributed networks functions abnormally. Mapping cortico-cortical evoked potentials (CCEPs) in response to direct electrical brain stimulation provides powerful insight into the organization of cortical networks. Despite many advances in quantifying cortical networks with CCEPs, the timing, direction, and magnitude of the responses are not fully understood. To better elucidate the relationship between CCEPs and cortical networks, we collected human electrocorticographic recordings from neurosurgical epilepsy patients while delivering single pulses of stimulation. We then created a directed adjacency matrix based on which electrodes responded. Next, we built directed functional adjacency matrices from at-rest data collected at least 12 hours separated from the stimulation session. We demonstrate that the estimated functional connections significantly overlap with the CCEPs and vice versa. Notably, one technique used to generate the functional adjacency matrices was able to capture the directionality of the connections while another technique predicted the onset and magnitude of the CCEPs. Using different metrics to predict the direction, timing, and magnitude of CCEPs is impactful as it provides new insights and tools for analyzing cortical networks. These results will enable more precise predictions of the responses to electrical brain stimulation furthering the development of targeted stimulation therapies.

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Poster

192. Network Interactions

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Topic: B.07. Network Interactions

Support: US Air Force Office for Scientific Research, Grant Number FA9550-19-1-0411

Title: Prediction in in-vitro neural networks, and its link to memory

Authors: M. LAMBERTI[1], S. TRIPATHI[2], S. MARZEN[3], *J. LE FEBER[1];
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Abstract: Several studies have suggested that memory and prediction are crucial neuronal functions that direct our actions and may be general features of neuronal networks. When an external input is perceived, its immediate registration is observed as short lasting activity changes, which can be seen as short-term memory (time scale of seconds), whereas long-term memory refers to activity or connectivity changes on a time scale of minutes to hours. Prediction can be defined as the ability to reduce uncertainty on future sensory input, and has been shown to critically depend on memory, particularly on the registration of recent sensory input. Recent work showed that retinal cells predict visual stimuli, but no proof has been provided yet that prediction is a general capability of neuronal networks. Here, we determined whether *in vitro* neural networks can predict external stimuli, and how prediction depends on (short-term and long-term) memory. We used rat primary cortical neurons plated on multi electrodes arrays (MEAs) with 59 recording electrodes. We subjected them to 20 hours of either electrical (10 cultures) or light (10 cultures) stimulation using interstimulus intervals (ISIs) taken from a known distribution. Repeated electrical stimulation at one electrode has been shown to induce long-term memory traces within one or a few hours, which can be detected as connectivity changes. In contrast, light stimulation did not induce significant long-term connectivity changes. We used mutual information to quantify to what extent recorded activity reduced the uncertainty on future stimuli (MI_{future}; prediction), or recent past stimuli (MI_{past}; short-term memory). Activity provided significant information on past stimulation, indicating that stimulus responses clearly deviated from spontaneous activity. MI_{future} reflected the distribution of ISIs, suggesting that it largely depended on these stimulus responses. In agreement with this notion, masking of stimulus responses largely reduced MI_{future}. MI_{future} almost linearly depended on MI_{past} throughout 20h of stimulation. However, during electrical (but not optogenetic) stimulation this dependency on short-term memory decreased with time, suggesting that other features gradually took over. Optogenetic stimulation did not induce long-term memory traces and showed unchanged dependency of MI_{future} on MI_{past}, suggesting long-term memory as a plausible candidate. We conclude that random neuronal networks are able to predict future stimuli, predominantly based on short-term memory of past stimuli. With the induction of long term memory traces, the dependency on short-term memory becomes less dominant.

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Poster

192. Network Interactions

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 192.14

Topic: B.07. Network Interactions

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BRAIN initiative Grant U19 NS107464-01

Title: Synchronized inter-hemispheric neuronal avalanches at cellular resolution

Authors: *A. VAKILI, T. RIBEIRO, P. KELLS, S. PAJEVIC, D. PLENZ;
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Abstract: At the core of neuronal avalanche dynamics, i.e., critical dynamics, lies the scale-invariant organization of many observable variables linked to cortex functions. Yet, this scale-invariant dynamics is not without its limitations, with most evidence of criticality in the brain exhibiting finite-size effects in scaling introduced by the limited size of our recording windows into the brain. Critical dynamics has been supported by results on a larger scale, i.e., the inter-areal scale, using e.g., MEG or fMRI research in humans. There is, however, limited evidence of neuronal avalanche dynamics on inter-areal level with cellular resolution. To overcome these limitations, in here we employ a Multiphoton Mesoscope (Thorlabs Inc.) to investigate inter-areal cortical functioning in rodent animal models. Specifically, we compared avalanche activity across hemispheres and studied synchronization of these spatio-temporal bursts of activity. We used Thy1 transgenic mice with global GCaMP6s expression. After a craniotomy procedure, a chronic 5-mm window was placed approximately on the midline. After a week of recovery, the animal was positioned on a running wheel with the head fixed under the Mesoscope. We simultaneously recorded from two homologue areas of ~450 μm × 450 μm on either side of the cortical midline using 2-photon imaging. Using an extra set of scanners, the Mesoscope is able to simultaneously image two planes from two inter-hemispheric field-of-views with a framerate of ~20 Hz. Simultaneous ongoing activity of the two hemispheres was recorded together with the mouse speed. After motion correction, 2-photon images passed through a machine learning based deep-interpolation technique to enhance signal-to-noise ratio (DeepInterpolation). Acquired calcium traces were deconvolved (MLspike) and the extracted spiking activity was used to compute neuronal avalanches, defined as periods of suprathreshold population activity. We observed synchronous, highly correlated activity within each hemisphere, as well as inter-hemispheric highly correlated cells. For each hemisphere, avalanche sizes and durations distributed as power laws. A power-law size-duration scaling relationship of exponent 2 was observed, as predicted for critical brain dynamics, together with parabolic avalanche shapes. When comparing the time series of avalanche propagation across the two hemispheres, very high correlation (~0.8) was present with zero lag, indicating co-propagation of population wide activity within our temporal resolution of ~50 ms. Our results suggest avalanche dynamics is coordinated across hemispheres and maintained near a critical phase transition.

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Poster

192. Network Interactions

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Topic: B.07. Network Interactions

Support: Alexander von Humboldt Foundation
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Title: Serotonergic versus noradrenergic control of pupil-linked arousal

Authors: *M. MAHEU[1,2], T. H. DONNER[1,3], J. S. WIEGERT[2,4];
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Abstract: The brain's arousal state profoundly shapes cortical computations underlying perception, memory, and decision-making. Non-luminance mediated variation of pupil size is an established peripheral proxy of central arousal state. Works in rodents, monkeys, and humans have established a link between pupil dynamics and the activity of the locus coeruleus (LC) noradrenergic (NA) and cholinergic basal forebrain systems. More recent evidence has begun to also implicate other neuromodulatory systems, such as hypothalamic orexin neurons and the serotonergic (5-HT) system of the dorsal raphe nucleus (DRN). The interpretation of these findings is complicated by the fact that different neuromodulatory populations tend to co-activate and brainstem nuclei often contain a mixture of neurons releasing distinct neurotransmitters. The present study had two aims: (1) to compare the pupil-linked arousal regulation by DRN-5HT and LC-NA systems; and (2) to determine whether the DRN-5HT regulation of pupil-linked arousal is direct or mediated by DRN-effects on the LC. To do so, we selectively manipulated and read-out DRN-5HT and LC-NA systems in the same animals thanks to a multiplexed genetic targeting approach. This strategy enables expression of optogenetic actuators and calcium activity indicators in various combinations. We observed that population activity in LC-NA and DRN-5HT, simultaneously recorded, both predicted pupil size but with different latencies, with pupil-predictive activity of LC-NA population occurring earlier than DRN-5HT population. Optogenetic activation of both populations induced pupil dilations but with smaller, more transient and more variable amplitude for DRN-5HT. Importantly, recording of LC-NA during DRN-5HT activation demonstrated a small yet reproducible increase in LC-NA activity preceding pupil dilation. However, pilot results of an experiment inhibiting LC during DRN activation suggest that DRN-induced pupil dilations remain unchanged upon LC silencing. Taken together, our results indicate that DRN-5HT also contributes to the control of pupil-linked arousal. The DRN-5HT control is however qualitatively distinct from the LC-NA control in several aspects. Strikingly, DRN-5HT control of pupil-linked arousal seems to occur, at least in part, independently of the LC-NA system.

Disclosures: M. Maheu: None. **T.H. Donner:** None. **J.S. Wiegert:** None.

Poster

192. Network Interactions

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Topic: B.07. Network Interactions

Support: JSPS KAKENHI Grant number 19K22883 and 19H04185.

Title: Functional connections in a cultured chimera network of neuronal cells derived from chick and rat cerebrum

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Abstract: In recent years, several attempts for medical technology using cells derived from different species of animals have been reported. It is important to confirm functional connectivity of the synaptic transmission between neuronal cells derived from different species for control of the planted neuronal system. In this study, we developed a heterogeneous neuronal network derived from chick and rat cerebrum cultured on a multi-electrodes-array-dish and elucidated of signal transduction between neurons from both species by combination of simultaneous Ca^{2+} imaging and measurement of spontaneous network activity. We defined a region of interest (ROI) in the Ca^{2+} imaging area and detected the peak time of the luminance values in the region. Simultaneously, the burst activities were detected the method with X-means clustering. The peak time stamps of the luminance of the ROIs were compared with the burst time stamps of all the electrodes, and the pair of a certain ROI and an electrode with the smallest time difference was identified as the same signals source. Then the chick cells were identified by superimposing the image of cells vitally stained with PKH26 and the image of ROIs. As a result, even yet preliminary data, the spontaneous electrical activity was synchronized between the neuron pair derived from chick and rat, indicated by correlation of burst timings in chick and rat neurons. The cultured chimera neuronal network contains not only neurons, but also glial cells derived from both chick and rat. We analyzed the effects of each glial cell derived from both species on spontaneous neuronal activity. We provided 4 types of cultured chimera neuronal networks combining glial cells and neurons, derived from chick and rat cerebrum, and analyzed spontaneous activity in these cultured neuronal networks. The results showed that the period with spontaneous electrical activity elongated in chick neuronal networks on the sheet of rat glial cells, while rat neurons on chick glial cells expressed the critical loss of activity much earlier. These results suggests that glial cells critically influenced on electrical network activity of neurons derived from other species.

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Poster

192. Network Interactions

Location: SDCC Halls B-H

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Program #/Poster #: 192.17

Topic: B.07. Network Interactions

Title: Features of hydropic changes and reorganization of rats of the sensorimotor cortex of neural complexes during ligation of common carotid arteries

Authors: *V. AKULININ[1], L. MAKARYEVA[2], S. STEPANOV[2], A. SHORONOVA[2], D. AVDEEV[2], M. KORZHUK[2];
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Abstract: We studied the correlation between the processes of hydropic dystrophy and the reorganization of neurons, gliocytes, and synaptic terminals in layers I, III and V of the sensori-motor cortex (SMC) of rats Wistar after prolonged incomplete cerebral ischemia. Ischemia was modeled in rats rats (n=36) by bilateral ligation of the common carotid arteries (LCCA). Animals without LCCA served as controls (n=6). At 1, 3, 7, 14 and 30 days after LCCA, the brain was fixed by perfusion of the fixative mixture through the aorta. For comparative morphometric evaluation of the layers I, III and V SMC were stained according to Nissl stain and hematoxylin-eosin for light microscopy. Primary antibodies to neuron NSE, glial GFAP, synaptophysin p38 and microglia IBA1 were used for immunofluorescence examination. Using plugins of the ImageJ 1.53 program, the numerical density of neurons, astrocytes, microgliocytes, and oligodendrocytes, the content of normo-, hypo-, and hyperchromic neurons, and the relative area of edema/swelling zones and terminals in the neuropil were determined. Statistical analysis was performed using non-parametric methods. After LCCA, destructive, compensatory-restorative changes in neurons, glial cells, and structures of interneuronal communication were revealed in SMC. Significant differences of all the studied morphometric parameters between the terms in the SMC layers were noted. For example, during the period of the strongest correlations between variables (3 days) in layer I, a change in the area of edema-swelling zones by 1% led to a change in the area of terminals by 0.57%, in layer III - by 0.31%, in layer V - by 0.72%. At the same time, the coefficient of determination of the models was 34% (p=0.02), 72% (p=0.03) and 80% (p=0.01). Consequently, in layer I, only 34% of the area of edema-swelling zones could be explained by changes in the terminals, and 66% were due to hydropic changes in the processes of astrocytes and small dendrites. In layers III and V, significantly more terminals changed in this way: 72 and 80%. We attribute this to the fact that layer I contains more processes of fibrous astrocytes, which provide water reabsorption from the edematous terminals, preventing their irreversible death by the light type of destruction. At the same time, in layer III, after 3 days, these mechanisms probably failed, followed by the destruction of the terminals, and in layer V, compensatory hypertrophy of the terminals prevailed. The results obtained demonstrate the pleiotropy of hydropic changes: on the one hand, manifestations of dystrophy, on the other hand, a necessary condition for the rehabilitation of ischemically altered nervous tissue.

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Poster

192. Network Interactions

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Program #/Poster #: 192.18

Topic: B.07. Network Interactions

Support: Support from Takeda plc

Title: Antibody targeting of the BTN3A sub-family of immunoglobulins regulates the synchronous activity of human cortical neurons

Authors: M. ALSAQATI[1], D. CABEZAS[1], J. HADDON[1], Y. ZHU[1], S. WAINWRIGHT[1], C. TIGARET[1], P. MAYCOX[2], M. PAPAKOSTA[2], R. HODGSON[2], J. SCHACHTER[2], M. LI[1], W. GRAY[1], A. HARWOOD[1], J. HALL[1], ***L. WILKINSON**[1];
[1]Cardiff Univ. Neurosci. and Mental Hlth. Innovation Inst., Cardiff, United Kingdom; [2]Takeda Develop. Ctr. Americas, Inc. (TDCA), La Jolla, CA

Abstract: The Cardiff-Takeda Drug Discovery Collaboration is tasked with finding new, genomically-informed, targets for brain disorders. As part of efforts to identify novel mechanisms impacting on synaptic function we have been focusing on the BTN3A sub-family of immunoglobulins. There are three closely related isoforms in the sub-family, BTN3A1, BTN3A2 and BTN3A3 all found in the human MHC region on chromosome 6. Variation in this region of the human genome is strongly linked with risk for psychopathology and BTN3A2 variants specifically have been associated with risk for schizophrenia. In this work we present data showing that antibodies interacting with the BTN3A immunoglobulins influence synaptic function in human neurons. For the experiments we used two anti-BTN3A monoclonal antibodies (mAbs); 20.1 mAb and mAb 103.2., developed originally for the manipulation of Vγ9Vδ2 T-cell function. mAb 20.1 was shown to promote Vγ9Vδ2 T-cell-mediated function, hence was described as ‘excitatory’ while mAb 103.2 was shown to inhibit Vγ9Vδ2 T-cell-mediated function, hence described as ‘inhibitory’. We assessed the effects of these two functional antibodies on human induced pluripotent stem cell (IBJ4 cell line) derived neurons grown in culture and then plated on to a multi-electrode array system. Following the establishment of stable synchronous activity patterns, neurons on MEAs were incubated with 10ug/ml inhibitory antibody and their activity monitored over 30min, 6hr and 24h. The inhibitory antibody led to a reduction in synchronous activity over time, culminating in a cessation of synchronous activity after 24h of incubation. These effects were reversable after washout and were specific to the disruption of synchronous activity, *viz* the neurons were able to generate action potentials but not in a co-ordinated way. 10ug/ml excitatory antibody had different effects dependent on the maturity of the neurons and the duration of antibody administration. An increase in synchrony was seen in less mature neurons with shorter administrations, whereas longer administrations in more mature neurons led to variable effects, encompassing increases, null, and rarely a reduction in synchrony seen in longer (72hr) incubations. Again, these effects were specific to synchronous activity but in contrast to the inhibitory antibody the effects were only partially reversable on washout. These data are the first demonstration of a role for BTN3A immunoglobulins in regulating the coordinated activity of

human neurons, they indicate a novel mechanism of potential relevance to synaptopathies such as schizophrenia, and offer new routes for drug discovery.

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Poster

192. Network Interactions

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Topic: B.07. Network Interactions

Support: T32HD007491

Title: The Role of Kirrel3-GABA neurons in Learning & Memory

Authors: *A. TUNON-ORTIZ, M. E. WILLIAMS;
Neurobio., Univ. of Utah, Salt Lake City, UT

Abstract: GABA neuron dysfunction is broadly implicated in neurological disorders. Different types of GABA neurons participate in distinct roles throughout the brain and specialize to meet specific neural circuit needs. However, the study of GABA neuron diversity is still young. Therefore, it is imperative to define the roles of specialized GABA neurons to better understand how GABA neuron dysfunction contributes to neurological disorders. Here, we identify a novel group of GABA neurons defined by expression of the synaptic cell adhesion protein, Kirrel3. Kirrel3-expressing GABA (Kirrel3-GABA) neurons comprise about 20% of all GABA neurons in the hippocampus but they do not fit into any commonly studied GABA neuron subtype. Thus, Kirrel3-GABA neurons are a unique group of inhibitory neurons whose function in learning and memory is unknown. Our lab previously discovered that, in the hippocampus, Kirrel3 protein is necessary for the formation of a specific type of excitatory synapse from dentate granule cells to GABA neurons in area CA3 (the mossy fiber filopodia synapse). This synapse was shown by our lab and others to be critical for constraining principal neuron activity and promoting memory discrimination. Thus, I propose that Kirrel3-GABA neurons are uniquely positioned to mediate memory discrimination by constraining hippocampal activity. To investigate this, I am using an intersectional chemogenetics approach to specifically silence and activate hippocampal Kirrel3-GABA neurons in mice and determine the effect on learning and memory. Here, I will present my work using the DREADD HM3Dq to activate Kirrel3-GABA neurons as mice are tested in a multi-context fear conditioning task.

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Poster

192. Network Interactions

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Title: Cross-frequency coupling detection and analysis of in-vitro human neural cultures by multi-electrode array

Authors: Y. SALIMPOUR[1], R. DASTGHEYB[2], S. LIU[2], H.-P. LEE[3], N. J. MARAGAKIS[2], W. ANDERSON[1], ***C. W. HABELA**[2];
[1]Neurosurg., [2]Neurol., Johns Hopkins Univ. SOM, Baltimore, MD; [3]Computer Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Human induced pluripotent stem cell (hIPSC) derived neurons offer the possibility of studying human specific neuronal behaviors in physiologic and pathologic states *in vitro*. However, it is unclear whether these cultured neurons can achieve the fundamental network behaviors that are required to process information in the human brain. The interactions between neural oscillations at different frequency bands, known as cross-frequency coupling (CFC), have been investigated as a mechanism for complex information processing in the central nervous system *in vivo*. Phase-amplitude coupling (PAC), which reflects the coupling of the amplitude of oscillations in a high-frequency range to the phase of oscillations in a lower frequency rhythm, is one of the most common forms of CFC and is modulated by both physiologic and pathologic changes in the brain. This study aimed to determine whether *in vitro* networks of human IPSC derived cortical neurons (hIPSC-CN) recapitulate the CFC that is present in *in vivo* networks. Microelectrode arrays (MEAs) provide a controlled framework to study populations of hiPSC-CN and enable monitoring of their electrical activity across time and in response to manipulation. We analyzed the electrical activity recorded from hiPSC-CN grown in culture with hiPSC derived astrocytes at day 218 of differentiation using a 24 well MEA plate with 16 electrodes per well. We employed the modulation index method for detecting PAC and used offline spike sorting to analyze the contribution of a single neuron's spiking activities to the spikes recorded by each electrode. We analyzed baseline activity as well as the changes occurring as a result of the addition of the GABA receptor antagonist, Bicuculine. Our preliminary results demonstrate that CFC is present mostly in the form of PAC in MEA recordings of hiPSC-CN. Our analysis demonstrates that the degree of PAC is specific to the network surrounding each electrode and is modulated by bicuculine administration. Additionally, the shift in PAC is not driven by a single neuron's properties but rather by network interactions. Together, this data suggests that CFC analysis provides additional information to explore communication and integration between groups of nearby cultured cells and dynamical changes across the entire network *in vitro*. CFC analysis has the potential to capture the effects of chemical agents and electrical or ultrasound stimulation on complex neuronal interactions. Using

hiPSC derived neuronal cultures *in vitro* to study how to modulate this activity may provide valuable information for the modulation of neural networks to treat nervous system disorders *in vivo*.

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Poster

192. Network Interactions

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Topic: B.07. Network Interactions

Support: NIH Grant DC015901

Title: Bushy cell dendrites exhibit dense local branching and form a small-world network via dendrite-dendrite contact

Authors: G. SPIROU[1], T. BAYLISS[2], M. BESHAI[1], P. B. MANIS[4], E. FULLER[5], S. PAYNE[1], C.-Q. ZHANG[3];
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Abstract: The dendrites of bushy cells (BC) in the cochear nucleus have, from their earliest investigation using the Golgi stain, been described as having dense and unique branching structures. Although labeling is sparse, dendrites of neighboring cells appeared to intertwine into hypothesized dendrite networks (Lorente de No, 1981). We have provided, using volume electron microscopy (EM), a more accurate rendering of branching complexity than is achievable by light microscopy (LM). Many branch locations yield daughter branches that extend in contact with each other and which bend at large, often obtuse, angles. Close examination of a subset of 10 BCs, whose dendrites were largely contained within a nearly cubic volume of about 120 µm length per edge, revealed that dendrites from neighboring cells also extended in contact with each other. We quantified the weight of dendrite-dendrite connections as the apposed surface area, which was measured directly on the serial EM sections. Self-contact among these 10 cells ranged from 5.7 - 173.7 μm^2 (μ = 32.2 (SD 44.14)). Contact between pairs of BCs ranged from 5.6 - 93.8 μm^2 (μ = 18.1 (SD 25.04)). After excluding 7 cells which had limited dendrite containment within the image volume, we investigated clustering of dendrites using the remaining 19 BCs, including the 10 BCs with complete dendrites. An adjacency matrix defined four clusters of dendrites, containing 5, 7, 7, and 6 cells. Two cells bridged clusters by having contact with cells in two clusters. Two additional BCs had 2 primary dendrites that further branched into non-overlapping domains (all other BCs had a single primary dendrite). Each dendrite contacted a different cluster, such that these two BCs also bridged between clusters.

Thus the overall group contact profile exhibited small-world network structure with a small number of highly-connected nodes bridging local communities. Two BCs did not contact other BCs. These data complement previous reports, using LM immunohistochemistry, of frequent gap junctions between BC cell bodies (Rubio and Nagy, 2015), and suggest mechanisms to correlate BC activity within the population of cells.

Disclosures: **G. Spirou:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IstoVisio, Inc.. **T. Bayliss:** None. **M. Beshai:** None. **P.B. Manis:** None. **E. Fuller:** None. **S. Payne:** None. **C. Zhang:** None.

Poster

192. Network Interactions

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Topic: B.07. Network Interactions

Support: MH127513-01

Title: Synaptic and mitochondrial abnormalities in the trisynaptic pathway in the hippocampus in schizophrenia: a postmortem ultrastructural study

Authors: *R. C. ROBERTS[1], C. B. FARMER[2], E. L. ROACH[2], L. R. BICE[2], M. E. FALGOUT[2], J. K. ROCHE[2];
[1]Univ. of Alabama, Birmingham, Univ. of Alabama, Birmingham, Birmingham, AL; [2]Psychiatry and Behavioral Neurobio., Univ. of Alabama, Birmingham, AL

Abstract: A preponderance of evidence suggests that the hippocampus is a key region of dysfunction in schizophrenia. Neuroimaging and other studies reveal a relationship between hippocampal dysfunction and the degree of psychosis. Preclinical and clinic data indicate hyperactivity in the hippocampus precedes the onset of psychosis, and is correlated with the severity of symptoms. Hippocampal hyperactivity in schizophrenia has been proposed by many and can arise in multiple regions/subfields and from cell specific changes. In this study we sought to identify circuitry at the electron microscopic level that could contribute to region-specific imbalances in excitation and inhibition in the hippocampus in schizophrenia. We used postmortem tissue from the anterior hippocampus from patients with schizophrenia and matched controls (n=11 and 12, respectively). Using stereological techniques we counted and measured synapses, PSDs, and mitochondria and in key nodes of the trisynaptic pathway. Compared to controls, the schizophrenia group had decreased numbers of inhibitory synapses in the dentate gyrus and CA3 and increased numbers of excitatory synapses in CA1. The thickness of the PSD was larger in excitatory synapses in CA3 and CA1, suggesting greater synaptic strength. The density of mitochondria was less in the DG in the schizophrenia group. Optical density, a measure of functional integrity, was decreased in CA1. Mitochondrial diameter was similar in all

regions between groups. The results suggest region specific increases in excitatory circuitry, decreases in inhibitory neurotransmission and fewer or damaged mitochondria. These results are consistent with the hyperactivity observed in the hippocampus in schizphrenia in previous studies.

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Poster

192. Network Interactions

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Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - 368482240/GRK2416 (RTG 2416 "MultiSenses-MultiScales")

Title: Neural manifolds are modulated by feedback in macaque primary visual cortex during resting state

Authors: *A. MORALES-GREGORIO[1,2], A. C. KURTH[1,3], J. ITO[1,4], A. KLEINJOHANN[1], F. V. BARTHÉLEMY[5,1], T. BROCHIER[5], S. GRÜN[1,4], S. J. VAN ALBADA[1,2];
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Abstract: High-dimensional brain activity is often organized into lower-dimensional neural manifolds, which can represent a plethora of behavioral variables, such as head direction, decision making, or hand movement. However, neural manifolds remain understudied in the visual cortex of primates, with studies rather focused on mice [1] or considering small samples of neurons in macaque [2].
Feedback communication in the cortex has been observed in specific frequency bands [3]. Moreover, the feedback to V1 from higher visual areas is known to mediate visual attention for figure-ground segregation and contour integration in macaque [4]. Computational modeling shows that feedback may also influence neural manifolds by rotating them in a context-

dependent manner to recover sensory inputs from different contexts [5]. However, whether feedback signals can modulate neural manifolds in the brain remains to be proven.
Here, we study the neural manifolds of macaque (*Macaca mulatta*, N=4) V1 during the resting state. The macaques were seated in a dark room and thus received virtually no visual input. We used extracellular multi-electrode (Utah array) recordings with unprecedented spatio-temporal resolution [6]. Our analysis reveals that resting-state neural manifolds of macaque V1 are organized as two distinct high-dimensional clusters. We show that these clusters are primarily correlated with the behavior (eye closure) of the macaques and that the dimensionality of each of these clusters is significantly different, with higher dimensionality during the eyes-open periods. In addition, we use LFP coherence and Granger causality to estimate signatures of feedback from V4 and DP to V1 (in the beta range) and find that feedback signatures are significantly stronger during the eyes-open periods. Finally, we simulate a cortical microcircuit under resting-state conditions and show that feedback signals can modulate the state space of our model: the presence and absence of feedback lead to distinct clusters in the state space, in agreement with the experimental observations. Taken together, the data analysis and simulations suggest that feedback signals actively modulate neural manifolds in the visual cortex of the macaque.
References:
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[4] Poort et al. 2012. *Neuron* 75 (1), 143-156
[5] Naumann et al. 2022. *eLife* 11, 76096
[6] Chen et al. 2022. *Scientific Data* 9 (1), 77

Disclosures: **A. Morales-Gregorio:** None. **A.C. Kurth:** None. **J. Ito:** None. **A. Kleinjohann:** None. **F.V. Barthélemy:** None. **T. Brochier:** None. **S. Grün:** None. **S.J. van Albada:** None.

Poster

192. Network Interactions

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 192.24

Topic: B.07. Network Interactions

Support: Marie Sklodowska-Curie Individual Fellowships 2018
ERC Grant 2016

Title: Locus coeruleus-mediated spontaneous changes in brain state in zebrafish

Authors: *E. MARACHLIAN, L. DANNATT, A. URIBE-ARIAS, G. SUMBRE;
École Normale Supérieure, Paris, France

Abstract: To adapt to the complex and ever changing environment, the brain needs to quickly shift between resting and working states. These global-brain-state shifts are linked to the brain-

wide release of neuromodulators. Is well known that escape responses is paired with changes in arousal states that are mediated by noradrenergic neurons in the Locus Cororus (LC-NA). However, the whole-brain mechanism underlying the switch in brain states, remains elusive. To address this open question, we used transgenic zebrafish larvae expressing GCaMP in combination with light-sheet microscopy, to monitor whole-brain dynamics with single-neuron resolution while simultaneously recording tail movements. We studied brain dynamics before and after the spontaneous or induced activation of the LC-NA.
Our results showed that LC-NA are activated when animals perform a strong escape behavior what induced a global brain state switch characterized by the shutting down of a vast number of active neurons and the activation of silent ones. Spontaneous activations of LC were preceded by a ramping activity starting around 15 sec before the onset of LC. This activity is abruptly silenced at the onset of LC. Interestingly, for the induced LC activations, the magnitude of LC activation appeared to be correlated with the number of raping neurons at the time the stimulus is presented. This suggests that the ramping cells trigger LC-NA.
We are now using optogenetics to directly stimulate the LC and specific cell-type fluorescence markers to further learn about the mechanisms underlying LC-mediated switches in brain state. Overall, we described a mechanism capable of spontaneously trigger the LC activation, and its implications at the whole-brain dynamics.

Disclosures: **E. Marachlian:** None. **L. Dannatt:** None. **A. Uribe-Arias:** None. **G. Sumbre:** None.

Poster

192. Network Interactions

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 192.25

Topic: B.07. Network Interactions

Support: CAPES Grant 23038.003069/2022-87
CNPq

Title: Phenomenological renormalization group analysis of cortical spiking data from urethane-anesthetized rats

Authors: *D. M. CASTRO, T. FELICIANO, M. COPELLI;
Univ. Federal de Pernambuco, Recife, Brazil

Abstract: The critical brain hypothesis has emerged in the last decades as a fruitful theoretical framework for understanding collective neuronal phenomena. Lending support to the idea that the brain operates near a phase transition, Beggs and Plenz were the first to report experimentally recorded neuronal avalanches, which are bouts of neuronal activity whose distributions coincide with those of a critical branching process, in which a phase transition occurs between an absorbing (silent) and an active phase. However, this hypothesis is highly debated, as neuronal

avalanches analysis and other common statistical mechanics tools may struggle with challenges ubiquitous in living systems, such as subsampling and the absence of an explicit model for the complete neuronal dynamics. In this context, a model-independent phenomenological renormalization group (PRG) method was recently proposed to analyze experimental data from neural networks (Meshulam et al., 2019). The procedure consists of repeatedly merging time series from maximally correlated units, in such a way that our description of the collective activity becomes increasingly coarse grained after each iteration. As in traditional applications of the renormalization group, we expect that, under a critical regime, non-trivial correlations and scale-free behavior will be unveiled as we simplify our description. This can be inferred from a series of statistical features of the data, such as the activity distribution of coarse grained variables, mean variance and autocorrelation decay time. Further investigation can be made with principal component analysis (PCA), which has been shown to play a role analogous to momentum space renormalization group in this context. Here, we apply the PRG technique to assess whether this scale-free, self-similar behavior, which is expected in critical dynamics, can be identified in spiking data recorded with 64-channel silicon probes in the primary visual cortex of urethane-anesthetized rats. . Additionally, we investigate how the scaling exponents found via PRG behave as we parse the data by the coefficient of variation (CV) of the population rate, and compare our results with those obtained via avalanche analysis (Fontenele et al., 2019).

Disclosures: **D.M. Castro:** None. **T. Feliciano:** None. **M. Copelli:** None.

Poster

192. Network Interactions

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Program #/Poster #: 192.26

Topic: B.07. Network Interactions

Support: CNPq Grant 301744/2018-1
CAPES Grant 23038.003069/2022-87
FAPESP Center for Neuromathematics Grant 2013/07699-0
CAPES Grant 88887.581360/2020-00

Title: Active-quiescent phase transitions in the stochastic Wilson-Cowan model

Authors: H. C. PIUVEZAM[1], B. MARIN[2], ***M. COPELLI**[1], M. A. MUÑOZ[3];
[1]Federal Univ. of Pernambuco, Recife, Brazil; [2]Federal Univ. of ABC, Santo André, Brazil;
[3]Univ. de Granada, Granada, Spain

Abstract: The original Wilson-Cowan model describes the collective behavior of a local population of neurons by using two coupled dynamic equations for the firing rates of excitatory and inhibitory subpopulations (Wilson & Cowan, 1972). These equations reproduce a large variety of possible dynamical regimes, including steady states and oscillations.
Decades later, a stochastic version of the Wilson-Cowan model was proposed to describe

microscopic single-unit dynamics (Benayoun et al, 2010), where the state of each neuron is represented by a binary variable (0 representing a quiescent neuron, 1 an active neuron). The transition rate from 0 to 1 depends nonlinearly on the sum of excitatory and inhibitory inputs, which is controlled by connection weights, as well as external stimuli. In the limit of a large number of neurons, the mean-field description of the stochastic model corresponds to the firing rate Wilson-Cowan model of 1972.
The stochastic Wilson-Cowan model is particularly useful for the theoretical study of neuronal avalanches, which are bouts of neuronal activity between periods of silence. These were first observed in cultured slices of rat cortex (Beggs & Plenz, 2003) and shown to follow scale-invariant statistics, such as power-law distribution of sizes and durations, which are characterized by critical exponents. In many experimental setups, the observed exponents were compatible with a critical branching process or, more generally, any phase transition belonging to the mean-field directed percolation (MF-DP) universality class. In this class, the phase transition is from a quiescent (absorbing) to an active phase. In other experimental setups, the compatibility of the critical exponents with MF-DP is less clear.
In the absence of external stimuli, the stochastic Wilson-Cowan model can present a phase transition in the MF-DP class and its corresponding avalanche statistics (de Candia, 2021). Exploring the phase diagram of the model, what we show here is that the model can also display a plethora of other phase transitions, including tricritical points, discontinuous transitions, and anomalous scaling. These transitions are characterized by exponents, which may or may not equal those of MF-DP, thus providing a set of statistical markers that can be used for comparison with experimental data.

Disclosures: **H.C. Piuvezam:** None. **B. Marin:** None. **M. Copelli:** None. **M.A. Muñoz:** None.

Poster

192. Network Interactions

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 192.27

Topic: B.07. Network Interactions

Support: German Research Foundation
Boehringer Ingelheim Foundation

Title: Beyond Correlation: Functional OPTO-MAgnetic Integration Concept (OPTOMAIC) to reveal the brain-wide signature of local neuronal signals-of-interest

Authors: *D. CLEPPIEN[1], F. AEDO-JURY[1], A. STROH[2,1];
[1]Leibniz-Institute for Resilience Res., Mainz, Germany; [2]Univ. Med. Ctr. Mainz, Mainz, Germany

Abstract: Assessing the functional network architecture on a brain-wide level is vital for discovering early dysfunctional brain states. fMRI affords imaging of brain-wide circuits, albeit

only via measuring vascular parameters, linked to neuronal activity by the process of neurovascular coupling. This fundamental problem limits the fine-grained and casual interpretation of BOLD fMRI patterns, and the identification of maladaptive early network dysregulation in patients at early stages of neuropsychiatric disorders. Optical fiber-based neuronal calcium recordings provide a specific and temporally highly resolved signal yet lacking brain-wide coverage. The cross-modal integration of both modalities holds the potential of unique synergies. The OPTO-MAgnetic Integration Concept (OPTOMAIC) extracts the very fraction of the BOLD response which reacts to optically recorded neuronal signals-of-interest. First, OPTOMAIC identifies the trials containing neuronal signal-of-interest in the optical recordings. The long duration of the BOLD response is considered by calculating and thresholding neuronal inter-event- intervals. The resulting optical regression vector is probed for a positive BOLD response with single-event and single-voxel resolution, generating a BOLD-response matrix containing only those events and voxels with both a neuronal signal-of-interest and a positive fMRI signal increase. Lastly, the onset of the BOLD response is being quantified, representing the section of the BOLD response most reliably reporting at least components of the neuronal signal. The seven OPTOMAIC steps result in a brain-wide BOLD signature reflecting the underlying neuronal signal-of-interest with utmost cross-modal integration depth and taking full advantage of the specific strengths of each method. In an experimental dataset, OPTOMAIC significantly outperformed the classical analysis scheme of averaging over all stimulation intervals. OPTOMAIC provides a novel tool with translational perspective, as this approach could also be applied in simultaneous EEG-fMRI recordings in humans. Therefore, OPTOMAIC affords to identify the spatiotemporal dynamics of a neurophysiological relevant signal of interest measured by the cross-modal approach of BOLD fMRI and simultaneous fiber-based calcium recordings and offers a promising diagnosis tool to investigate the integrity and functionality of neuronal networks.

Disclosures: **D. Cleppien:** None. **F. Aedo-Jury:** None. **A. Stroh:** None.

Poster

192. Network Interactions

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Program #/Poster #: 192.28

Topic: B.07. Network Interactions

Support: German Research Foundation
Boehringer Ingelheim Foundation

Title: Stress resilience is associated with an increased accuracy of the cortical representation of visual afferents and a unique adaptive local network signature

Authors: *H. BACKHAUS[1], S. ALTAHINI[1], A. WIERCZEIKO[1], N. RUFFINI[1], A. STROH[2,1]; [1]Leibniz Inst. for Resilience Res., Mainz, Germany; [2]Johannes Gutenberg-University Mainz, Univ. Med. Ctr. Mainz, Mainz, Germany

Abstract: We and others identified changes in cortical microcircuit activity and connectivity in mouse models of Multiple Sclerosis and Huntington´s disease in very early stages, prior to irrevocable neurodegeneration. These early, and putatively maladaptive dysregulations suggest an ongoing effort of the brain to compensate for the initial pathophysiological event, adopting a new, temporarily meta-stable attractor state, ensuring the preservation of overall network stability. Studying the effects of chronic stress on neuronal networks has been up to now mainly focused on the dmPFC. Whether sensory cortical microcircuits are affected, has not been investigated yet. Sensory circuits provide the basic prerequisite for efficient sensory discrimination, which is discussed to represent a resilience mechanism. Here, we ask, whether chronic stress exposure can lead to a distinct shift in cortical microcircuit activity and connectivity patterns.We subjected male mice to a chronic social defeat (CSD) paradigm to mimic severe stress exposure. The experimental animals were present with an aggressor over 10 consecutive days. To assess behavioral phenotypes, a social interaction (SI) test was carried out and experimental animals were classified as rather stress resilient or non-resilient. We employed two-photon calcium imaging in awake mice.Non-stress-resilient mice exhibited higher spontaneous activity levels in the primary visual cortex, and the social interaction score correlates with the shift in cortical microcircuit activity. Non-resilient microcircuits display a reduction in local connectivity despite overall higher activity. Non-traumatized animals exhibit activity dynamics close to non-resilient animals. We then asked, if the network activity signature in resilient mice is adaptive or maladaptive? We found, that resilient microcircuits surpass non-traumatized microcircuits in the accuracy of the representation of visual afferents. Resilient mice display even stronger and modular connectivity than non-traumatized circuits upon visual stimulation. We found, that upon chronic stress exposure, stress resilient animals, as classified by the behavioral outcome, undergo an adaptive shift towards a microcircuit with higher connectivity, and increased discrimination ability. These findings suggest, that while in models of neuropsychiatric diseases, new network states were accompanied by a decreased functionality, here, stress resilient animals transition to an adaptive state. We could conceptualize stress resilience as the ability to undergo plastic changes of cortical sensory circuits.

Disclosures: H. Backhaus: None. **S. Altahini:** None. **A. Wierczeiko:** None. **N. Ruffini:** None. **A. Stroh:** None.

Poster

192. Network Interactions

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Program #/Poster #: 192.29

Topic: B.07. Network Interactions

Support: German Research Foundation
Leibniz Association

Title: Identifying the brain-wide signature of slow wave events on single-subject level in human EEG-fMRI assessments: an early and individualized biomarker of early network dysregulations?

Authors: *M. ILHAN-BAYRAKCI[1], O. TÜSCHER[2,1], A. STROH[2,1];
[1]Leibniz Inst. for Resilience Res., Mainz, Germany; [2]Univ. Med. Ctr. Mainz, Mainz, Germany

Abstract: The slow wave state is a brain state occurring in slow wave sleep and in certain anesthetic regimens, characterized by network quiescence interrupted by sudden bursts of activity or so-called slow wave events (SWEs). Neurophysiologically well-defined SWEs can be found in mice and humans, and their main features seem to be highly preserved across species. SWE occurrence and propagation are highly susceptible to excitability changes and states of the neuronal network. Early stages of neurodegenerative and neuroimmunological disorders are marked by ensembles of hyperactive neurons and seem to alter the propagation of SWEs. This susceptibility makes SWEs an ideal target for studying brain (dys-)function across species and across diseases. Resolving distinct BOLD fMRI patterns of SWEs in sleeping healthy humans is a critical prerequisite for any study examining SWE alterations reflecting early network dysregulations caused by pathological conditions in humans. In rats, we have assessed the relationship between SWEs and BOLD fMRI signals and have observed a cortex-wide BOLD pattern directly related to SWEs. In healthy humans, we have analyzed simultaneous EEG-fMRI data during non-REM sleep. SWEs individually detected in the EEG data were used as predictors in event-related fMRI analyses on single subject level to examine the relationship between SWEs and fMRI signals. For all subjects we identified significant changes in BOLD activity associated with SWEs covering substantial parts of the gray matter.By means of three distinct datasets comprising simultaneous EEG-fMRI data of sleeping healthy humans (N = 60) we took our analyses one step further. We classified SWEs by the vigilance stages they occurred in and examined their corresponding BOLD fMRI signatures, respectively. Preliminary results showed distinct patterns of significant BOLD fMRI signal changes related to wake SWEs, N2 SWEs and N3 SWEs. Importantly, the resulting BOLD fMRI patterns for each respective vigilance stage varied between subjects in terms of spatial extent and amplitude of the BOLD signal. Still, the brain regions recruited by SWEs of each individual vigilance stage were highly consistent. BOLD fMRI signatures related to SWEs need further thorough specifications and classifications to create a systematic catalogue of existing patterns in healthy humans. Only such a differential and profound catalogue will allow to distinguish between physiological and pathophysiological SWE network signatures, emerging in a variety of neuropsychiatric diseases long before the onset of clinical symptoms.

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Poster

192. Network Interactions

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 192.30

Title: WITHDRAWN

Poster

193. Oligodendrocyte Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 193.01

Topic: B.09. Glial Mechanisms

Support: MEXT Grant 20K07756
MEXT Grant 20KK0188

Title: Teneurin-4 forms a molecular complex with actin-regulating proteins in oligodendrocytes and promotes myelin sheath formation in the CNS

Authors: *M. IWASE, C. HAYASHI, B. SASAKI, M. YAMADA, N. SUZUKI;
Tokyo Med. and Dent. Univ., Tokyo, Japan

Abstract: In the CNS, oligodendrocytes (OLs) form multi-lamellar myelin sheaths around axons, which enable the rapid conduction of action potential. The initial step of myelination requires the assembly of actin filaments for the extension of OL processes and for the ensheathment of axons. In contrast, the subsequent step of myelin wrapping requires the disassembly of actin filaments. The mutant mice that lack the expression of a type II transmembrane protein teneurin-4 (Ten-4) display the tremor phenotype because of hypomyelination in the CNS. Our previous study revealed that Ten-4 facilitated the cell adhesion through its extracellular domain and promoted OLs differentiation. In this study, we analyzed the expression pattern and the function of Ten-4 via its intracellular domain during myelination. Immunohistochemistry of mouse spinal cords showed Ten-4 expression in myelin sheath at P7, when the state of actin is shifted from polymerization to depolymerization. In Ten-4 deficient mice at P7, the shift from the initial stage of actin polymerization to the later stage of actin depolymerization was inhibited. To analyze the molecular mechanism, we screened Ten-4 binding proteins and obtained 2', 3'-cyclic-nucleotide 3'-phosphodiesterase (CNP), OL-specific actin binding protein, and Arpc1a, an Arp2/3 complex component highly expressed in OLs. In proximity ligation assay on mouse primary OLs, Ten-4 and CNP were colocalized at the branching points of the processes, where myelin-like membrane sheets are formed. When Ten-4 and CNP were overexpressed in COS-7 cells, these two proteins were co-immunoprecipitated and downregulated actin assembly. Arpc1a also exhibited the similar activity with Ten-4 as CNP. From these results, the formation of the molecular complex of these proteins interferes their activity for actin polymerization. In addition, we analyzed the function of Arpc1a in OLs, since it was unknown. The intense expression signal of Arpc1a was detected at the initial stage of myelination in immunohistochemistry of mice spinal cords. Knockdown and overexpression experiments of Arpc1a in OLs showed that Arpc1a was required for the proper formation of cell processes and myelin-like membrane sheets in OLs. Taken together, Ten-4 formed a molecular complex with CNP and Arpc1a and positively regulated CNS myelination through the actin cytoskeletal organization in OLs.

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Poster

193. Oligodendrocyte Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 193.02

Topic: B.09. Glial Mechanisms

Support: K01NS126813 NIH-NINDS
Startup Package
T34GM137854 NIH-NIGMS

Title: Voluntary activity levels are regulated by mature myelin in models of neurofibromatosis type 1

Authors: *C. MARTINEZ, D. CRUZ, D. HERNANDEZ, P. DE LEON, S. LOPEZ, L. MANOJ, T. ALVAREZ, A. LOPEZ-JUAREZ;
Hlth. and Biomed. Sci., The Univ. of Texas Rio Grande Valley, Brownsville, TX

Abstract: ABSTRACTRationale and Objective Neurofibromatosis type 1 (NF1, 1/3000 live births worldwide), is an autosomal dominant disease caused by mutations in the gene coding for neurofibromin (*NF1*). NF1 patients develop diverse conditions ranging from aesthetic issues to life-threatening tumors. Moreover, most NF1 patients present with neurological issues including learning deficits, delayed motor skills, attention deficit, and increased risk for depression. Interestingly, NF1 patients also present multiple abnormalities in the brain WM and myelin; although proposed decades ago, myelin-behavior links remain obscure in NF1. Hence, our objective is to provide robust experimental evidence for the impact of *Nf1* mutation on behavior. **Methods and Results**Mice carrying a mutation known to cause NF1 (*Nf1*+/- mice) showed compromised learning curves in a voluntary/myelin-regulated motor learning test, the Complex Wheel (CW; a running wheel with irregularly spaced rungs). This phenotype was caused by compromised activity levels without affecting capability to run (max speed). To test the actual contribution of myelin to this phenotype, we conditionally induced *Nf1* mutation in mature myelin-producing cells using a tamoxifen inducible system *(PlpCreEr;Nf1flox* or *pNf1flox*). Our results show that *pNf1flox* mice show subnormal learning curves in a gene-dose-dependent manner and that, similar to *Nf1*+/- mice, this phenotype is driven by decreased activity levels without affecting their capability to run. Whether motivation issues drive this phenotype is under investigation.**Scientific rigor measures** To establish robust and relevant results, both germline and myelin producing cell-specific *Nf1* mutants we tested in the CW. Adult WT (control), hemizygous, and homozygous *Nf1* mutant mice (n=10/genotype) were subjected to the CW test. A fully automated recording system was used to avoid observer bias and data was processed by personnel blinded to the genotypes.**Conclusion**Our results shed light onto the roles of myelin in the control of behavior, particularly on the regulation of voluntary activity that is relevant for attention deficit and motivation/depression issues in NF1.

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Poster

193. Oligodendrocyte Development and Function

Location: SDCC Halls B-H

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Program #/Poster #: 193.03

Topic: B.09. Glial Mechanisms

Support: NICHD ZIAHD000713

Title: Title: Activity-dependent Plasticity of the Node of Ranvier

Authors: E. N. SANTOS, W. HUFFMAN, M. FOOTE, P. R. LEE, ***R. FIELDS**;
NICHD, Bethesda, MD

Abstract: Myelin is a multilayered lipid membrane structure that ensheaths axons to promote rapid transmission of action potentials via saltatory conduction. Changes in the geometry of the gaps between myelin segments, known as nodes of Ranvier, can impact the conduction speed of neuronal signals to alter neural synchronization and circuit function. The geometry of the node of Ranvier can be altered by perinodal astrocytes that regulate thrombin proteolysis of the axo-glial adhesion protein neurofascin155; however, it is unknown whether such nodal remodeling is impacted by sensory experience and activity-dependent signaling between neurons and astrocytes. Here, we show that the process of nodal widening that occurs in mice with transgenic impairment of astrocyte exocytosis proceeds normally in the optic nerve following long-term binocular visual deprivation initiated during adulthood. Nodal recovery, which occurs when normal astrocyte function is restored following early impairment, indicates that mechanisms driving nodal narrowing are also not impacted by binocular visual deprivation. To address the possibility that the influence of the astrocyte-mediated mechanism of nodal widening could obscure other possible mechanisms of activity-dependent nodal plasticity, we separately determined that binocular visual deprivation had no effect on nodal gap length in adult animals in which astrocyte exocytosis was not manipulated. Prior to these experiments, studies on sensory experience have implicitly assumed that myelin remains unchanged following visual deprivation. The absence of plasticity of the node of Ranvier in response to binocular visual deprivation supports this understanding. Furthermore, in parallel with understanding of visual system synaptic plasticity, competition between inputs, such as is created by monocular deprivation, may be necessary to drive mechanisms of activity-dependent plasticity at the node of Ranvier. This hypothesis is tested by examining nodal gap changes in the optic tract following monocular suture and inhibition of neuronal activity in the retina using a viral construct in adult mice.

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Poster

193. Oligodendrocyte Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 193.04

Topic: B.09. Glial Mechanisms

Title: Role of Tim-2 in Central Nervous System Myelination

Authors: *Q. WADE[1], E. NEELY[1], J. R. CONNOR[2];
[2]Neurosurg., [1]Penn State Col. of Med., Hershey, PA

Abstract: Myelin, which is formed by oligodendrocytes (OLs) in the central nervous system (CNS), is crucial for efficient signal transduction and neuronal function. Iron is a critical micronutrient for OLs due to its involvement in energy production and its role as a cofactor for myelin synthesis, and it is well-known that OLs are the highest iron staining cells in the brain. Inadequate iron delivery to OLs frequently results in severe and long-lasting neurological deficits that are attributed to hypomyelination. Our laboratory and others have identified H-ferritin (Fth), traditionally considered an iron storage protein, as the primary iron delivery protein to OLs. We have also found that Fth utilizes a novel receptor on rodent OLs, T-cell immunoglobulin and mucin domain-containing protein-2 (Tim-2). To determine the importance of Fth-mediated iron delivery through Tim-2 on OLs for myelination, we have generated *Timd2*$^{fl/fl}$ *Plp1-Cre/ERT*conditional knockout mice to eliminate Tim-2 expression specifically from mature OLs (*Plp1*+) following consecutive tamoxifen injections. We hypothesize that removal of Tim-2 from OLs will lead to decreased Fth uptake, which will, in turn, lead to reduced iron uptake and consequently, insufficient myelination. In this study, we observed that *Timd2*$^{fl/fl}$*Plp1-Cre/ERT* mice injected with tamoxifen, on post-natal days 7-9 to induce the knockout, displayed a trend towards poorer motor performance on the rotarod as measured by decreased latency to fall. Moreover, there was a statistically significant reduction in myelin basic protein (MBP) and proteolipid protein (PLP) in addition to a decrease in myelin staining in the corpus callosum of Tim-2 knockout animals compared to controls. Essentially, the data from this study indicates that myelin is negatively affected following Tim-2 knockout from OLs during development.

Disclosures: Q. Wade: None. **E. Neely:** None. **J.R. Connor:** None.

Poster

193. Oligodendrocyte Development and Function

Location: SDCC Halls B-H

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Program #/Poster #: 193.05

Topic: B.09. Glial Mechanisms

Support: 21J20080

Title: Metabolites from gut microbiota regulate oligodendrocytes gene expression.

Authors: *J. KAMBE[1], K. USUDA[2], M. ITO[4], K. HIRAYAMA[5], R. INOUE[6], G. WATANABE[3], N. KENTARO[1];
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Abstract: Myelin formed by oligodendrocytes has been known to be involved in jumping conduction, however, recent studies revealed that myelin also plays an important role in various brain functions such as memory consolidation and recall. On the other hand, other studies indicated that gut microbiota participated in the myelin formation of the central nervous system, however, the molecular mechanisms of how gut microbiota mediate the myelin formation and affect the brain function remain unclear. We previously reported that mice depleted L-amino acid oxidase 1 gene (*LAO1* KO mice) had altered gut microbiota composition during infancy and also displayed the differences in myelin protein and myelin-related gene expression in the hippocampus. In the present study, we aimed to elucidate the candidate connection between the gut microbiota and hippocampal myelin formation using *LAO1* KO mice together with germ-free (GF) mice. Firstly, we conducted fecal transplantation to GF mice using cecal feces collected from the WT or *LAO1* KO pups and found that the expression of the myelin-related gene, such as *Plp1*, was decreased in GF mice inoculated with feces from *LAO1* KO pups. Secondly, to explore a key molecule that could connect the gut microbiota and the myelin-related gene expression, we profiled the metabolite in feces and serum from the inoculated GF mice, and in serum from the WT or *LAO1* KO pups. This profiling revealed that D-Glucaric acid was commonly presented in serum and feces from GF mice inoculated with feces from *LAO1* KO pups and in serum from the *LAO1* KO pups. In addition, PICRUSt analysis using microbiome metagenomic data also showed that D-Glucaric acid metabolism was regulated by the gut microbiota. To confirm the effect of D-Glucaric acid on the myelin-related gene expression in the pup's hippocampus, 100 mg/kg/day D-Glucaric acid was orally administrated on the WT pups from postnatal day 1 to day 10. As a result, administration of D-Glucaric acid induced decreasing expression of *Mbp* and *Plp1* in the hippocampus compared to the control mice. Finally, to investigate whether D-Glucaric acid affects the differentiation of Oligodendrocyte precursor cells (OPCs), we isolated OPCs from mice hippocampus and stimulated the differentiation with or without D-Glucaric acid. It resulted that D-Glucaric acid treatment decreased the level of *Mbp* and *Plp1* expression, suggesting that D-Glucaric acid could suppress the differentiation of OPCs *in vitro*. In conclusion, our results firstly report that D-Glucaric acid produced by the gut microbiota might directly act on the differentiation of oligodendrocytes lineage cells by suppressing the myelin-related gene expression during mouse infancy.

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Poster

193. Oligodendrocyte Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 193.06

Topic: B.09. Glial Mechanisms

Support: NIH F31 NS124282-01
NIH T32 GM007250 MSTP
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Title: Pervasive environmental chemicals impair oligodendrocyte development

Authors: *E. COHN, B. L. L. CLAYTON, M. MADHAVAN, S. YACOUB, P. J. TESAR; Case Western Reserve Univ., Cleveland, OH

Abstract: Prenatal and childhood exposure to environmental chemicals can impair neurodevelopment and contribute to adverse cognitive and neurological outcomes. Oligodendrocytes, which play an essential role in the central nervous system to support and myelinate neurons, are at unique risk for chemical exposure because their maturation spans from fetal development to adulthood. Here we performed a cellular phenotypic screen of ~1850 environmental chemicals and identified two significantly enriched chemical classes, consisting of eight compounds in total, that impair oligodendrocyte development through distinct cellular mechanisms. Quaternary ammonium and phosphonium compounds, commonly found in disinfecting agents and personal care products, were potently toxic to developing oligodendrocytes, whereas organophosphate flame retardants, which decrease flammability of building materials and consumer products, prematurely arrested oligodendrocyte maturation. The detrimental effects of these chemical classes also extended to a three-dimensional organoid model of human prenatal brain development. Finally, using epidemiological data we identified associations between childhood organophosphate flame retardant exposure and increased odds of cognitive and motor dysfunction. Critically, this work identifies novel toxicological liabilities specific to oligodendrocyte development and highlights two chemical classes with significant environmental contamination that warrant deeper scrutiny for their impact on human health.

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Poster

193. Oligodendrocyte Development and Function

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Program #/Poster #: 193.07

Topic: B.09. Glial Mechanisms

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Title: Regulation of oligodendrocyte process extension and myelination by the lysosomal cation channel TRPML1

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[1]Univ. of Pennsylvania, Univ. of Pennsylvania, Philadelphia, PA; [2]Children's Hosp Philadelphia, Children's Hosp Philadelphia, Philadelphia, PA

Abstract: Ca^{2+} signaling is a dynamic second messenger system that links extrinsic signals to complex intracellular changes. Within the oligodendrocyte (OL) lineage, Ca^{2+} signaling has been shown to be indispensable for oligodendrocyte precursor cell (OPC) proliferation, OL differentiation, and myelination. While the focus on Ca^{2+} signaling in OPCs and OLs has been on plasma membrane channels and receptors, the role of intracellular Ca^{2+} stores, such as the lysosome, needs to be considered. Emerging evidence has demonstrated the lysosome's essential role in nutrient sensing, control of energy metabolism, and plasma membrane repair, all of which are dependent on lysosomal Ca^{2+} efflux. The main Ca^{2+} exporter on the lysosomal membrane is TRPML1; mutations in this protein lead to the rare disease mucolipidosis type IV, which is characterized by pronounced hypomyelination and delayed OL maturation. Further highlighting the potential importance of TRPML1 on OL function, reduction of its endogenous agonist, PI(3,5)P2, significantly impairs OL differentiation and myelination. Based on this, we sought to examine the mechanism underlying the effects of TRPML1 on OL function, including maturation and myelination, utilizing two different in vitro paradigms. For differentiation, OPCs were treated at the time of differentiation with either MLSA1 or MLSI1, synthetic agonist and antagonist of TRPML1 respectively, and stained for markers of OPCs, immature OLs, and mature OLs. While no alterations were observed in the number of mature OLs, cells treated with MLSI1 had significantly shorter and fewer processes compared to vehicle and MLSA1-treated cells. Additionally, when nanofiber scaffolds were used to access in vitro myelination, significant decreases in mature OL number, as well as the length and number of myelinating processes, were seen in MLSI1-treated cultures. Intriguingly, when cells were allowed to differentiate and myelinate for 72 hours prior to MLSI1 treatment, no changes were observed in length or number of processes. Together, these data suggest a role for TRPML1 activation, and subsequently lysosomal Ca^{2+} efflux, in remodeling of the actin cytoskeleton during differentiation and the early stages of myelination. Ongoing studies in the lab are currently investigating the temporal dynamics of lysosomal Ca^{2+} efflux and its relationship to actin polymerization and depolymerization. Taken together, our work highlights a previously unknown function of TRPML1 in modulating OL function and has implications not only for homeostatic regulation but also disease states where lysosomal function is known to be dysregulated

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Poster

193. Oligodendrocyte Development and Function

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Title: Role of PAK1 in promoting oligodendrocyte precursor cell proliferation

Authors: *Y. WANG[1,2], B. KIM[1,2], V. DOAN[1,2], F. GUO[1,2];
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Abstract: Oligodendrocyte precursor cells (OPCs) are the main proliferative cells in the developing and mature central nervous system (CNS). OPCs give rise to oligodendrocytes that generate myelin, which ensures fast signal transmission and provides physical, trophic, and metabolic for neurons. p21-activated kinase 1 (PAK1) is a serine/threonine kinase known to be activated by the Rho family small GTPases and to control cell polarity, invasion and actin cytoskeletal organization. Clinical studies have demonstrated that children with PAK1 gain-of-function mutations display a very broad range of abnormalities, such as developmental delay, macrocephaly, seizures, and intellectual disability. Some of the affected brains display white matter hyperintensity, which is suggestive of white matter hypomyelination. These clinical data suggest that PAK1 may influence OPC development. In this study, we investigated the role of PAK1 in OPCs proliferation during mouse brain development. Group I PAK activity inhibition by specific inhibitors significantly decreased OPC proliferation in vivo and in vitro, without causing OPC apoptosis or death. We next employed constitutive knockout (KO) and Cre-loxP-based conditional KO (cKO) to elucidate PAK1's role in OPC proliferation. The number of proliferative OPCs was significantly decreased in the corpus callosum of postnatal day 7 PAK1 KO or cKO mice. Together, PAK1 and its activity promote OPC proliferation in vivo and in vitro. Mechanistically, the role of PAK1 in OPC proliferation is partially through binding and regulating PDGFRα and its activity.

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Poster

193. Oligodendrocyte Development and Function

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Topic: B.09. Glial Mechanisms

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NIH/NIA 2RF1AG043640-06

Title: Epigenetic regulation changes in oligodendrocyte differentiation in a monkey model of aging

Authors: *C. DIMOVASILI, A. FAIR, I. GARZA, T. MOORE, D. ROSENE;
Boston Univ. Sch. Med., Boston, MA

Abstract: Normal cognitive aging is free of neuronal death, but shows cognitive deficits strongly associated with myelin loss in the monkey brain. This myelin damage is multifactorial, with previous studies showing that aging compromises the differentiation potency of oligodendrocyte precursor cells (OPCs), creating a bottleneck to remyelination. In addition, recent findings have revealed that epigenetic factors are key players in fine tuning gene expression during remyelination. Here we screened an array of epigenetic regulators that are known to affect OPC differentiation, to determine which of those change with aging in the Rhesus monkey brain.
For this, we used fresh frozen white matter from 3 young (<10 years) and 5 old (>20 years) cognitively characterized male rhesus monkeys and performed quantitative PCR analysis using primers against epigenetic regulators. To verify whether the changes observed were specific to oligodendroglia, we applied *in situ* hybridization on a similar cohort of male and female, young and old monkeys, using fixed frozen tissue cut in 30μm slices. Results were analyzed relative to age and our Cognitive Impairment Index (CII).
We found that the expression of DNA methyltransferase DNMT1 is reduced in aging OPCs, potentially allowing for the aberrant activation of genes that are normally suppressed. This decrease correlates with an overall decline in cognitive function. Together with our previous results showing decreased OPC differentiation potency in aging, we hypothesize that increased epigenetic gene activation in aging might trap OPCs in the progenitor state, preventing their differentiation in to mature myelinating oligodendroglia.
These results collectively support the hypothesis that with aging, OPCs fail to differentiate into new mature oligodendrocytes, likely impairing myelin maintenance in the aging brain. Interventions to stimulate OPC differentiation and promote remyelination could potentially reduce or reverse the accumulation of age-related myelin and damage associated cognitive decline.

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Poster

193. Oligodendrocyte Development and Function

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Topic: B.09. Glial Mechanisms

Support: National Multiple Sclerosis Society RG 1807-32053

Title: Influenza infection suppresses myelin transcripts in the cortex during remyelination in the cuprizone mouse model.

Authors: *A. Y. LOUIE[1], J. DRNEVICH[2], A. J. STEELMAN[3];
[1]Neurosci. Program, [2]Roy J. Carver Biotech. Ctr., [3]Animal Sci., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: A hallmark feature of multiple sclerosis (MS) is the partial but incomplete regeneration of myelin following inflammatory demyelination. Incomplete remyelination is attributed, in part, to the failure of oligodendrocyte progenitor cells to differentiate into mature, myelinating oligodendrocytes within the inflammatory microenvironment of MS lesions. The effect of peripheral viral infection, which induces inflammatory glial profiles, on the process of remyelination has yet to be described. We previously found that influenza infection downregulated genes associated with myelination in an otherwise healthy adult mouse. Here, we hypothesized that infection would induce alterations to oligodendrocyte-specific transcripts during remyelination. C57BL/6J mice were fed 0.2% cuprizone-laced diet for 5 weeks and inoculated at 4.5 weeks with saline or influenza virus (A/PR8/34). Brains were harvested, bisected, and immediately frozen at time points corresponding to peak demyelination and partial remyelination. Sagittal sections were placed on slides containing spatially-barcoded oligonucleotide probes in a 6.5 x 6.5 mm area. Tissues were fixed and permeabilized on the slide, and cDNA libraries were synthesized from captured RNA, resulting in spatially-resolved brain transcriptomes of mice from four conditions (n=3): “Normal” (control diet), “Demyelinated” (peak demyelination), “Saline” (PBS-inoculated, partial remyelination), and “Flu” (influenza-inoculated, partial remyelination). Analysis resulted in eight clusters corresponding to specific brain regions of which we have identified hypothalamus, cortex, corpus callosum/fornix, thalamus, hippocampus, habenula, and choroid plexus. Within the cortex, differentially expressed genes ($q < 0.05$) associated with the ontology term “immune system process” were increased in Demyelinated mice compared to Normal mice, whereas genes involved in “myelination” were decreased. Genes associated with the terms “myelination” and “axon development” were increased in the cortex of Saline mice undergoing remyelination compared to the Demyelinated group. However, genes involved in “fatty acid biosynthesis”, “lipid metabolic process” and “myelination” were decreased in the cortex of Flu mice compared to that of Saline mice during remyelination. These data confirm our previous findings that influenza infection affects oligodendrocyte homeostasis and suggest that in the cuprizone mouse model of MS, infection impedes remyelination at the transcriptional level in the cortex.

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Poster

193. Oligodendrocyte Development and Function

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Topic: B.09. Glial Mechanisms

Support: ARSEP
Fondation Jérôme Lejeune

NEURATRIS
Sorbonne University/Paris Brain Institute-ICM

Title: Pak1 inactivation triggers myelin formation through actin disassembly in oligodendrocytes

Authors: L. BAUDOUIN[1], N. ADÈS[1], K. KANTÉ[1], C. BACHELIN[1], K. DUARTE[2], J.-V. BARNIER[2], B. NAIT OUMESMAR[1], ***L. BOUSLAMA-OUEGHLANI**[1];
[1]Sorbonne University/Paris Brain Institute-ICM, Paris, France; [2]CNRS Université Paris-Sud, Paris, France

Abstract: Myelin is a multilayered membrane formed by oligodendrocytes (OLs) in the central nervous system (CNS). It is essential for increasing the speed of action potentials and for the metabolic support of axons. Its alteration leads to disabling diseases such as Multiple sclerosis. The formation of myelin relies on actin dynamics; actin polymerization supports the ensheathing phase, when OL processes contact and surround the axon. A dramatic change in actin depolymerization is then required to allow the wrapping phase and the formation of multilayered myelin sheaths. The molecular mechanisms that trigger this actin depolymerization in OLs are still unknown. P21-activated kinase 1 (PAK1), a downstream effector of the Rho GTPases Rac1 and Cdc42, regulates actin disassembly through its kinase activity. We showed that PAK1 expression increases during OL maturation while its phosphorylated form decreases, indicating its inactivation. We also provided compelling evidence demonstrating that strengthening PAK1 inhibition in OLs increases actin depolymerization and the subsequent formation of pseudo-myelin membranes. Conversely, preventing PAK1 inhibition decreases actin depolymerization in OLs and restrains the generation of pseudo-myelin. Interestingly, we showed that PAK1 loss-of-function in OLs increases myelin thickness *in vivo* and that pharmacological inhibition of PAK1 in cerebellar slices enhances myelin formation. Overall, our data highlight PAK1 as a key regulator of CNS myelination through the modulation of actin cytoskeleton disassembly in OLs. Our results may pave the way for therapeutic approaches in myelin diseases by targeting PAK1.

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Poster

193. Oligodendrocyte Development and Function

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Support: K01NS126813 NINDS-NIH
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Title: Abnormal mature myelin impacts learning in Costello Syndrome model

Authors: *D. CRUZ, D. HERNANDEZ, R. GARZA, C. REGALADO, P. DE LEON, S. LOPEZ, L. MANOJ, T. ALVAREZ, A. LOPEZ-JUAREZ;
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Abstract: ABSTRACT
Rationale and objective: Increasing evidence indicates that myelin regulates high order brain functions including learning and that defective or insufficient myelin contributes to the onset of neurological conditions including Intellectual Disability (ID), Autism Spectrum Disorders (ASD), and Attention Deficit and Hyperactivity Disorder (ADHD). Per myelin's extensive and prolonged plasticity, it is believed that restoring or improving myelin function holds therapeutic potential beyond neurodevelopmental phases of disease. Here, we explore the links between abnormal myelin and behavior in a mouse model of Costello Syndrome (CS), as CS patients present with abnormal white matter and myelin in correlation with neurological issues including ID, ASD and ADHD.**Methods and Results:** Adult mice carrying a mutation known to cause CS (*HRasG12V*) conditionally induced in myelin-producing cells (*PlpCreER;frHRasG12V*), were subjected to a myelin-regulated voluntary motor learning test, the Complex Wheel (CW; a running wheel with unevenly spaced rungs). Our results show that subnormal learning curves in CS mice, in a mutant gene-dose-dependent manner. Specifically, the normal daily improvement in the average speed and total distance run is affected in CS mice. This phenotype is mediated by decreases in maximum speed, without affecting the levels of activity, as compared with wild type mice. Our results establish that the disease-causing mutation *HRasG12V* in myelinating cells compromises learning of motor skills and suggest chronic roles of abnormal myelin in the neuropathophysiology of CS.**Scientific rigor measures:** To avoid an abnormal genetic environment precluding key CS features, the strain *FR-HRas*^{G12V} was used to switch the endogenous wild type (WT) *HRas* gene for a *HRasG12V* mutant version, following an induction treatment with tamoxifen. Adult WT, hemizygous, and homozygous *HRasG12V* mutant mice (n=7/genotype) were subjected to two CW protocols. Fully automated recording system was used to avoid observer bias and data was processed by personnel blinded to the genotypes.**Conclusions:** Together with our previous data on the roles of *HRasG12V* in hyperactivating the RAS/MAPK pathway and causing myelin decompaction, our data unveils that maintenance of mature myelin structure/function plays important roles in regulating brain function and suggest chronic roles of defective myelin in the neuropathophysiology of Costello Syndrome.

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Poster

193. Oligodendrocyte Development and Function

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Title: Characterization Of Oligodendroglial Lineage Cells In 3D Cortical Microtissues

Authors: *A. DEL TORO[1], D. HOFFMAN-KIM[1], S. R. MAYORAL[2];
[1]Dept. of Neurosci., [2]Neurosci., Brown Univ., Providence, RI

Abstract: Oligodendrocytes take part in one of the most intimate cell-cell interactions within the brain, which is the wrapping of myelin around neuronal axons. Myelination is a critical process that allows oligodendrocytes to produce an insulting lipid-rich sheath to facilitate supportive brain functions such as electrical impulse propagation, metabolic support, and neuroprotection. Demyelination can lead to neurodegenerative diseases like Multiple Sclerosis (MS), where the immune system attacks oligodendrocytes and causes inflammation and damage to the myelin sheath and surrounding nerve fibers. Oligodendrocytes (OLs) arise from self-renewing oligodendrocyte progenitor cells (OPCs). Currently, there is a lack of understanding on the processes of proliferation, differentiation, and myelination from oligodendroglial lineage cells within 3-Dimensional (3D) cell culture systems. Here, we demonstrate the presence of oligodendrocyte progenitor cells, mature oligodendrocytes, and myelinating oligodendrocytes in our 3D cortical spheroid microtissue. Spheroids were generated from primary postnatal rat cortical cells, seeded at 4000 cells/well density in a 2% agarose hydrogel, and cultured for up to 35 Days In Vitro (DIV). Immunohistochemistry, confocal microscopy, and transmission electron microscopy (TEM) were methods employed to understand the state of oligodendroglial lineage cells. The molecular markers neural-glial antigen 2 (NG2), adenomatous polyposis coli clone (CC1), oligodendrocyte transcription factor 2 (OLIG2), and myelin basic protein (MBP) were used to assess lineage specific cell states. Our results indicate that OPCs (NG2+ cells) are distributed throughout the microtissue, exhibit similar morphology compared to cortical tissue, and are present up to DIV 35. CC1+ cells were co-labeled with Olig2, a transcription factor required for the differentiation of oligodendroglial cells, from DIV 3 to DIV 21 to suggest that mature OLs are derived from the oligodendroglial lineage. MBP-expressing OLs was surprisingly low in DIV 16 and DIV 20 yet exhibited unique morphology compared 2-dimensional MBP+ OLs. Our TEM analysis confirmed myelin can begin to form as early as DIV 3 and can persist up to DIV 21. Taken together, our 3D cortical microtissues contain populations of cells within the oligodendroglial lineage and myelin formation is permissible in a 3D microenvironment. One of our future goals will be to investigate neuron-oligodendrocyte interactions and incorporate drug screening for therapeutics that promote remyelination and assess the impacts of myelin following a neural injury. Funding: NIEHS U01ES028184, US ONR N000142112044.

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Poster

193. Oligodendrocyte Development and Function

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Support: Penn State Clinical and Translational Science Institute Translational Research Training Award (TL1)

Title: The impacts of HFE H63D and iron overload on glial cells

Authors: *M. NOLT[1], E. NEELY[2], J. R. CONNOR[2];
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Abstract: Iron homeostasis is an integral component of a healthy central nervous system (CNS). Iron is a cofactor for proper myelin formation which makes oligodendrocytes (OLs) one of the highest iron-containing cells in the CNS. Additionally, the mammalian target of rapamycin (mTOR) pathway is integral for proper myelin formation since it plays a role in iron homeostasis. Further, astrocytes, a support glial cell in the CNS, tightly regulates how iron is distributed through the CNS. These factors have led our lab to study OLs and astrocytes in relation to mTOR and a polymorphism in the homeostatic iron regulatory gene, *HFE* H63D, which causes iron overload. We have previously published data showing *HFE* H63D cognitively normal patients and *HFE* H67D (ortholog to H63D) mice have reduced white matter in major association areas such as the arcuate fasciculus, longitudinal fasciculus, and corpus callosum. To better understand how OLs and astrocytes may be affected by *HFE* H67D, we utilized primary cell culture to isolate H67D and wild type (WT) OLs and astrocytes then performed immunocytochemistry (ICC), western blot, scratch wound migration assay, and Alamar blue cell viability assays. We hypothesized that H67D OLs and astrocytes would have impaired maturation, migration, and viability compared to WT. Some data we have collected has shown primary enriched H67D OLs cultures had a statistically significant reduction in myelin basic protein. Furthermore, analysis of GFAP, a cytoskeletal protein in astrocytes, had shown H67D had a statistically significant increase of GFAP over time from 3 days in vitro (DIV) to 14DIV. These data points indicate that H67D primary OLs and astrocytes have altered maturation patterns.

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Poster

193. Oligodendrocyte Development and Function

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Title: Maladaptive myelination promotes generalized epilepsy progression

Authors: *J. K. KNOWLES[1], H. XU[2], G. CHAU[3], T. SAUCEDO[2], A. BATRA[2], C. SOANE[2], L. T. TAM[2], D. FRAGA[2], L. NI[2], J. MCNAB[3], J. R. HUGUENARD[4], M. MONJE[4]; [1]Neurol., Lucile Packard Children's Hosp. / Stanford Chil, Palo Alto, CA; [2]Neurol., [3]Radiology, Stanford Univ. Sch. of Med., Palo Alto, CA; [4]Neurol., Stanford Univ., Stanford, CA

Abstract: Activity-dependent myelination modulates neural network function, enabling adaptation. Myelin plasticity involves oligodendrogenesis (proliferation and maturation of oligodendrocyte precursor cells, OPCs), requiring brain-derived neurotrophic factor (BDNF) signaling through its receptor, TrkB, on OPCs as well as epigenetic changes that can be blocked with histone deacetylase inhibitors. The relationship between pathological neuronal activity (seizures) and myelination is unknown. We hypothesized that generalized absence seizures might induce aberrant activity-dependent myelination that contributes to pathological network change and epilepsy progression. We used Scn8a+/mut mice (bearing a loss of function mutation in Nav1.6), which develop absence seizures during well-defined periods of seizure progression (increasing seizure frequency). We used unbiased stereology, electron microscopy and MRI (quantitative magnetization transfer with diffusion imaging) to assess oligodendrogenesis and myelin structure within the thalamocortical seizure network. We generated Scn8a+/mut mice with deletion of TrkB specifically from OPCs (Scn8a+/mut; TrkBfl/fl; PDGFRA::Cre-ER mice, designated as Scn8a+/mut OPC cKO), induced with tamoxifen. In separate studies, Scn8a+/mut mice were treated with the histone deacetylase inhibitor trichostatin A (TSA), 10mg/kg daily. Seizures were quantified with EEG. Callosal OPC proliferation was increased in association with seizures in P45 Scn8a+/mut mice; these changes were absent at earlier time-points before seizure onset. Decreased callosal g-ratios, indicative of thicker myelin sheaths per axon diameter were observed in Scn8a+/mut mice with established seizures. Genetic blockade of activity-dependent myelination in Scn8a+/mut cKO mice prevented aberrant myelination, thalamocortical hypersynchrony assessed with EEG coherence, and significantly decreased seizure progression. Pharmacological blockade of activity-dependent myelination in Scn8a+/mut mice with TSA, initiated after seizure onset at P28, prevented aberrantly increased oligodendrogenesis and decreased subsequent seizure burden. Myelination induced by absence seizures can become maladaptive and contribute to epilepsy progression. Maladaptive myelin plasticity may be a novel therapeutic target to decrease seizure burden in some types of epilepsy.

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Poster

193. Oligodendrocyte Development and Function

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Title: Myelin phagocytosis by oligodendroglia promotes lineage progression and maturation

Authors: C. PEIRÓ, J. CHARA, M. DOMERCQ, ***C. MATUTE**;
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Abstract: Oligodendrocytes (OLs) are the myelinating cells of the CNS. In addition to building up the myelin sheath, OLs provide energy substrates to axons to support action potential propagation. Here we tested the idea of whether OLs have phagocytic capacity and may recycle myelin as an energy source using Alexa488-labelled myelin and time-lapse imaging. We observed that cultured OL's readily endocytose myelin particles that are thereafter transported into the cytoplasm, an effect that it is enhanced by selective activation of P2X7 and NMDA receptors. Surprisingly, the addition of myelin induced an increase in the total number of MBP^+ OLs and $NG2^+$ oligodendrocyte progenitor cells. In turn, stereotaxic injections of labelled myelin into the cerebral cortex of adult mice resulted in a robust proliferative response within and around the injection site with $Iba1^+$ microglial cells migrating towards injected myelin. Our preliminary in vivo data show fluorescent myelin debris within phagocytic microglia and other neighboring cells including oligodendrocytes. We conclude that OLs have phagocytic capacity and that myelin behaves as an energy reservoir that can fuel oligodendrocyte proliferation, differentiation and survival.Funded by MINECO, CIBERNED and Gobierno Vasco. C.P. holds a fellowship from MINECO.

Disclosures: **C. Peiró:** None. **J. Chara:** None. **M. Domercq:** None. **C. Matute:** None.

Poster

193. Oligodendrocyte Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 193.17

Topic: B.09. Glial Mechanisms

Support: NINDS grant R01NS104021
NYSTEM Stem Cells in Regenerative Medicine Fellowship #C302090G

Title: Oscillatory Store-Operated Calcium Signaling Regulates Human Oligodendrocyte Progenitor Stem Cell Fate

Authors: *R. SEIDMAN[1], J. J. POLANCO[2], J. E. BROOME[1], F. J. SIM[2];
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Abstract: In multiple sclerosis (MS), destruction of myelin sheaths and insufficient repair contributes to axonal degeneration and neurological dysfunction. Endogenous remyelination requires the differentiation of adult oligodendrocyte progenitor cells (OPCs). As such, pharmacologic interventions for MS requires an understanding of molecular regulators of human OPC (hOPC) maturation. We have previously shown that the $G\alpha_q$ muscarinic M_3 receptor (M_3R) inhibits hOPC differentiation, and conditional deletion of M_3R in OPCs promotes remyelination. We hypothesized that intracellular Ca^{2+} signals ($[Ca^{2+}]_i$) downstream $G\alpha_q$-coupled-receptor (G_qR) signaling regulates hOPC cell fate. We exposed hOPCs to a panel of selective ligands that target G_qRs identified by RNA-seq. Only muscarinic agonist oxotremorine (Oxo-M) and the metabotropic glutamate receptor 5 ($mGluR_5$) agonist CHPG induced prolonged oscillatory $[Ca^{2+}]_i$ in hOPCs and blocked hOPC differentiation while another G_qR agonist had no effect. Oscillatory $G\alpha_q$-$[Ca^{2+}]_i$ was also dependent on store operated calcium entry (SOCE) and was reduced following treatment with SOCE antagonists and siRNA-mediated knockdown (KD) of either STIM1 or STIM2. Interestingly, STIM2 KD reduced $[Ca^{2+}]_i$ oscillatory frequency and abolished effects of muscarinic agonist on differentiation. To further examine the role of oscillatory SOCE, we used optogenetic STIM lentivirus to directly induce SOCE by blue light stimulation at various frequencies. Following long-term live cell imaging, we observed a frequency-dependent effect of SOCE activation resulting in blockade of hOPC differentiation and stimulation of proliferation. Lastly, in our preliminary *in vivo* studies using conditional deletion of SOCE components *Stim1/2* in $NG2^+$ OPCs, we found *Stim1/2* ablation increases oligodendrocyte (OL) generation following demyelination. These findings afford insights into mechanisms that regulate hOPC proliferation and differentiation, suggesting a key role for SOCE in the regulation of OL cell fate.

Disclosures: R. Seidman: None. **J.J. Polanco:** None. **J.E. Broome:** None. **F.J. Sim:** None.

Poster

193. Oligodendrocyte Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 193.18

Title: WITHDRAWN

Poster

193. Oligodendrocyte Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 193.19

Topic: B.09. Glial Mechanisms

Support: Department of Defense grant W81XWH-18-1-0525
NIH Grant R25 NS 80686

Title: The role of mechanical memory on oligodendrocyte progenitor cells differentiation

Authors: *Z. WANG[1], A. TABASSUM[1], A. ALCANTARA[2], C. V. MELENDEZ-VASQUEZ[2]; [1]Dept. of Biol. Sciences, Hunter College, City Univ. of New York, New York, NY; [2]Mol. Cell and Developmental Biology, Biochem. and Behavioral and Cognitive Neuroscience, The Grad. Center, City Univ. of New York, New York, NY

Abstract: Research in mechanically-driven differentiation of mesenchymal stem cells (MSC) have shown that the subcellular localization of YAP/TAZ changes in response to matrix stiffness. Thus, prolonged exposure of MSC to either soft or stiff matrices, leads to the acquisition of a mechanical memory. YAP/TAZ subcellular localization in MSC is correlated with this epigenetic memory and their final cell fate. Specifically in stiffer substrates YAP/TAZ is retained in the nucleus and cells become osteocytes, while YAP/TAZ is translocated out of the nucleus in softer substrates and MSCs become adipocytes.Oligodendrocyte progenitor cells (OPC) are essential for myelination and remyelination of axons. Previous research found that YAP/TAZ are necessary for normal OPC differentiation into mature oligodendrocytes (OL) in response to mechanical cues. Our lab has also shown that increased extracellular matrix (ECM) stiffness such as that found in chronic demyelinated lesions inhibits OPC differentiation.To investigate if OPC acquire a mechanical memory in response to changes in ECM stiffness, we have examined the subcellular localization of YAP/TAZ in primary cultures of rodent and iPSC-derived human OPC grown in conditions that mimic healthy or chronically-demyelinated brains. Initial data obtained from myelinating co-cultures of rat OPC and DRG neurons indicate that at high stiffness conditions (30 kPa), there is less MBP expression and YAP is mainly localized in the nucleus of OPC. By contrast, cultures grown in soft hydrogels, mimicking a healthy brain (1.5 kPa); exhibit higher MBP expression and translocation of YAP outside the nucleus. Collectively, our findings suggest that nuclear retention of YAP by OPC in stiffer matrices can contribute to remyelination failure in chronically demyelinated lesions.

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Poster

193. Oligodendrocyte Development and Function

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 193.20

Topic: B.09. Glial Mechanisms

Support: Academy of Finland, Grant 316282

Title: An in vitro stress model of primary oligodendrocytes from anxious and non-anxious inbred mouse strains

Authors: *A. GIGLIOTTA[1], J. MINGARDI[2], S. CUMMINGS[3], R. KOTHARY[3], I. HOVATTA[1];
[1]SleepWell Res. Program and Dept. of Psychology and Logopedics, Fac. of Med., Univ. of Helsinki, Helsinki, Finland; [2]Sch. of Med. and Surgery, Univ. of Milano-Bicocca, Monza, Italy; [3]Regenerative Med. Program, Ottawa Hosp. Res. Inst. and Univ. of Ottawa, Ottawa, Ottawa, ON, Canada

Abstract: Anxiety disorders are prevalent psychiatric disorders that greatly affect life quality. Predisposition to anxiety disorders involves a combination of genetic and environmental factors, such as psychosocial stress. Our group recently identified myelin plasticity as a major brain stress response using a mouse model of chronic social defeat stress (CSDS). Differences in myelin thickness were brain-region dependent, influenced by the genetic background of the mice, and varied between animals that developed social avoidance after CSDS (susceptible) and mice that did not (resilient). To investigate the cellular and molecular effects of stress on oligodendrocytes (OLs), we have set up an in vitro stress model of primary OLs from both C57BL/6NCrl (B6) and DBA/2NCrl (D2) mouse strains. Immunohistochemical characterization after acute corticosterone exposure led to a 45% increase in myelin production by OLs isolated from D2, but not from B6 mice. Chronic exposure to the glucocorticoid dexamethasone reduced OL maturation, OPC proliferation, and myelin production in OLs isolated from the two strains but these effects were observed at different time points. Taken together, our results reveal a strain-specific OL sensitivity to glucocorticoids, which depends on the cellular maturation stage. This may be one mechanism underlying the strain differences observed in stress-induced myelin plasticity.

Disclosures: A. Gigliotta: None. **J. Mingardi:** None. **S. Cummings:** None. **R. Kothary:** None. **I. Hovatta:** None.

Poster

193. Oligodendrocyte Development and Function

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Program #/Poster #: 193.21

Topic: B.09. Glial Mechanisms

Support: Research Grant Council ECS25104220
Food and Health Bureau HMRF06173836
PolyU Start-up Fund P0030307

Title: Bridging integrator 1 (BIN1)-mediated cell cycle regulation - bridging ATM and Myc functions in oligodendrocyte

Authors: I. MA[1], S. YEUNG[1], G. CHENG[1], K. MOK[1], K. HERRUP[2], **K.-H. TSE**[1]; [1]Dept. of Hlth. Technol. and Informatics, Hong Kong Polytechnic Univ., Hung Hom, Hong Kong; [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Bridging investigator 1 (*BIN1*) is the second most important genetic risk loci for sporadic Alzheimer's disease identified by the genome-wide association studies. Despite the reported linkage with misfolded proteins aggregations, BIN1 is unexpectedly enriched in the oligodendrocyte (OL) population, but its OL-specific functions remain unknown. Given its well-established roles on cell cycle control by its interaction with ATM kinase and c-myc in cancer cells, we propose that BIN1 may serve as the bridge between DNA damage repair and cell cycle control in the OLs. To investigate, we first confirmed the cell type specificity of BIN1 expression in the postmortem human frontal cortex of multiple sclerosis (MS, n =11) and in a cuprizone-based MS mouse model (n = 4). To test our hypothesis, OL lineage in two strains of ATM-deficient mice, *Atm*$^{Awb-/-}$ (B6.129S6-Atm^{tm1Awb}/J) and *Atm*$^{Bal-/-}$ (B6;129S4-Atm^{tm1Bal}/J) with distinct ATM mutation sites were examined *in vivo* and *in vitro*. First, we confirmed that BIN1-immunoreactivity is OL-specific. Importantly, BIN1 signal is lost in defined myelin lesion of the gray and white matter. This finding was recapitulated in cuprizone-treated mice where the Bin1 signal was significantly reduced across brain regions, particularly at the cingulum and somatosensory cortex ($P = 0.0011$). In the genu of wildtype mice (n = 6), the density of Olig2$^+$ OL and the Bin1$^+$ Olig2$^+$ population gradually increase from 1 to 4 months old. In the same period, OL density remained steady, but the Bin1$^+$ Olig2$^+$ population sharply decreased from 43.9 to 15.1% and 32.7 to 5.1% in *Atm*$^{Awb-/-}$ and *Atm*$^{Bal-/-}$ (n = 4), respectively. This reduction was correlated with a significant reduced density of Nkx 2.2$^+$ preOL and ASPA$^+$ CC1$^+$ mature OL in the *Atm*$^{Bal-/-}$, but not *Atm*$^{Awb-/-}$ animals, suggesting a differential effect of ATM mutation types on Bin1 expression and OL differentiation. Furthermore, transient *Bin1* silencing inhibited the *Mag* and *Myrf* mRNA expression during differentiation of a OL progenitor cell model, Oli-Neu. The ATM-BIN1-Myc signaling axis in regulating OL cell cycle with or without chemically induced DNA damage is being investigated on primary OL culture derived drom *Atm*-deficient models, and the effects of ATM activity on the interactions between BIN1-Myc is being revealed by immunopreciptations. The preliminary findings support our hypothesis that ATM-BIN1-Myc signalling axis may be intrinsically involved in the differentiation program of OL, and this study will idenfity if this axis is associated with etopic cell cycle re-entry in mature OL.

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Poster

193. Oligodendrocyte Development and Function

Location: SDCC Halls B-H

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Program #/Poster #: 193.22

Topic: B.09. Glial Mechanisms

Support: Australian Research Council Special Research Initiative
CASS Foundation
Australian Research Council Future Fellowship
Metal Manufactures Ltd

Title: High Efficiency Pharmacogenetic Ablation of Oligodendrocyte Progenitor Cells in the Adult Mouse CNS

Authors: *Y. L. XING[1], B. H. A. CHUANG[1], J. POH[1], K. MORADI[1], S. MITEW[1], W. D. RICHARDSON[2], T. J. KILPATRICK[3], Y. OSANAI[1,4], T. D. MERSON[1];
[1]Australian Regenerative Med. Inst., Monash Univ., Clayton, Australia; [2]Wolfson Inst. for Biomed. Res., Univ. Col. London, London, United Kingdom; [3]Florey Inst. of Neurosci. and Mental Hlth., The Univ. of Melbourne, Parkville, Australia; [4]Dept. of Anat., Jichi Med. Univ., Tochigi, Japan

Abstract: Introduction: Adult oligodendrocyte progenitor cells (OPCs) have been studied principally for their role in generating new oligodendrocytes in the central nervous system (CNS). However recent studies have suggested that OPCs could play additional roles besides oligodendrogenesis. Particularly, the role of OPCs in the regulation of neuronal function remains unclear. Attempts to investigate the function of OPCs by targeted cell ablation in the adult CNS have been limited by methodological challenges resulting in only partial and transient OPC ablation.**Aim:** To overcome these limitations, we have developed a novel pharmacogenetic mouse model of conditional OPC ablation.**Methods:** Combining diphtheria toxin-mediated cell ablation with the intracisternal delivery of an antimitotic drug into *PdgfraCreER^{T2};Sox10-DTA* transgenic mice resulted in the elimination of 98.6% of all OPCs throughout the brain for up to 10 days.**Results:** This OPC ablation initiated a complex sequence of cellular responses characterised by inflammatory and neurovascular changes accompanied by the presentation of an anxiety-like phenotype. Restoration of normal behaviour after OPC loss coincided with the return of normal microglial densities and the onset of PdgfRα+ cell regeneration, which arose first in regions near the subventricular zone (V-SVZ). By combining recombinase-based transgenic and viral strategies for targeting of OPCs and V-SVZ-derived neural precursor cells (NPCs), we found that new PdgfRα-expressing cells from V-SVZ repopulated the OPC-deficient brain starting 12 days after OPC ablation. This indicates that OPC depletion induced V-SVZ-derived NPCs to generate new OPC-like cells with the capacity to migrate and proliferate extensively throughout the dorsal anterior forebrain.**Conclusion:** Further application of this novel OPC ablation approach will provide fundamental understanding of the functions that OPCs and NPCs serve in the adult CNS in health and disease.

Disclosures: Y.L. Xing: None. **B.H.A. Chuang:** None. **J. Poh:** None. **K. Moradi:** None. **S. Mitew:** None. **W.D. Richardson:** None. **T.J. Kilpatrick:** None. **Y. Osanai:** None. **T.D. Merson:** None.

Poster

193. Oligodendrocyte Development and Function

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Topic: B.09. Glial Mechanisms

Support: University of Birmingham Biomedical Sciences BSc bench fees
University of Birmingham iDTP bench fees
University of Birmingham COVID support fund

Title: Modelling oligodendrocyte myelination, plasticity and injury responses in slice cultures of neonatal mouse forebrain

Authors: N. BESTALL[1], F. BAINMAHFOUZ[2], L. DABBS[3], M. KIRKPATRICK[3], ***D. FULTON**[1];
[1]Inst. of Inflammation and Ageing, [2]Integrated PhD in Life Sci. Doctoral Training Programme, [3]Biomed. Sci. BSc, Univ. of Birmingham, Birmingham, United Kingdom

Abstract: Organotypic slice culture (OSC) models are a key tool for oligodendrocyte (OL) research. Information on OSC models preserving myelination of both cortical (Ct) and subcortical white matter (SCWM) areas are scarce. These structures are synonymous with higher CNS functions, and are loci for neurological and neurodevelopmental pathology, thus myelinating OSC featuring these areas offer opportunities to model OL functions in health and disease. Towards this we examined OL myelination, and injury and plasticity responses, in forebrain OSC prepared from postnatal PLP::dsRED mice of both sexes. Analysis involved qualitative (blind ranking) and quantitative (morphological) assessments of $dsRED^+$ mature OL, MBP staining of myelin, and qPCR for OL genes.
Under standard conditions (20% O_2, 41 mM glucose, 3.9 mM KCl) OSC exhibited myelination in both SCWM and Ct areas. MBP signals at 2, 7 and 15 days in vitro (DIV) revealed a pattern of myelination reminiscent of in vivo development, with anterior to posterior, and ventral to dorsal (SCWM > deep Ct > outer Ct) developmental gradients. Analysis between 3 and 7 DIV revealed increased MBP^+ segment lengths (8.1 vs 38.2 µm, $p<0.0001$, N = 10 cells / group), while levels of PDGFRα mRNA decreased ($p<0.001$, N=3 slices), and MOG mRNA increased ($p<0.0001$, N=3 slices), between 3 and 12 DIV, implying OL lineage progression and myelination.
OSC could be used to screen OL-protective drugs for perinatal conditions involving hypoxic-ischemia (HI). Thus, we explored the response of OL to HI (2% 0_2, 4 mM glucose, 24h). HI at 2 DIV reduced PDGFRα ($p<0.05$, N=3 slices) and MOG mRNA ($p<0.05$, N=3 slices) levels measured at 5 DIV suggesting a loss of OPC/OL. Similarly, analysis of dsRED signals at 7 DIV revealed a reduction in OL maturation ($X^2 = 12.81$, $p<0.001$) and a modest decrease in $dsRED^+$ segment lengths (35.5 vs 29.2 µm, $p<0.05$, N=10 to 13 cells / group). We continue to explore OL and microglial responses to HI to check relevance to in vivo perinatal OL injury.
OSC are suited to multi-site electrophysiology and live-imaging, thus they could be used to answer questions relating OL plasticity to circuit functions. Therefore, we explored OL responses following treatments designed to block (1µM TTX) or increase (4mM KCl) neural activity. TTX reduced PDGFRα ($p<0.05$, N=3 slices) and MOG ($p<0.0001$, N=3 slices) mRNA levels, and dsRED OL maturation ranking scores ($p<0.05$, N=4 to 5 slices), while MOG expression was elevated by treatment with KCl ($p<0.05$, N=3 slices). Our work continues to

explore the use of OSC as models for myelin plasticity and perinatal OL injury by expanding these data sets, and extending them with electrophysiological and live-imaging approaches.

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Poster

193. Oligodendrocyte Development and Function

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Program #/Poster #: 193.24

Topic: B.09. Glial Mechanisms

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Monash University Departmental Top up Stipend

Title: Voluntary exercise potentiates myelin repair in a mouse model of CNS demyelination

Authors: *J. POH[1], Y. L. XING[2], H. COLEMAN[3], H. PARKINGTON[3], T. D. MERSON[4]; [1]ARMI, Monash Univ., Clayton, Australia; [2]Australian Regenerative Med. Inst., Monash Univ., North Melbourne, Australia; [3]Monash Univ., Clayton, Australia; [4]Natl. Inst. of Mental Hlth., NIH, Natl. Inst. of Mental Hlth. (NIMH), Bethesda, MD

Abstract: Central nervous system demyelination is a hallmark of several neurological and neurodegenerative diseases, an archetype being multiple sclerosis (MS). Studies with MS subjects suggest that the efficiency of remyelination following a demyelinating episode is correlated with improved clinical prognosis. On this basis, it is believed that clinical interventions that potentiate remyelination efficiency are likely to significantly improve disease outcomes. In this study, we aimed to investigate whether a naturalistic behavior that increases the activity of specific neural pathways can drive remyelination by potentiating the generation of new myelin-forming oligodendrocytes. To investigate this question, we challenged mice for 5 weeks with cuprizone to induce demyelination of the corpus callosum and then asked whether voluntary running for 2 weeks on a complex wheel (CW) after cuprizone withdrawal could potentiate myelin repair by activating transcallosal axons. We also aimed to differentiate between the effects of CW running on myelin repair mediated by oligodendrocyte progenitor cells (OPCs) and neural progenitor cells (NPCs) that derive from the ventricular-subventricular zone (V-SVZ), two populations of progenitor cells that we and others have previously demonstrated are engaged in myelin repair. Our data reveal that compared to non-runners, CW runners exhibited improved motor performance evaluated on the rotarod test (157.2 ± 5.7s vs 230.2 ± 12.9s; $p<0.0001$). Runners also exhibited an increase in synchronous neuronal firing of transcallosal axons examined by acute brain slice multielectrode array recordings. *In vivo* fate-mapping of NPC- and OPC-derived oligodendroglia using *PdgfRαCreER^{T2};Rosa26-mTmG* and

NestinCreER^{T2};Rosa26-mTmG mice revealed that CW running enhanced oligodendrogenesis in the corpus callosum reflected by an increase in the density of newly-generated GFP$^+$/EdU$^+$/PdgfR$^+$ OPCs and GFP$^+$/EdU$^+$/ASPA$^+$ mature oligodendrocytes in the *NestinCreER^{T2};Rosa26-mTmG* mice. Immunoelectron microscopy using the Tokuyasu method demonstrated that compared to non-runners, the myelin ensheathing the callosal axons of CW runners was thicker (lower g-ratio), despite an increase in myelin compaction due to a significant increase in the number of myelin lamellae. Together these observations suggest that voluntary physical exercise following demyelination enhances functional remyelination, at least in part via a preferential increase in oligodendrogenesis mediated by NPCs originating in the V-SVZ.

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Poster

193. Oligodendrocyte Development and Function

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ARO W911NF-12-1-0594
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NIH/NIMH U01 MH114824
NIH/NINDS R01NS104926

Title: Evidence of physical interactions between oligodendrocyte precursor cells, microglia and oligodendrocytes - a volume electron microscopy serial section study

Authors: *J. BUCHANAN[1], L. ELABBADY[1], F. C. COLLMAN[1], S. SESHAMANI[5], C. OTT[6], J. C. GLATZER[7], N. L. JORSTAD[2], T. BAKKEN[2], A. A. BLECKERT[1], A. L. BODOR[1], D. BRITTAIN[2], D. J. BUMBARGER[1], G. MAHALINGAM[1], C. M. SCHNEIDER-MIZELL[8], M. M. TAKENO[8], R. TORRES[1], W. YIN[1], R. D. HODGE[3], E. LEIN[4], J. LIPPINCOTT-SCHWARTZ[6], D. E. BERGLES[9], H. S. SEUNG[10], R. C. REID[1], N. M. DA COSTA[1];
[1]Neural Coding, [3]Cell Types Program, [4]Human Cell Types, [2]Allen Inst. for Brain Sci., Seattle, WA; [5]Allen Inst., Allen Inst., Seattle, WA; [6]Janelia Res. Campus, Ashburn, VA; [7]Cell. and Mol. Med. Grad. Program, Johns Hopkins Univ., Baltimore, MD; [8]Neural Coding, Allen Inst. For Brain Sci., Seattle, WA; [9]Johns Hopkins Univ. Sch. Med., Baltimore, MD; [10]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: The importance of glial cells in the brain cannot be overstated. Now, with the advent of modern microscopy techniques, we have tools to analyze their complex morphology and interactions with neurons and each other. We used computationally reconstructed datasets created from large volume serial section electron microscopy(SSEM) samples of mouse visual cortex, ages P36, P54 and P81, to find evidence of physical contacts between glia. Our focus was on contacts between satellite microglia, oligodendrocytes(OLs) and oligodendrocyte precursor cells(OPCs), examining their ramified branches and morphological features at large-scale and fine structural levels- https://www.microns-explorer.org/. In each dataset,microglia, OLs and OPCs were often found in satellite positions with their nuclei closely opposed to their neuronal host's soma. In the P54 mouse, their numbers measured:OPC 49%, OLs, 33%, microglia 43%. All five premyelinating OLs in P54 were in satellite positions. Every glial cell maintained its distinct territory, avoiding contact with their own kind. However, branches of microglia and OPCs frequently intermingled, and their processes extended up to 50 μm and contacted 10-20 neuronal somas. Infrequently, we observed microglia and OPCs in satellite positions adjacent to each other, each contacting the neuronal soma with their branches intertwining. These events were rare and observed in all three datasets, two in P36 and two in P54.OPCs are highly dynamic cells and are the most proliferative cells in the brain. When an OPC dies or transitions to an OL, it is quickly replaced. In all datasets, there was evidence of newly divided asymmetric OPCs in satellite positions. In the P54 dataset, there were 12 split cells and nine in P81. In the largest dataset P81, we found a rare combination of a newly divided OPC, a microglia and an OL nearby (Fig.1). These findings provide a snapshot of relationships between three types of glia in the mammalian cortex and raise questions about roles microglia might play in the morphological transformation of OPCs and their proximity to newly formed OLs.

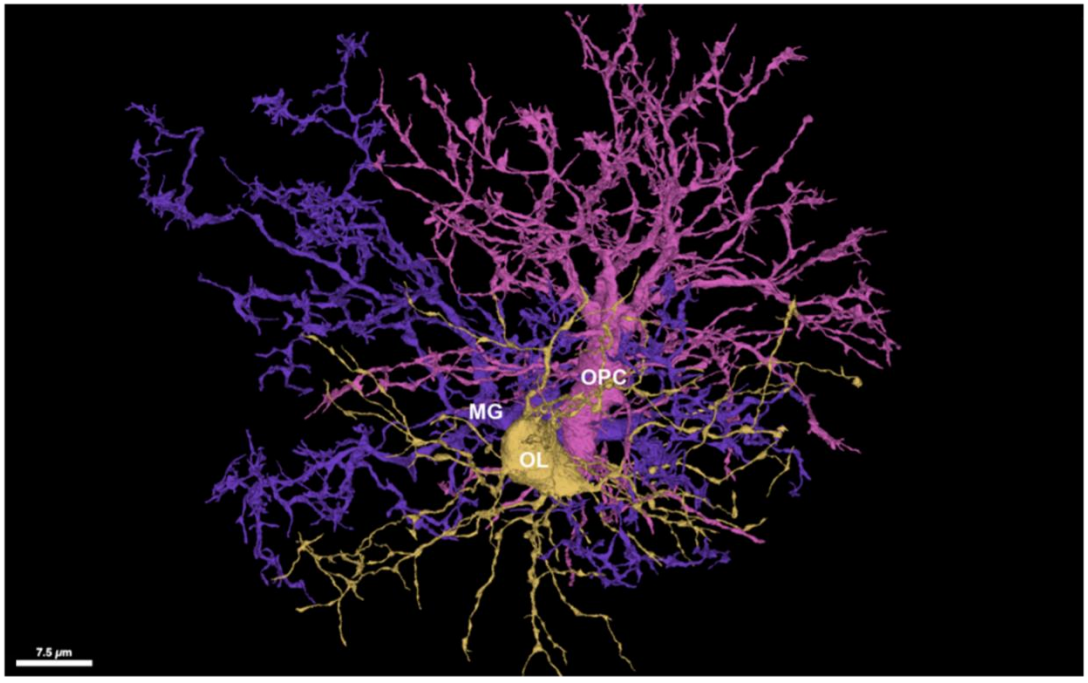

Fig.1. A recently divided OPC (pink) is adjacent to an oligodendrocyte (OL)in gold and a microglia(MG) in purple. Scale bar 7.5 μm.

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Poster

193. Oligodendrocyte Development and Function

Location: SDCC Halls B-H

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Program #/Poster #: 193.26

Topic: B.09. Glial Mechanisms

Support: MEXT Grant 20K07756
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Title: The extracellular matrix protein fibulin-7 positively regulates oligodendrocyte differentiation through the interaction between myelin and neuronal axons

Authors: *M. YAMADA[1], M. IWASE[1], C. HAYASHI[1], S. DE VEGA[2], N. SUZUKI[1];
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Abstract: Fibulin-7 (Fbln7) is an extracellular matrix (ECM) protein composed of an N-terminal sushi domain, three EGF-like motifs, and a C-terminal fibulin-type module and binds to cell surfaces through integrin β1 and heparan sulfate proteoglycan, among others. According to RNA-Seq analysis, Fbln7 is specifically expressed in oligodendrocyte (OL)-lineage cells among the CNS cell types. The normal expression and function of Fbln7 in the CNS have not been analyzed yet though Fbln7 is overexpressed and causes angiogenesis in glioblastoma. Therefore, we aimed the elucidation of the expression pattern and the function of Fbln7 in the CNS.First, we performed immunohistochemical analysis of the spinal cord from postnatal (P) 3 and P7 mice. Fbln7 was expressed at both P3 and P7 stages and was localized on myelin and between myelin and axon at P7. In primary cell-culture, Fbln7 expression was visualized along the processes and around the tips of protrusions in OLs. Since these results indicated the Fbln7 function related to OL differentiation and axon myelinization, we examined it by using co-culture of OLs and neurons. As the result, the number of GalC-positive OLs was increased when the co-culture was incubated in the medium including recombinant Fbln7 (rFbln7). Moreover, the number of GalC-positive OLs adhering to neurites was also increased in the presence of soluble rFbln7. Further, we tested synthetic peptides derived from N-terminal and C-terminal regions of Fbln7, and analyzed their effect on the OL differentiation in co-culture. Similar to the result using rFbln7, GalC-positive OLs were increased in the medium with the Fbln7 peptides. In conclusion, the expression of Fbln7 from OL lineage cells is accumulated between myelin and axons at postnatal

myelination stages and Fbln7 positively regulates OL differentiation through active sites at its N- and C-terminal regions. Our findings will be useful for better understanding of the ECM functions in OL biology.

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Poster

193. Oligodendrocyte Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 193.27

Topic: B.09. Glial Mechanisms

Support: MH098742
MH128135

Title: Hiv pre-exposure prophylaxis (prep) inhibits oligodendrocyte differentiation

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Abstract: Adolescents comprise one-fourth of individuals newly diagnosed with HIV in the United States each year. PrEP, a combination of emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF) two antiretrovirals (ARV) of the nucleoside reverse transcriptase inhibitor (NRTI) class, is an effective method to prevent the transmission of HIV in adolescents at substantial risk for acquiring HIV. However, data from our lab has demonstrated that even in the absence of HIV, select antiretroviral drugs of the integrase strand transfer inhibitor (INSTI) and protease inhibitor (PI) classes inhibit OL differentiation *in vitro*. The CNS of adolescents taking PrEP is particularly susceptible to impairment due to ongoing myelination and synaptic plasticity during this phase of development. Using our well-established cell culture system for purification and differentiation of primary rat OLs, we have demonstrated that treatment with FTC and TDF, alone or in combination, inhibited OL differentiation. OL were treated at the time of differentiation with concentrations of FTC or TDF equivalent to that of the plasma C_{max} in patients on PrEP, 7uM and 0.6uM respectively, reduced the number of GalC and PLP positive cells after 3 days of differentiation. Furthermore, using an adolescent rat model, we demonstrated that adolescent rats treated with PrEP from 3 to 6-weeks of age had significantly fewer mature OLs in the corpus callosum at 6-weeks-old compared to vehicle treated controls. Other ARV drugs inhibit OL differentiation by deacidifying lysosomes due their slightly alkaline pH. FTC and TDF are also weak bases. Treatment with FTC and TDF, alone or in combination, significantly decreased the number of acidic lysosomes, decreased the number of functional lysosomes, and increased the pH of lysosomes in a dose dependent manner. Reacidifying

lysosomes restored OL differentiation in the context of PrEP treatment. As lysosomes are critical for processing and transporting large quantities of lipids and proteins necessary for the generation of the myelin membrane, we are investigating the effects of PrEP- induced lysosome deacidification on lipid and myelin protein content. These data suggest that FTC and TDF inhibit OL differentiation *in vitro* and *in vivo* via impairment of lysosome function. Our findings address a gap in our understanding of the effects of PrEP on myelination in uninfected adolescents *in vivo*. Identifying the mechanisms underlying myelin changes in uninfected adolescents taking PrEP will allow for the development of treatments or new prophylactic therapies that protect this uniquely vulnerable population.

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Poster

193. Oligodendrocyte Development and Function

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Topic: B.09. Glial Mechanisms

Support: 1R01NS122800-01
5R00NS099469-05

Title: Intravital mitochondrial dynamics during oligodendrocyte generation

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Abstract: Myelinating oligodendrocytes have a readily available pool of resident progenitors called NG2 glia. Oligodendrocyte generation from NG2 glia persists throughout life and allows for oligodendrocyte replacement in neurodegenerative pathologies and aging. The cellular and molecular mechanisms that guide certain subpopulations of NG2 glia to generate oligodendrocytes while others engage in self renewal are not fully understood. Intracellular metabolism and organelle dynamics are likely to play key roles in regulating this decision. Using high resolution intravital optical imaging techniques to trace fluorophore labeled-oligodendrocyte lineage cells and their mitochondria, we determined how mitochondrial distribution, shape, and dynamic movement throughout the cell change as single NG2 glia transform into myelinating oligodendrocytes. We observed that NG2 glia contained a denser network and tubular mitochondria while mature oligodendrocytes contained fewer and more fragmented mitochondria. The transition from progenitor to fully myelinating oligodendrocyte involved key checkpoints in mitochondrial shape, localization within the cell, and overall content indicating a relationship between mitochondrial dynamics and oligodendrocyte generation. This work provides insight into how mitochondrial dynamics affect NG2 glia fate and introduces a potential target to modulate oligodendrocyte generation in the live intact brain.

Disclosures: **X. Bame:** None. **R.A. Hill:** None.

Poster

193. Oligodendrocyte Development and Function

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Topic: B.09. Glial Mechanisms

Support: NIH Grant R00NS099469
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Title: Oligodendrocyte death initiates synchronous remyelination by local NG2-glia

Authors: *T. W. CHAPMAN, G. E. OLVEDA, X. BAME, E. PEREIRA, R. A. HILL;
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Abstract: Demyelination is widespread in neurodegenerative disease and aging. In early stages of these conditions, homeostatic repair mechanisms initiate oligodendrocyte replacement by resident progenitor cells called NG2-glia. To investigate the cellular dynamics of this repair, we developed a novel demyelination model by combining intravital myelin imaging with a targeted single-cell ablation technique called 2Phatal. Oligodendrocyte 2Phatal activated a stereotyped degeneration cascade which triggered remyelination by local NG2-glia. Remyelination efficiency and timing was dependent on initial myelin patterning. Longitudinal imaging revealed a synchronous repair mechanism, resulting in near-seamless transitions between myelin loss and repair. A subset of morphologically complex NG2-glia executed this remyelination, pointing towards unrecognized functional diversity within this local glia population. Age-related demyelination mirrored the degenerative cascade observed with oligodendrocyte 2Phatal, although remyelination in aging was defective, due to failed oligodendrogenesis. Thus, oligodendrocyte 2Phatal is a powerful new model for non-inflammatory demyelination, allowing the discovery of cellular diversity within the oligodendrocyte lineage and a novel form of rapid repair called synchronous remyelination.

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Poster

193. Oligodendrocyte Development and Function

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Topic: B.09. Glial Mechanisms

Support: NIH Grant 1R01NS122800-01
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Title: Dynamic clearance of dying oligodendrocytes by single microglia

Authors: *G. E. OLVEDA, X. BAME, R. A. HILL;
Biol. Sci., Dartmouth Col., Hanover, NH

Abstract: Myelin is a complex multilamellar structure, generated by oligodendrocytes, that ensheaths axons and is a vital component for neural processing. Degeneration of the myelin sheath is a common pathology associated with many neurodegenerative diseases and aging. The factors involved in myelin degeneration across different diseases vary, however the failure to efficiently remove myelin debris generated as a result of this degenerative cascade contributes to disease progression and inhibits tissue repair. Removal of myelin debris is thought to be carried out by microglia, the primary phagocyte of the brain. To investigate the cellular dynamics underlying microglia phagocytosis and clearance of myelin debris, we have developed a novel model for titratable and inducible cortical demyelination called oligodendrocyte 2Phatal. After targeted single cell cortical demyelination, we observed microglia engaging with both the targeted oligodendrocyte and its myelin sheaths followed by rapid and efficient removal of both. Following the removal of the myelin sheath we observed rapid remyelination, suggesting that efficient clearance of myelin debris plays a vital role in successful remyelination. Thus, we describe a new model for in vivo optical imaging of demyelination and phagocyte engagement and reveal the underlying cellular dynamics involved in myelin debris clearance and repair.

Disclosures: G.E. Olveda: None. **X. Bame:** None. **R.A. Hill:** None.

Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

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Program #/Poster #: 194.01

Topic: B.10. Demyelinating Disorders

Support: HICI 851693
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IZKF Erlangen

Title: The PD-1/PD-L1 axis regulates pathogenesis in relapsing-remitting Multiple Sclerosis

Authors: *T. TSAKTANIS, M. LINNERBAUER, L. LÖßLEIN, O. VANDREY, A. PETER, V. ROTHHAMMER;
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Abstract: Objective: The PD-1 / PD-L1 (programmed cell death protein 1/ programmed cell death ligand 1) axis plays an important role in the adaptive immune system and has influence on neoplastic and inflammatory diseases. However, the role of thePD-1 / PD-L1 axis in multiple sclerosis (MS) has not yet been investigated in detail. Here, we aimed to delineate distinct expression patterns in peripheral immune cell subsets and to evaluate the role of soluble PD-1 and PD-L1 in the context of MS. **Methods:** In depth immunophenotyping by flow cytometry was performed on Peripheral Blood Mononuclear Cells (PBMCs) of 49 patients with relapsing-remitting MS (RRMS) and 27 controls. Soluble PD-1 and PD-L1 serum levels were analyzed in a cohort of 84 patients with RRMS and 36 controls. To evaluate the therapeutic potential of soluble PD-L1, 600 µg/kg recombinant protein was systemically administered daily starting from symptom onset in Experimental AutoimmuneEncephalomyelitis (EAE). **Results:** Patients with RRMS displayed distinct cellular PD-1 / PD-L1 expression patterns in peripheral immune cell subsets as well as an association of soluble PD-L1 with MS subtype and disease severity. Systemic administration of recombinant PD-L1 in EAE resulted in ameliorated disease severity and reduced infiltration of peripheral immune cells into the CNS. **Interpretation:** The marked expression of PD-1 / PD-L1 in immune cell subsets of patients with RRMS, as well as the correlation of soluble PD-L1 with disease-severity offers promising potential for the use as a biomarker in the context of MS. Furthermore, restoring the lack of coregulatory PD-1 / PD-L1 signaling by exogenous supplementation of PD-L1 may serve as a novel therapeutic strategy for severe stages of acute neuroinflammation.

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Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

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Program #/Poster #: 194.02

Topic: B.10. Demyelinating Disorders

Support: MSTP Training Grant: National Institute of General Medical Sciences (T32 GM 65841)
Mayo Clinic Center for MS and Autoimmune Neurology
NCAA Postgraduate Scholarship

Title: Relative expression of brain-enriched cerebrospinal fluid proteins in multiple sclerosis

Authors: *L. I. WURTZ[1], E. E. KNYAZHANSKAYA[1], D. SOHAEI[2,3], I. PRASSAS[3], S. PITTOCK[1], M. WILLRICH[1], R. SAADEH[1], R. GUPTA[1], E. P. DIAMANDIS[3], I. A. SCARISBRICK[1];
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Abstract: Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system commonly affecting young adults, predominantly females. Individuals with MS are broadly categorized into two clinical subtypes, relapsing remitting (RR) and primary progressive (PP), based on symptom time-course and severity of disease. In this study, we analyzed the cerebrospinal fluid (CSF) from 40 individuals with diagnosed MS (65% female, median age of 48) of both subtypes (n=20 RR, n=20 PP), as well as 14 headache controls (78.6% female, median age of 45). Using liquid-chromatography tandem mass spectrometry, we report the relative expression of 53 brain-enriched proteins in patients with diagnosed PP vs. RR vs. non-MS headache controls. We determined a subset of 22 proteins that showed significant ($p < 0.05$) differential expression via Wilcoxon signed-rank test in MS vs. control (13 proteins), RR vs. control (20 proteins), PP vs. control (1 proteins), or RR vs. PP (1 protein). Logistic regression analysis revealed 19 of these proteins could predict disease ($p < 0.05$). This set includes known players in disease pathology, such as kallikrein 6. These findings provide new insights into the potential molecular disease process that drives MS pathogenesis and unveil targets for further research and for the development of new treatment strategies.

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Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 194.03

Topic: B.10. Demyelinating Disorders

Title: Characteristics of MOGAD patients meeting McDonald's criteria

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Abstract: Myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) is an autoimmune inflammatory demyelinating disorder of the central nervous system, characterized by manifestation and relapse as encephalitis, transverse myelitis, and optic neuritis either alone or in combination. Because MOGAD has a potential to involve multiple brain regions through multiple time points, patients with MOGAD may also meet the McDonald's criteria—dissemination in time and space—that is a gold standard for the diagnosis of the other demyelinating disease, multiple sclerosis (MS). Although these MOGAD patients who satisfied McDonald's criteria may have distinctive disease characteristics, only a few relevant studies have been conducted. Herein, we investigated the prevalence of MOGAD patients who meet the McDonald's criteria with brain and spinal MRI findings, and examined their clinical characteristics, as compared to those who did not. During the study period, we included a total of 37 MOGAD patients with a follow-up period ≥12 months and one or more follow-up MRI scans.

Of these patients, 8 (21.6%) met the McDonald's criteria. In their MRI scans, 4 patients (50%) had Dawson's fingers, 5 patients (62.5%) had lesions adjacent to the body of lateral ventricles and in the inferior temporal lobe, and 2 patients (13%) had curved juxtacortical lesions. Lesions of 6 patients (75%) were found as enhanced, while 2 (25%) patients had rim or open-ring enhancement. Three patients (20%) had central-vein signs. A total of 4 patients (50%) had more than two of the above-described characteristics. In comparison of clinical characteristics between patients who met McDonald's criteria and those who did not, there were no significant differences in female sex (5 [62.5%] vs 17 [58.6%], p = 0.506), onset age (36.0 [12.4] vs 46.5 [14.8] years, p = 0.392), annual recurrence rate (0.291 [0.64] vs 0.32 [0.39], p = 0.741), and the frequency of optic neuritis (p = 0.749). Taken together, a significant number of MOGAD patients met the McDonald's criteria that is specific for MS, and these patients did not differ significantly from those who did not meet the criteria in their clinical characteristics. These findings suggest that MOGAD and MS may share some imaging and clinical characteristics, thus anti-MOG antibody should be chekced for diagnosis even in patients who met the McDonald's criteria.

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Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 194.04

Topic: B.10. Demyelinating Disorders

Title: Relationship between serum biomarkers and clinical variables according to lesion acuteness in patients with CNS demyelinating disorders

Authors: *S. KANG[1,2], S. KIM[1], E.-J. LEE[2,3], J. SO[3], D. SEO[3], J. KIM[3], H. KIM[3], K.-K. KIM[3], Y.-M. LIM[3];
[1]Depeartment of Neurol., Veterans Hlth. Service Med. Ctr., Seoul, Korea, Republic of; [2]Col. of medicine, The graduate school, Univ. of Ulsan, Seoul, Korea, Republic of; [3]Dept. of Neurol., Asan Med. Ctr., Seoul, Korea, Republic of

Abstract: Neurofilament-light chain (NfL) and glial fibrillary acidic protein (GFAP) have been reported to be useful blood biomarkers in patients with multiple sclerosis (MS) and neuromyelitis optica spectrum disorders (NMOSD), which are inflammatory CNS demyelinating diseases with different mechanisms. However, the association between these serum biomarkers and MRI lesion characteristics has rarely been investigated particularly during acute relapses. Herein, we evaluated the relationship between serum biomarkers (NfL and GFAP) and T2-weighted fluid attenuated recovery imaging findings (lesion volume and number). Between June 2018 and July 2021, consecutive patients with MS or NMOSD who experienced acute relapses in brain and/or spinal cord within 2 months were prospectively enrolled in a tertiary medical

center. Included patients underwent single molecule array analysis with blood samples. Lesion volumes were calculated by integrating cross-sectional area of 2D-lesions that were manually demarcated. During the study period, a total of 31 patients (MS, n = 14; NMOSD, n = 17) with acute relapses were enrolled. T2-weighted lesion volumes (median (interquartiles), MS vs. NMOSD, 5.68 (1.95-13.07) cm^3 vs. 6.00 (3.76-8.15) cm^3, p = 0.874), and lesion numbers (31.5 (10.5-38) vs. 20 (10-31), p = 0.565) were comparable between patients with MS and those with NMOSD. Levels of GFAP/volume (26.20 (6.47-55.04) vs. 182.10 (31.33-269.40) $pg/ml/cm^3$, p = 0.004), but not those of NfL (4.30 (1.21-8.95) vs. 5.64 (2.03-9.78) $pg/ml/cm^3$, p = 0.475)), were significantly higher in patients with NMOSD. In regard of relationships between lesion characteristics and serum biomarker levels, both the volume and number of lesions were not correlated with serum NfL or GFAP levels in patients with MS. Meanwhile, the number of lesions, but not the volume, was significantly correlated with serum GFAP levels in patients with NMOSD (Spearman rho, 0.669, p = 0.003 for the lesion number; 0.374, p = 0.230 for the lesion volume). Serum levels of NfL tended to be correlated with the number of lesions, but not with the volume (rho, 0.443, p = 0.075 for the number; 0.331, p = 0.195 for the volume) in patients with NMOSD. Taken together, during acute relapse phase, serum biomarker levels and lesion volume did not reveal strong associations in both the MS and NMOSD groups. Notably, the degree of brain damage in NMOSD appears to be proportional to T2-weighted lesion numbers, wheras that in MS might occur more generally throughout the CNS in addition to T2-weighted lesions. These findings suggest that the pattern of serum biomarkers during acute phase in demyelinating diseases may be different according to pathogenesis.

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Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 194.05

Topic: B.10. Demyelinating Disorders

Title: Antibody response to COVID vaccination, booster and infection in MS patients on anti-CD20 therapies

Authors: *J. LIU, G. FEUER, V. KIRSCHNER, J. LEI, M. MALIN, M. ROCHE, J. LIN, R. ALFONSO, S. A. SADIQ;
Tisch MSRCNY, NY, NY

Abstract: Multiple sclerosis (MS) patients on anti-CD20 treatments may have compromised abilities to generate an antibody response to SARS-CoV-2 due to a depletion of B-cells, which could make them more susceptible to COVID-19 infections. At Tisch Multiple Sclerosis Research Center of New York (MSRCNY), we investigated the antibody response of MS patients on anti-CD20 therapy to COVID-19 vaccines, boosters and/or infections. Blood samples

were collected from MS patients who were on anti-CD20 therapies for at least 6 months and who were vaccinated and/or naturally infected with COVID. Longitudinal samples were collected when patients returned after 6 months for their therapies. As a part of patients' routine clinical management, CD19 B-cell counts were obtained prior to each therapy which allowed us to access B-cell counts at the time of serum analysis. Control samples were collected from non-MS staff at Tisch MSRCNY. IgG antibody levels were assessed from serum samples with an in-house ELISA detecting IgG antibodies against the spike protein's receptor binding domain of the SARS-CoV-2 virus. In addition, a commercial FDA-approved SARS-CoV-2 neutralization antibody detection kit was used. For a positive antibody response, longitudinal samples must show an increase in antibody levels from our in-house ELISA. If longitudinal samples were not available, then the sample must have positive neutralization. A total of 206 MS patients were studied and 44 had antibody response. In contrast, all 43 non-MS controls were responsive to vaccination and/or infection. To assess whether recovering B-cell levels were correlated with antibody response, we looked at MS responders' CD19 counts prior to sample collection. Out of the 44 MS responders, 6 patients had samples with normal CD19 counts and the other 37 responders had abnormally low CD19 counts, suggesting that the ability to mount a protective antibody response may involve more than just the presence of circulating B-cells. Of 145 patients with longitudinal samples, 57 received a booster between the two sample collections. An increase in antibody response post-booster was only seen in 8 patients. There were 8 out of 206 MS patients (3.88%) with breakthrough infections, which is higher than the CDC reported rates of 0.005% to 1.48% from April 2021 to April 2022. Our results indicate an impaired antibody response to COVID vaccination, booster and/or infection in MS patients on anti-CD20 but that may not correlate completely with CD19 B-cell counts. From our data, MS patients seem to have higher risk for breakthrough cases, but it is difficult to analyze the variables involved, such as time between vaccination and infection and others.

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Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

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Program #/Poster #: 194.06

Topic: B.10. Demyelinating Disorders

Title: Lysophosphatidylcholine (LPC) -induced demyelination model in the corpus callosum of CD rats: diffusion tensor MRI (DTI), myelin staining and serum neurofilament light chain (NfL) analysis

Authors: J. TOIVANEN[1], T.-K. STENIUS[1], K. LEHTIMÄKI[1], J. OKSMAN[1], D. MISZCZUK[1], ***T. HUHTALA**[1], A. BHATTACHARYA[2];
[1]Charles River Discovery Services, Kuopio, Finland; [2]Janssen R&D, San Diego, CA

Abstract: Myelin is essential for nervous system ensuring the transfer of impulses along myelinated fibers and provides protection and nutrients to neurons. Damage or loss of myelin sheaths, demyelination, and impaired endogenous remyelination, are common features in multiple sclerosis, Alzheimer's disease, and diffused traumatic brain injury. The objective of this study was to characterize the corpus callosum (CC) -targeted, lysophosphatidylcholine (LPC) -induced demyelination model in rats using diffusion tensor MRI (DTI), serum neurofilament light chain readout (NfL) and myelin staining histology.
Male CD rats were infused with 1.5 µl of LPC (1% solution) or vehicle (PBS) to the right hemisphere CC. Induction of the lesion was verified at D3 after the infusion, when animals were DTI scanned (11.7 Tesla Bruker, SE-EPI, 60 directions, b-values 0 and 970 s/mm^2) and the tensor was analyzed for fractional anisotropy (FA), axial, radial and mean diffusions. This baseline DTI was used to ensure that study-enrolled rats had lesion in anatomically correct location, at proper size and without infusion related mechanical damages such as hemorrhages or ventricle abnormalities. DTI was performed again at D15 to assess lesion development and the extent of remyelination in the CC. Brain and blood samples were collected at D15. Histological analysis was done to assess the terminal level of myelination in the CC by Sudan black staining. Serum samples were used for Quanterix Simoa^{TM} NfL analysis of axonal damage.
LPC infusion resulted in significant demyelination in the CC, as observed by DTI at D3 and D15 and by Sudan black staining of CC at D15. Highly significant reduction of fractional anisotropy (>30% decrease) was observed in the CC lesion as compared to anatomically matched areas in sham animals. Minor non-significant recovery both in the ipsilateral FA and the %-difference between the sides was observed from D3 to D15. Also, lesions in the ipsilateral CC in LPC-treated animals were detected at the targeted region using Sudan black staining of brain samples collected at D15. NfL analysis from D15 serum showed significant increase in the LPC infusion group, suggesting axonal damage. However, model induction that results in sufficiently sized lesions without hemorrhage is challenging. DTI-based screening at D3 has proven to be critical to achieve this.
In conclusion, we have characterized a focal lesioning model in which lesion size and demyelination can be quantified and used a basis for balancing experimental groups after initial lesion maturation. Remyelination can be conveniently followed in a non-invasive way in vivo allowing also for the assessment of therapy efficacy.

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Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

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Topic: B.10. Demyelinating Disorders

Support: National Institute of Neurological Disorders and Stroke of the National Institutes of Health R01NS103940
Janssen Research & Development LLC

Title: Preventive Effects of Ponesimod in Cuprizone Demyelination Model of Multiple Sclerosis

Authors: *A. SHAHNAEE[1], D. JONNALAGADDA[2], C. PALMER[3], C. S. LIU[4], V. TAN[2], Y. KIHARA[2], J. CHUN[2];
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Abstract: Sphingosine 1-phosphate (S1P) is a bioactive lysophospholipid that can bind to five cognate G Protein-coupled receptors (GPCRs). There have been identified five subtypes of S1P receptors, $S1P_{1\text{-}5}$. S1PR modulators include FDA-approved medicines ponesimod, ozanimod, fingolimod and siponimod, which are approved for treating multiple sclerosis (MS). S1PR modulators function as agonists and functional antagonists. Ponesimod selectively modulates $S1P_{1,}$ towards determining pharmacological brain properties of this S1P receptor subtype and assessing ponesimod's mechanism of action in MS, including myelination. Determine the effects of ponesimod on demyelination in cortex, corpus callosum (CC) and cingulum (Cg) using toxin-induced demyelination (cuprizone model) as an animal model of MS. C57BL/6 male mice were fed a 0.2% cuprizone diet for five weeks with concurrent preventative treatment with vehicle or ponesimod (30 mg/kg) for five weeks. A histological assessment was conducted to validate demyelination by black-gold staining II and cellular profiles. Furthermore, single nucleus RNA-sequencing (snRNA-seq) was conducted in the cortices, CC, and Cg of mice fed with a cuprizone diet with Ponesomid or vehicle. Ponesimod protected demyelination particularly in Cg. Moreover, ponesimod treatment increased $Olg2^+$ oligodendrocyte progenitor cells in both CC and Cg, and suppressed $Iba1^+$ microglial accumulation in the Cg. The snRNA-seq identified neuronal cell types (neurons, astrocytes, and oligodendrocytes) and accumulation of myeloid cells in CC and Cg. Ponesimod effectively inhibited demyelination and accelerated remyelination in Cg that have been associated with several neurologic impairments including MS fatigue. Thus, may explain the ponesimod's efficacy in fatigue reported in a completed phase 3 clinical trial.

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Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 194.08

Topic: B.10. Demyelinating Disorders

Title: A high throughput platform to identify myelin inducers for CNS repair

Authors: X. LIU[1], X. HE[1], L. ZHANG[1], A. DOURSON[1], M. A. SMITH[2], M. P. JANKOWSKI[1], ***R. LU**[1];
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Abstract: Demyelinating diseases of the central nervous system such as multiple sclerosis (MS) are among the most devastating and disabling neurological disorders that can arise across the lifespan and lead to severe handicaps. A major feature of demyelination lesions is the block of the remyelination capacity of oligodendrocytes and their precursors (OPCs). Single-cell transcriptomics analysis in human adult MS brains reveals the presence of committed OPCs and premyelinating oligodendrocytes, but they fail to remyelinate the axons in the demyelinaitng lesions. Recent study using ^{14}C tracing in MS brain tissues indicates that remyelination failure is likely in part due to the loss of capacity of residual or surviving oligodendrocytes to myelinate naked axons rather than an impairment in the formation of OPCs. Currently, the molecular blocks that prevent the myelin-producing capacity of mature oligodendrocytes and their remyelination in demyelinating lesions are poorly understood. Although most previous screens focused on the compounds that promote OPC differentiation, the factors that promote the myelin-producing potential and late-stage transition from pre-myelinating to myelin-producing oligodendrocytes remain poorly defined. To identify small molecule compounds that inhibit the myelin-producing capacity of premyelinating oligodendrocytes, we have established a transgenic mouse line (CNP-Luc) carrying a CNP-promoter-driven luciferase reporter to specifically label CNP+ postmitotic mature oligodendrocytes. The transgenic platform allows a high throughput screen of the functional compounds that activate expression of myelin genes such as CNP. We carried out a chemical genetic screen with a library of small molecule chromatin modifying modulators using O4+/GalC+ pre-myelinating oligodendrocytes isolated from CNP-Luc transgenic mice. We identified a set of chromatin modulators that potently enhance myelin-producing potential. Treatment with the lead candidate robustly promotes oligodendrocyte myelination in the developing brain and remyelination in the lesions induced by lysolecithin-or experimental autoimmune encephalomyelitis (EAE)-induced demyelination as well as myelination of regenerated axons after optic nerve crush injury. Thus, our studies established a platform for high-throughput screen of compounds that can induce myelin-producing potential from mature oligodendrocytes and provide a better understanding of the inhibitory cues that limit myelination or remyelination and inform potential future therapeutic treatment for demyelinating diseases.

Disclosures: X. Liu: None. **X. He:** None. **L. Zhang:** None. **A. Dourson:** None. **M.A. Smith:** None. **M.P. Jankowski:** None. **R. Lu:** None.

Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

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Topic: B.10. Demyelinating Disorders

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UofS CoMGRAD

Title: Novel non-invasive therapy that significantly enhances intrinsic repair in the experimental autoimmune encephalomyelitis model of Multiple Sclerosis

Authors: *N. TOKARSKA[1], S. RATHNAYAKA KORALAGE[1], S. J. DONKERS[2], V. M. K. VERGE[1];
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Abstract: Multiple sclerosis (MS) is an inflammatory, autoimmune disease of the central nervous system (CNS) that is characterized by segmental demyelination and variable degrees of axonal and neuronal degeneration. MS also disproportionately affects females (3:1), however males typically have more severe symptoms. Because males and females differ in their physiology and immune responses, they also may respond differently to therapies. In both sexes, effective remyelination and prevention of neurodegeneration can mitigate disability in MS but currently, there is a limited number of MS therapies available with broad neuroreparative impacts. Evidence in early MS demonstrates that endogenous repair mechanisms exist as efficient remyelination can occur even prior to treatment. Thus, by targeting already existing repair mechanisms in MS, we have the ability of enhancing that intrinsic repair. Previously we have shown that acute intermittent hypoxia (AIH) is a highly effective, novel non-invasive therapy for peripheral nerve repair, including remyelination. But its potential for repair in MS was unknown. As a result, we examined the capability of AIH to enhance intrinsic repair in both sexes using the MOG_{35-55} experimental autoimmune encephalomyelitis (EAE) mouse model of MS. One week of daily AIH treatment (10 cycles of 5 min 11% oxygen [hypoxia] alternating with 5 min 21% oxygen [normoxia]) begun at near peak disease significantly improved clinical scores and associated histopathology relative to normoxia control EAE animals. AIH enhanced correlates of myelination, axon protection and recruitment of oligodendrocyte precursor cells to the demyelinated areas. AIH also effected a dramatic reduction in inflammation, polarizing remaining macrophages/microglia towards a pro-repair state. These results were evident in parallel in both male and female mice suggesting that AIH demonstrates beneficial effects in a similar manner in both sexes. Together, this research provides evidence to support that AIH generates a favourable environment for significant repair and holds promise as a novel non-invasive neuroreparative strategy for MS in both males and females.

Disclosures: N. Tokarska: None. **S. Rathnayaka Koralage:** None. **S.J. Donkers:** None. **V.M.K. Verge:** None.

Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

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Program #/Poster #: 194.10

Topic: B.10. Demyelinating Disorders

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Title: Luman (CREB3) is a novel regulator of survival and myelinating capacity of myelinating glia

Authors: *J. M. A. NANIONG[1], V. MISRA[2], V. M. K. VERGE[1];
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Abstract: Multiple sclerosis (MS) is an immune-mediated demyelinating disorder that leads to axonal and neuronal degeneration. The accumulation of misfolded myelin protein triggers the unfolded protein response (UPR) and cholesterol biosynthesis pathways to promote the activation of cytoprotective genes in an attempt to alleviate endoplasmic reticulum (ER) stress. Failure to resolve the stress, however, can lead to continual activation of the aforementioned pathways and eventual cell death. Here, we demonstrate that the ER stress-associated transcription factor Luman/CREB3, which has previously been shown to play a major role in axonal regeneration by regulating adaptive low-level stress responses in injured sensory neurons through the UPR and cholesterol biosynthesis pathways, plays a role in regulating survival and myelinating capacity of Schwann cells (SCs) and oligodendrocytes (OLs). Knockdown of Luman in primary SCs and OLs by siRNA led to a marked decrease in both cell viability (22% decrease in SCs and 25% decrease in OLs compared to naive and control siRNA-transfected cells, $n = 6$ exp replicates) and the expression of brain-derived neurotrophic factor (BDNF) (70% reduction in SCs and 50% reduction in OLs, $n = 3$ exp replicates), a known positive regulator of myelination. The effect of Luman knockdown in SCs and OLs on the expression profiles of other known UPR regulators is currently being studied. An adenoviral rat Luman construct was employed to overexpress Luman in myelinating glia and examine its impact on myelination in mixed cultures of P3 Wistar rat sensory neurons and SCs or OLs. Increased myelination and myelin production was observed in myelinating glia with elevated Luman levels, as determined by quantifying myelin basic protein (MBP) levels as a function of numbers of axons present. This study uncovers an essential role of Luman in the survival and myelinating capacity of myelinating glia. Thus, a potential connection between activating adaptive ER stress pathways and inducing intrinsic cytoprotective mechanisms in myelinating glia may exist, both of which open avenues for designing non-invasive treatments for demyelinating disorders such as MS.

Disclosures: J.M.A. Naniong: None. **V. Misra:** None. **V.M.K. Verge:** None.

Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 194.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Discovery of remyelination therapeutics that overcome the effects of inflammatory macrophages on oligodendrocyte formation

Authors: *K. I. LORRAIN[1], A. R. BROADHEAD[2], M. M. POON[2], D. S. LORRAIN[2], A. CHEN[3];
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Abstract: Multiple sclerosis (MS) is an inflammatory demyelinating disease that results in the disruption of neuronal transmission and ultimately neurodegeneration. Current treatments focus on suppressing the immune system to limit inflammation and the further loss of the myelin sheath. The next advance in the treatment of MS has focused on molecules that promote remyelination in inhibitory environments. One important question is how inflammatory factors released over the course of MS impact OPC differentiation. Macrophages are an innate immune cell known to infiltrate the CNS and accumulate in MS lesions. Macrophages release numerous factors that negatively impact OPC differentiation and remyelination efficiency. Among other things, they express choline acetyltransferase (ChAT) and autotaxin (ATX), the enzymes responsible for producing acetylcholine and lysophosphatidic acid (LPA), respectively. Previous work from our lab has shown that direct application of either ligand as single agents inhibit OPC differentiation. Pipeline Therapeutics has developed brain penetrant, selective, small molecule antagonists against M1 (PIPE-307) or LPA1 (PIPE-791). In the presence of their respective ligands, both small molecules antagonize their receptors, and permit OPC differentiation. To understand the impact of macrophages on OPC differentiation, we turned to a more complex, transwell culture system whereby OPCs are cultured in one compartment, macrophages in another, but media is freely exchanged. There are two key advantages this system has over an isolated OPC culture, 1) invading immune cells release a multitude of factors at various concentrations into the OPC environment and thus better represents the disease state in contrast to OPCs alone, 2) some of the secreted inhibitory/ factors are labile and the physical presence of macrophages provides a constant, renewable source unlike conditioned media which can be depleted. Using this system, we observed a significant increase in the number of oligodendrocytes with either PIPE-791 or PIPE-307, demonstrating that even in the presence of inhibitory macrophages, either mechanism alone is sufficient to overcome the repression of differentiation. Both are promising therapeutics to promote remyelination in people with Multiple Sclerosis. PIPE-307 is currently advancing in phase 2 clinical trials while PIPE-791 is entering IND enabling toxicity studies.

Disclosures: K.I. Lorrain: None. **A.R. Broadhead:** None. **M.M. Poon:** None. **D.S. Lorrain:** None. **A. Chen:** None.

Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 194.12

Topic: B.10. Demyelinating Disorders

Title: Selective oligodendrocyte death in the optic nerve alters RGC axonal conduction and organization of alpha-RGC and cholinergic amacrine cells in the retina

Authors: *A. BALRAJ, R. H. MILLER;
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Abstract: The myelin sheath, produced by oligodendrocytes, provides insulation for signal conduction along retinal ganglion cell (RGC) axons and when disrupted, can delay or block visual signals. Patients with optic neuritis (characterized by the demyelination and inflammation of the optic nerve) experience sudden deficits in visual acuity, color perception, and contrast sensitivity followed by spontaneous recovery of the visual field. However, persistent deficits in contrast sensitivity and thinning of retinal layers (including retinal nerve fiber, ganglion cell (GCL), and inner plexiform layers (IPL)) indicate a complex relationship between myelination, nerve conduction, and retinal connectivity which is not yet fully understood. This study evaluates optic nerve function and retinal organization in an inducible demyelinating mouse model (MBP-iCP9) using extracellular nerve recordings of compound action potentials (CAPs) and immunohistochemistry. In MBP-iCP9 mice, selective oligodendrocyte ablation in the optic nerve is induced by injection of the chemical inducer of dimerization (CID) into the eye at P14, which triggers apoptosis of a population of oligodendrocytes expressing myelin basic protein (MBP) and the inducible caspase-9 (iCP9) sequence. Two weeks post-injection, transgenic mice injected with CID were compared with Vehicle-injected (Veh) or untreated (Naïve) transgenics as controls. We find that CID-treated MBP-iCP9 mice have a loss of oligodendrocytes in the optic nerve ($n>5$) and altered CAP responses, including fewer functional axons and a loss of the slowest-conducting axon populations ($n>8$). In the retina, lower GCL density and thinner IPL and ON-sublamina thickness were observed in CID-injected MBP-iCP9 mice compared to controls. Quantification of alpha-RGC and amacrine cell density using molecular markers Brn3a and choline acetyl transferase identified a reduction in alpha-RGCs in temporal regions of the retina ($n=3$) and a significant loss of displaced cholinergic amacrine cells in central regions of the retina ($n>5$). Preliminary analysis found altered distribution of RGC subtypes, including reductions in ON-sustained and OFF-transient alpha-RGCs in CID-treated transgenic retinas ($n>4$). These results show that induced oligodendrocyte loss that disrupts conduction in a subset of RGC axons also affects the organization of alpha-RGCs and cholinergic amacrine cell populations. This suggests that functionally-relevant demyelination in RGC axons can alter retinal networks, demonstrating the regulatory role of myelination in the maintenance of upstream neural networks.

Disclosures: A. Balraj: None. **R.H. Miller:** None.

Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 194.13

Topic: B.10. Demyelinating Disorders

Title: Taurine chloramine interrupts the microtubule hyper-polymerization in a tubulinopathy rat model: an in silico search for new therapeutic strategies

Authors: *V. VARGAS-CASTRO[1,2], J. R. EGUIBAR[1], B. A. LEON-CHAVEZ[1], J. M. SOLANO-ALTAMIRANO[1];
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Abstract: The *taiep* rat is an animal model of tubulinopathy, which shows progressive hypo-myelination and demyelination of the central nervous system (CNS). The hypo-demyelination renders neuroinflammation and motor behavior dysfunction and is caused by an abnormal accumulation of microtubules in oligodendrocytes due to the Ala302Thr single-point mutation in the β-Tubulin 4a protein. Our research group has previously demonstrated (Vargas-Castro et al., 2021) that taurine administration might improve the *taiep* pathology by stimulating cell proliferation and promoting myelination, which alleviates motor impairment. In this work, we use an in silico approach to demonstrate that the Ala302Thr mutation induces structural changes in the β-Tubulin 4a protein, which increase the microtubule's hyper-polymerization. In addition, we found that taurine and its metabolite (taurine chloramine) might prevent hyper-polymerization, hence improving *taiep*'s motor pathology. We used homology modeling to obtain the structures of mutated (*taiep*) and native (Sprague Dawley, SD) β-tubulins, using the primary structure B4F7C2_RAT (UniprotKB) as a template. The models for β/β-Tubulin 4a dimers were built and then analyzed with molecular dynamics simulations under physiological conditions (310K, pH=7). The last 20 conformations, for each dimer, were docked with taurine or taurine chloramine and the Gibbs free energy of interaction was calculated. The radius of gyration (which measures the compactness of a protein) is 2Å smaller in the *taiep*'s dimer than in the SD's, i.e., the mutated *taiep* β/β-Tubulin 4a dimers are more compact than the SD counterparts. The RSMD showed that the *taiep* dimers move less (12Å), relative to the SD dimers. These results suggest that the *taiep* mutated dimer is more rigid and less dynamic than the native SD dimer. We also found that the M loop of the *taiep* β-Tubulin 4a protein (amino acids 282-289, which regulate the β/β lateral interactions) shifts (RMSD 98.76Å) compared with the SD dimer. This structural change strengthens the coupling between lateral β-Tubulins, which might be responsible for the hyper-polymerization in the *taiep* rat. While assessing the role of taurine or taurine chloramine on the dimer, we found that the lowest binding energy state occurs when taurine chloramine binds to the M Loop, with an affinity of -4.43 ± 0.086 kcal/mol. In summary, our findings suggest that a) Ala302Thr mutation promotes structural changes in the M Loop, causing *taiep* microtubule's hyper-polymerization, and b) taurine chloramine weakens the β-β tubulin interactions caused by the mutation, which might normalize tubulin polymerization processes in the *taiep* rat.

Disclosures: **V. Vargas-Castro:** None. **J.R. Eguibar:** None. **B.A. Leon-Chavez:** None. **J.M. Solano-Altamirano:** None.

Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 194.14

Topic: B.10. Demyelinating Disorders

Support: DFG 270949263/GRK2162

Title: Influence of promyelinogenic molecules on MAPK-mediated signal transduction and metabolic profile during oligodendroglial myelination

Authors: L. MESZAROS, Y. SCHNEIDER, F. DÖRJE, *J. WINKLER;
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Abstract: Oligodendrocytes, the myelinating cells of the central nervous system (CNS), concentrically wrap axons with multi-lamellar sheets of their plasma membrane both to enable rapid saltatory conduction of action potentials as well as to provide nutritional support for axons. In inflammatory or neurodegenerative CNS diseases like multiple sclerosis (MS) and multiple system atrophy (MSA) loss of myelin sheaths is an important neuropathological hallmark. In order to protect axons from severe degenerative processes myelin is newly generated or compensated by local proliferation and differentiation of oligodendrocyte precursor cells (OPCs). Since intrinsic remyelination is limited and often fails to restore functional compact myelin, the exploration of remyelinating strategies is crucial. Several antimuscarinics and thyroid hormone receptor agonists have shown promyelinogenic effects in *in vitro* high-throughput screens in rodent OPCs and murine disease models. The underlying pathways driving myelination, however, remain poorly understood. The mitogen-activated protein kinase (MAPK) pathway, especially the extracellular signal-related kinase (ERK) 1 and 2, have been implicated in modulating oligodendroglial myelination. Interestingly, oligodendroglial activation of ERK 1 and 2 increases myelin sheath thickness during development. Here, we first examined the link between MAPK-mediated myelination and different antimuscarinic small molecules to gain more insights into the underlying molecular mechanisms using rodent-derived primary OPCs. We observed a synergistic effect of antimuscarinic compounds, but not thyroid hormone receptor agonists combined with the treatment of an ERK inhibitor. We furthermore investigated the metabolic profiles of oligodendrocytes upon different treatments, which revealed that promyelinogenic compounds regulate glutamate and glycerophospholipid as well as sphingolipid pathways differentially in primary rodent-derived OPCs. We next examined the rescue effect of these molecules in the context of MSA using murine OPCs derived from mice overexpressing human α-synuclein specifically in oligodendrocytes. Interestingly, thyroid hormone receptor agonists were indeed able to rescue α-synuclein-mediated demyelination. Overall, by investigating compound-driven mechanisms for myelination, this study contributes to the

discovery of new therapeutic approaches for inflammatory and neurodegenerative demyelinating diseases (e.g. MS and MSA).

Disclosures: **L. Meszaros:** None. **Y. Schneider:** None. **F. Dörje:** None. **J. Winkler:** None.

Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

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National Taiwan University Hospital: 111-UN0007
National Taiwan University: NTU-CC-111L892705

Title: Identification of pathophysiologically relevant pathways potentially targeted by MSA, one type of atypical Parkinsonian, patient associated plasma microRNAs

Authors: *H.-H. LIN-WANG[1], C.-C. LU[2], M. KUO[3], Y.-T. TSAI[2], J.-W. HUANG[4], P.-J. KUNG[2], K. PHOA[4], Y.-H. LIN[2], C.-C. WU[5], T. OCHIYA[6], R.-M. WU[3], S.-P. LIN[2];
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Abstract: Multiple system atrophy (MSA) is an atypical Parkinson's disease and lacking diagnostic biomarkers and therapies. Accordingly, we collected blood plasma from MSA patients for small non-coding RNA profiling in comparison with that of healthy control (HC), and found some miRNAs significantly associated with the disease by using Biomedical Oriented Logistic Dantzig (BOLD) selector. These miRNAs have been analyzed with miRNA target prediction tools to assess their implication in the potential pathological mechanism of MSA. As a result, 55 of the KEGG pathways are found, with some of which are directly relevant to the physiological function of oligodendrocytes, the suspected neuroglia cell lineage primarily affected in the MSA brain. Especially regulatory pathways for cholesterol homeostasis, which may respond to the PI3K/AKT-mTOR coupled pathways, are essentially highlighted because of this sterol lipid constituting a main part of myelin membranes. Therefore, we speculate that the MSA-associated miRNAs may disrupt myelination by silencing the expression of cholesterol-related genes such as *LDLR* and *HMGCS*. Moreover, we also found our miRNAs may potentially

target *TDP-43* to downregulate SPREBP2, which is a master cholesterol regulator, for example, by triggering autophagy in cholesterol depletion condition. On top of PI3K/Akt-mTOR pathways, some miRNAs are directly related with the autophagy pathway through targeting autophagy-related genes like *LC3A* and *ULK1*, thereby interrupting the process of α-Synuclein degradation. With these lines, we have hypothesized a miRNA-target gene regulatory network that knits cholesterol homeostasis and autophagy pathways together for the elucidation of the pathophysiological mechanism underlying MSA. Finally, the research provides a chance to build the feasibility for developing miRNA therapeutics through discovering miRNAs and their novel targets in MSA disease.

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Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

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Program #/Poster #: 194.16

Topic: B.10. Demyelinating Disorders

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Title: A humanized mouse model of Pelizaeus-Merzbacher Disease and its rescue by glial replacement

Authors: P. M. MADSEN[1,2], ***D. KESMEN**[1], S. J. SCHANZ[1], L. J. ZOU[1], Z. NEVIN[3], M. S. WINDREM[1], P. J. TESAR[3], A. BENRAISS[1], J. N. MARIANI[1], S. A. GOLDMAN[1,2,4]; [1]Ctr. for Translational Neuromedicine, Univ. of Rochester Med. Ctr., Rochester, NY; [2]Ctr. for Translational Neuromedicine, Univ. of Copenhagen Fac. of Hlth. and Med. Sci., Copenhagen, Denmark; [3]Dept. of Genet. and Genome Sci., Case Western Reserve Sch. of Med., Cleveland, OH; [4]Sana Biotech., Cambridge, MA

Abstract: Pelizaeus-Merzbacher disease (PMD) is a rare X-linked leukodystrophy caused by mutations in oligodendrocytic PLP1, and characterized by CNS hypomyelination and attendant neurologic dysfunction. Due to its genetic heterogeneity, PMD remains a difficult candidate for molecular therapeutics, suggesting the potential utility of cell-based approaches. To explore this possibility, we generated PLP1-mutant human glial progenitor cells (hGPCs) from iPSCs derived from a 4- year-old boy with connatal PMD (c.T254G; the PLP1-L85R line of Nevin et al, 2017: PMID 28366443), and transplanted these into neonatal *shiverer* x *rag2*$^{-/-}$ hosts to establish human glial chimeric mice. When assessed 19-weeks later, the PMD hGPCs were found to have

engrafted well, but generated fewer MBP^+ oligodendrocytes (7.3 ± 1.0%) than wild-type (WT; an unrelated healthy iPSC line) (18.1 ± 1.0%) or isogenically-corrected (ISO) (19.2% ± 4.4%) hGPCs. The mice engrafted with PMD hGPCs survived no longer than untreated *shiverer* mice, whereas transplantation of WT or ISO hGPCs significantly extended their lifespans (PMD vs. WT: $p<0.0001$; PMD vs. ISO: $p\leq0.01$). Electron microscopy of PMD hGPC-engrafted white matter revealed more frequent myelin delamination ($p<0.01$), and larger periaxonal spaces ($p<0.001$), relative to the compact myelin of WT and ISO hGPC chimeras. scRNA-seq of the callosa of WT and PMD hGPC chimeras revealed that PMD glia manifested differential expression of a number of oligodendrocytic genes, suggesting that mutant PLP1-linked dysmyelinogenesis triggered a broader process of transcriptional dysregulation. Accordingly, when *shiverer* mice were neonatally co-transplanted with PMD and WT hGPCs, the WT hGPCs outcompeted the PMD hGPCs for axonal engagement and callosal myelination (WT: 20.6 ± 1.6%, PMD: 4.0 ± 1.2%; $p<0.0001$). scRNA-seq of these co-engrafted hGPCs revealed preferential expansion of the co-engrafted WT hGPC pool, accompanied by higher expression of proliferation-associated genes than when engrafted alone. On that basis, we asked if WT hGPCs might outcompete already-resident PMD hGPCs and their derived oligodendrocytes, and hence potentially rescue mice already established with PMD myelin. To that end, we transplanted WT hGPCs into young adult mice that had been earlier engrafted as neonates with spectrally-distinct PMD hGPCs. The WT hGPCs dispersed widely in the PMD-chimeric hosts, and differentiated as MBP^+ oligodendrocytes, with local replacement of PMD mutant hGPCs. Together, these findings suggest the potential of glial cell replacement as a therapeutic strategy for PMD, as well as for similar primary disorders of hypomyelination.

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Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 194.17

Topic: B.10. Demyelinating Disorders

Support: The Legacy of Angels Foundation
MidAtlantic Neonatology Associates

Title: Cell modeling and galactocerebrosidase protein assays for preclinical drug development in Krabbe Disease

Authors: A. HERDT[1,2], M. GELB[3], ***C. W. LEE**[1,2];
[1]Biomed. Res. Inst. of New Jersey, Cedar Knolls, NJ; [2]Atlantic Hlth. Syst., Morristown, NJ;
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Abstract: Krabbe disease (KD) is a lysosomal storage disorder caused by a functional deficiency of the galactocerebrosidase (GALC) protein. KD patients inherit loss-of-function mutations on both alleles of the *GALC* gene. Although GALC missense mutations (MMs) are common in KD patients, their molecular impact on GALC protein is unclear.Many KD-MMs are thought to cause GALC protein misfolding and mis-trafficking defects, thus inhibiting GALC secretion and lysosomal delivery. Using a human oligodendrocytic cell line with GALC knockout background (MO3.13/GALC-KO), we performed an expression analysis to interpret the molecular defects of GALC carrying various KD-MMs. We generated a panel of 42 GALC cDNA expression constructs that carry polymorphisms alone, MMs alone or MMs in the presence of relevant polymorphic background. We also developed immunoassays to quantitatively detect exogenous GALC protein. Analysis of our KD-MMs panel revealed there is a strong positive association between GALC activity and mature (cleaved) GALC levels, consistent with its catalytic role. While mature GALC was also positively associated with secreted GALC levels, it was not correlated to intracellular precursor (uncleaved) GALC levels. Overall, MMs-GALC lead to various molecular abnormalities that include a reduction in GALC secretion, mature cleaved GALC and enzymatic activity, and an increase intracellular GALC to extracellular GALC ratio.Using the KD-MMs cell models, we have evaluated the effect of α-lobeline (LB) on various GALC variants. Working with a 2-dose (30μM and 120μM), 3 days treatment protocol, about half of the cell lines (21 out of 42) respond positively to LB. At the 30μM doses, LB significantly increased levels of secreted GALC in 14 KD-MMs lines. While we did not detect a significant increase in lysate GALC activity in LB treated cells, 8 KD-MMs lines had increased levels of mature GALC protein. These results suggest LB may increase lysosomal function of GALC carrying certain KD-MMs.

Disclosures: A. Herdt: None. **M. Gelb:** None. **C.W. Lee:** None.

Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 194.18

Topic: B.10. Demyelinating Disorders

Support: NF2 BioSolutions

Title: Development of therapeutic approaches for the treatment of neurofibromatosis type 2(NF2) using a novel mutation specific in vitro model

Authors: *P. BISWAS[1,2], K. ALCANTARA[1], M. SCHWARTZ[1], S. SINHA RAY[1], C. DENNYS[3], S. LIKHITE[1], L. CHANG[1], K. FLANIGAN[1], K. MEYER[1,2];

[1]Ctr. for Gene Therapy, The Res. Inst. at Nationwide Children's Hosp., COLUMBUS, OH; [2]The Ohio State Univ., Columbus, OH; [3]Alcyone Therapeut. Inc, Columbus, OH

Abstract: Neurofibromatosis type 2 (NF2) is a rare autosomal dominant disorder characterized by bilateral vestibular schwannomas and other nervous system tumors. Although these tumors are usually benign and slow-growing, tumor burden in the brain and spine ultimately leads to increased morbidity and reduced life expectancy. Current treatment options are limited to surgery and radiotherapy, leaving a high unmet need to develop effective strategies for NF2. In this study, we explored both gene replacement therapy and antiretroviral drug (ARD) treatment as complementary treatment approaches for NF2. For our gene replacement strategy for NF2 loss-of-function pathology, a healthy *NF2* gene copy is delivered to cell types that are highly susceptible to *NF2* downregulation. We have generated multiple *NF2* promoter-green fluorescent protein cDNA reporters (AAV.*NF2*.GFP) and *NF2* promoter-*NF2* cDNA (AAV.*NF2*.*NF2* cDNA) with phosphorylation resistant -AAV.*NF2*.NF2 S518A constructs for increased stability of the expressed protein. *In vivo* testing of the AAV.*NF2*.GFP and AAV.*NF2*.*NF2* cDNA constructs in wild-type mice showed high GFP reporter transgene and relevant markers co-localization and upregulation of NF2 expression in whole brain lysates of the injected mice. Further efficacy and safety testing are currently ongoing for our two lead candidate vectors using *in vitro* and *in vivo* systems in preparation for Investigational New Drug (IND)application-enabling studies. We have also developed a novel human NF2 *in vitro* model to study disease mechanisms in a mutation-specific manner and to evaluate potential therapies more efficiently. Here, we successfully reprogrammed four healthy and three patient skin fibroblast cell lines into induced Schwann cells (iSCs) through direct chemical conversion. Molecular characterization of the healthy and NF2-mutant iSCs suggests that NF2 patient cell lines have reduced differentiation and maturation capacity even in the pre-tumoral stage. To mimic the complete loss of NF2 expression in tumor-forming cells in human patients, we generated NF2 knockdown (shRNA) and NF2 knockout (CRISPR/CAS genome editing) patient iSCs that showed significantly higher cell proliferation rates than healthy iSCs, and we are currently evaluating their ability to form tumors *in-vitro* and *in-vivo*. We are currently using these cells to test therapeutic approaches AAV based gene replacement therapy and ARD treatment individually and in combination. Our new model system allows the testing of therapeutics in the context of patient mutations to evaluate the impact of various mutations on the efficacy of different treatment options.

Disclosures: P. Biswas: None. **K. Alcantara:** None. **M. Schwartz:** None. **S. Sinha Ray:** None. **C. Dennys:** None. **S. Likhite:** None. **L. Chang:** None. **K. Flanigan:** None. **K. Meyer:** None.

Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 194.19

Topic: B.10. Demyelinating Disorders

Support: Midwestern University One-Health Research Stimulus Award to M.T.
Midwestern University Startup fundings to M.T. and M.E.

Title: Schwann cell ablation in DTA mice causes peripheral neuropathy and triggers robust Schwann cell regeneration in the cochlea

Authors: J. GEORGOPULOS[1], E. MARKUSON[1], M. EBEID[1,2,3], ***M. TRAKA**[1,2];
[1]Chicago Col. of Osteo. Medicine, Midwestern Univ., Downers Grove, IL, IL; [2]Anat., Col. of Grad. Studies, Midwestern Univ., Downers Grove, IL, IL; [3]Col. of Dent. Med. Illinois, Midwestern Univ., Downers Grove, IL, IL

Abstract: We have previously shown that ablation of oligodendrocytes can be induced through tamoxifen administration in young adult *PLP/CreER^T;ROSA26-eGFP-DTA* (DTA) mice, which as a result develop a demyelinating disease characterized by significant motor and physiological defects by 5 weeks post-induction (5w p.i), followed by a full recovery of symptoms a few weeks later, at ~ 10w p.i. (Traka., *et* al. 2010). However, less is known about the effects of Schwann cell (SC) loss that also occurs in the peripheral nervous system (PNS) of these animals. We hypothesize that depletion of SCs in DTA mice will cause peripheral nerve demyelination, which will be followed by robust SC replenishment and remyelination of axons, as demonstrated in other animal models of demyelinating neuropathy. To test this, we analyzed the impact of SC loss on myelinated fibers in sciatic nerve, as well as in auditory nerve of the mature adult (4-6 months of age) DTA mice (n=6) that were treated with tamoxifen for four continuous days to induce recombination and expression of the diphtheria toxin A subunit (DT-A) and subsequent death in SCs as compared to untreated *PLP/CreER^T;ROSA26-eGFP-DTA* littermate mice (n=5) that were used as normal controls. We checked for motor coordination, forelimb and hindlimb strength defects in DTA mice as compared to controls by testing them weekly on the rotarod and grip strength devices and by assessing their motor symptoms with a clinical scoring system (Traka, 2019) for 5 continuous weeks. All mice were sacrificed at 5w p.i. and their sciatic nerves were collected and processed for immunohistochemical analysis for myelin and node of Ranvier markers and for transmission electron microscopy (TEM analysis) to examine the impact of SC loss on sciatic nerve myelinated fibers. In addition, the cochleae were dissected from these mice into apical and basal whole mount samples and stained with SOX10, a marker for SCs, and TUJ1, a neuronal marker, as well as with myelin and node of Ranvier markers to investigate how SC loss affects myelinated fibers in the auditory nerve. Our results demonstrate both forelimb ($p<0.01$) and hindlimb ($p<0.001$) grip strength were decreased in DTA mice as compared to controls, indicating that SC loss in sciatic nerve causes demyelination and associated peripheral neuropathy symptoms by 5w p.i. Consistently, normal nodes of Ranvier were less frequently observed in sciatic nerve fibers of the DTA mice as compared to controls ($p<0.05$). Furthermore, preliminary data show that the density of SCs within the spiral ganglion and auditory nerve in DTA mice is similar to controls, indicating that SC loss in cochleae is followed by a robust SC regeneration.

Disclosures: J. Georgopulos: None. **E. Markuson:** None. **M. Ebeid:** None. **M. Traka:** None.

Poster

194. Mechanisms and Therapeutics of Demyelinating and Peripheral Nerve Disorders

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 194.20

Topic: B.10. Demyelinating Disorders

Support: CNPQ
FAPERJ

Title: Gene Expression Profile of Mycobacterium leprae Contribution in the Pathology of Leprosy Neuropathy

Authors: *B. J. DE SOUZA[1], M. A. MENDES[1], G. M. S. DA SILVA[2], R. O. PINHEIRO[1], M. M. R. JARDIM[1], M. O. MORAES[1], P. SAMMARCO-ROSA[3], E. N. SARNO[1], B. S. MIETTO[4]; [1]IOC, [2]INI, FIOCRUZ, Rio de Janeiro, Brazil; [3]Lab. Animal House, Lauro Souza Lima, Sao Paulo, Brazil; [4]Biol. Sci., Federal Univ. of Juiz de Fora, Juiz de Fora, Brazil

Abstract: Peripheral neuropathy is the main cause of physical disability in leprosy patients. Importantly, the extension and pattern of peripheral damage has been linked to how the host cell will respond against *Mycobacterium leprae* (*M. leprae*) infection, in particular, how the pathogen will establish infection in Schwann cells. Interestingly, viable and dead *M. leprae* have been linked to neuropathology of leprosy by distinct mechanisms. While viable *M. leprae* promotes transcriptional modifications that allow the bacteria to survive through the use of the host cell's internal machinery and the subvert of host metabolites, components of the dead bacteria are associated with the generation of a harmful nerve microenvironment. Therefore, understanding the pathognomonic characteristics mediated by viable and dead *M. leprae* are essential for elucidating leprosy disease and its associated reactional episodes. Moreover, the impact of the viable and dead bacteria in Schwann cells is largely unknown and their gene signature profiling has, as yet, been poorly explored. In this study, we analyzed the early differences in the expression profile of genes involved in peripheral neuropathy, dedifferentiation and plasticity, neural regeneration, and inflammation in human Schwann cells challenged with viable and dead *M. leprae*. We substantiated our findings by analyzing this genetic profiling in human nerve biopsies of leprosy and non-leprosy patients, with accompanied histopathological analysis. We observed that viable and dead bacteria distinctly modulate Schwann cell genes, with emphasis to viable bacilli upregulating transcripts related to glial cell plasticity, dedifferentiation and anti-inflammatory profile, while dead bacteria affected genes involved in neuropathy and pro-inflammatory response. In addition, dead bacteria also upregulated genes associated with nerve support, which expression profile was similar to those obtained from leprosy nerve biopsies. These findings suggest that early exposure to viable and dead bacteria may provoke Schwann cells to behave differentially, with far-reaching implications for the ongoing neuropathy seen in leprosy patients, where a mixture of active and non-active bacteria are found in the nerve microenvironment.

Disclosures: B.J. de Souza: None. **M.A. Mendes:** None. **G.M.S. da Silva:** None. **R.O. Pinheiro:** None. **M.M.R. Jardim:** None. **M.O. Moraes:** None. **P. Sammarco-Rosa:** None. **E.N. Sarno:** None. **B.S. Mietto:** None.

Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 195.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Ed and Ethel Moore Research Program, Florida Department of Health and Schmidt Family Foundation

Title: Evidence of Oxidative Stress and Inflammatory Activation following Genetic Elimination in Mice of Mblac1, an Alzheimer's Disease Risk Gene

Authors: *J. LAMAR[1], M. J. GROSS[1], S. J. MCGOVERN[1], P. RODRIGUEZ[1], H. A. MESA[1], Q. ZHANG[2], M. K. HAHN[2], R. D. BLAKELY[2];
[1]Dept. of Biomed. Science, Charles E. Schmidt Col. of Med., Boca Raton, FL; [2]Dept. of Biomed. Science, Charles E. Schmidt Col. of Medicine, and Stiles-Nicholson Brain Inst., Jupiter, FL

Abstract: The etiology of many neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, is complex, with a small fraction of subjects arising from highly penetrant, heritable mutations, whereas more common forms of the disorder arise from a constellation of genetic and environmental risk factors. We recently identified a previously unstudied *C. elegans* gene, *swip-10*, in a screen for genetic determinants of dopamine (DA) signaling, finding that glial expression of *swip-10* is required to limit glutamate-dependent DA neuron excitability and excess DA release. Further studies demonstrated that *swip-10* missense and deletion mutations result in the degeneration of glial ensheathed DA and glutamate secreting neurons, with reporter and genetic studies providing evidence of systemic oxidative and metabolic stress. The putative mammalian ortholog of *swip-10* is the gene *Mblac1*, whose endogenous activity has been found to involve 3' RNA processing of replication-dependent histones required for cell cycle control. Importantly, MBLAC1 has been recently identified in GWAS studies as a risk factor for neurodegenerative disease, specifically Alzheimer's disease with cardiovascular disease (ADCD). Moreover, frontal cortex mRNA levels of MBLAC1 were found to be significantly reduced in those dying with ADCD. As neurons in the adult worm (and human brain) are largely post-mitotic, the physiological, behavioral and neurodegenerative impact of *swip-10* suggests a contribution to cellular programs, beyond cell cycle control, that contribute to neuronal signaling and health. Here, we initiate studies of neurodegenerative disease risk using tissues and cultured glia/neurons from MBLAC1 KO mice. Our preliminary qPCR analysis of 20-week old male mouse prefrontal cortex suggest the elevation of genes linked to neuroinflammation and oxidative stress, comparing KOs vs WT littermates. Cultured mouse embryonic fibroblasts also indicate the presence of oxidative stress as detected by ratios of reduced to oxidized glutathione. Future studies will extend these findings to other brain regions, assessments in older animals of both sexes, and an evaluation of contributions of candidate pathways beyond cell cycle control, including pathways currently being assessed in *swip-10* mutant worms. Moreover, as MBLAC1 protein has been shown to be a CNS target of the beta lactam antibiotic ceftriaxone, a molecule

with neuroprotective function, we hypothesize that ceftriaxone interactions may induce neuroprotective pathways.

Disclosures: **J. LaMar:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Ed and Ethel Moore Research Program, Florida Department of Health and Schmidt Family Foundation. **M.J. Gross:** None. **S.J. McGovern:** None. **P. Rodriguez:** None. **H.A. Mesa:** None. **Q. Zhang:** None. **M.K. Hahn:** None. **R.D. Blakely:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Ed and Ethel Moore Research Program, Florida Department of Health and Schmidt Family Foundation.

Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 195.02

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: Ed and Ethel Moore Alzheimer's Research Foundation, Florida Dept. of Health

Title: Glial-expressed swip-10supports neuronal signaling and health in Caenorhabditis elegans via a novel copper reductase pathway that regulates global mitochondrial function and metabolic activity

Authors: *P. RODRIGUEZ, Jr.[1], Z. GICHI[1], V. KHALIA[3], J. A. LAMAR[4], C. D. MATIER[5], C. J. CHANG[5], G. W. MILLER[3], A. T. PEZACKI[5], A. KHAMOUI[2], R. D. BLAKELY[4]; [1]Florida Atlantic Univ., Jupiter, FL; [2]Florida Atlantic Univ., Boca Raton, FL; [3]Columbia, New York, FL; [4]Biomed. Sci., Florida Atlantic Univ. Grad. Neurosci. Training Program, Jupiter, FL; [5]Univ. of California, Berkeley, Berkeley, CA

Abstract: Through a forward genetic screen in the nematode *C. elegans* designed to reveal molecular determinants of dopamine (DA) signaling, we identified the previously unstudied enzyme *swip-10* whose disrupted expression in glia results in DA-dependent swimming-induced paralysis (Swip) as well as premature DA neuron degeneration (Hardaway et al, 2015; Gibson et al, 2018). Recently, the putative mammalian ortholog of *swip-10, MBLAC1* was found to encode a 3’ endoribonuclease, processing replication-dependent histone pre-mRNA transcripts to allow for nuclear export (Pettinatti et al, 2018). As glia supporting *swip-10* expression are postmitotic, we hypothesized that SWIP-10/MBLAC1 protein support an activity beyond that of histone-dependent cell cycle control. Recently, H3 histones have been found to encode a copper reductase (Cu^{2+}◊Cu^{1+}) activity, independent of their direct role in chromatin modelling. Cu dyshomeostasis has been linked to numerous neurodegenerative diseases (ND’s) for more than

50 years. Moreover, MBLAC1 has been implicated via GWAS studies as a risk factor for Alzheimer's disease with cardiovascular disease comorbidity (ADCD) with reduced MBLAC1 expression detected in ADCD frontal cortex (Broce et al, 2019). Using whole worm metabolomic approaches, reporter strains, biochemical assays and transgenic reporter lines, we report that *swip-10* effects extend well beyond their glial sites of expression, including global support of mitochondrial respiration and prevention of oxidative stress. As Cu^{1+} is required to sustain mitochondrial function and suppression of oxidative stress, we hypothesized that SWIP-10/MBLAC1 exerts control over glial, neuronal and systemic metabolic function via regulation of histone-dependent Cu^{1+} production and export. In support of this hypothesis, we demonstrate that *swip-10* mutants exhibit reduced staining with the Cu^{1+} selective dye CF4. Moreover, worms grown on the Cu^{1+} selective chelator bathocuproinedisulfonic acid display Swip and DA neuron degeneration. Additionally, both treatment of *swip-10* mutants with the Cu^{1+} chaperome elesclomol and glial-specific expression of WT *swip-10* into *swip-10* mutants rescues Swip and neural degeneration. Normalization of selected mRNAs and genetic/chemical reporters for systemic oxidative stress impacted by *swip-10* mutation can also be normalized by these manipulations. Studies in cells and tissues derived from MBLAC1 KO mice and crosses of these animals to AD mutant lines are underway to seek translation of our model of Cu^{1+} dyshomeostasis in *swip-10* to mammals and their potential significance for human neurodegenerative diseases and their treatments.

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Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 195.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG054214
NIH/NIA
Rainwater Charitable Foundation

Title: Trem2-apoe pathway in alzheimer's disease: r47h exerts differential effects on the apoe3 and apoe4 background in a tauopathy model

Authors: *G. CARLING, P. YE, M. WONG, L. FAN, R. HOROWITZ, K. NORMAN, S. GONG, S. SINHA, W. LUO, L. GAN;
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Abstract: Alzheimer's disease (AD) is the most common form of dementia. Inflammation prompted by microglia may precede the spread of pathogenic tau, and most AD risk genes are

highly expressed in microglia, supporting a significant role for inflammation in disease. The *R47H* variant of the microglial *Trem2* receptor elevates AD risk by 2-4 fold. Inflammation requires metabolic reprogramming to provide the energy and substrates needed for activation, and mitochondrial dysfunction could contribute to aberrant inflammatory responses in AD. APOE, encoded by the strongest AD risk gene, is a known ligand of the *Trem2* receptor, and *Trem2* activation robustly increases microglial *APOE* expression, as well as affecting cellular metabolism and increasing inflammation. However, little is known about the mechanistic interactions between risk variants *R47H* and *APOE4*. We investigated the effects of *R47H* on tau-triggered inflammatory and metabolic response in primary microglia on either the *APOE4* or *APOE3* background. Our data showed that *R47H* elevates inflammatory responses with increased mitochondrial respiration on the E3 background but lowers inflammatory response and impairs mitochondrial function on the E4 background. In addition, inhibition of Akt, a kinase downstream of *TREM2* activation that regulates both metabolism and inflammation, reverses the elevated inflammatory responses and mitochondrial metabolism in R47H-E3 *in vitro* and rescues synaptophysin loss in a *PS19 R47H/+* mouse model. Single nuclei RNA-sequencing of mouse hippocampal tissue reveals that *R47H* differentially regulates subclusters on the E3 and E4 background in multiple cell types including microglia, astrocytes, and oligodendrocytes, and confirms a decrease in genetic signatures of inflammation in an R47H-E4-enriched microglial subcluster. Further investigation of the differential effects of *R47H* on E4 or E3 microglia will shed new light into *APOE* isoform-specific effects on inflammation and metabolism in AD and inform on the mechanistic involvement of Akt in this pathway.

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Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 195.04

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIA 5R01AG071785
NINDS 5T32NS061788
Alzheimer's of Central Alabama (ACA) Pre-doctoral Fellowship
NIGMS 5T32GM008361

Title: The long non-coding RNA neat1 in aging and Alzheimer's disease

Authors: *A. IRWIN[1], R. BAHABRY[2], R. STUCKEY[2], F. D. LUBIN[3];
[1]Med. Scientist Training Program, UAB, Birmingham, AL, [2]Univ. of Alabama at Birmingham, Birmingham, AL; [3]Dept. Neurobiol, Univ. Alabama Birmingham, Birmingham, AL

Abstract: Normal aging and Alzheimer's disease (AD) are associated with alterations in epigenetic mechanisms in the hippocampus; yet cell-type specific changes in long non-coding RNAs (lncRNAs) underlying aging and AD related memory impairments are not well understood. We have previously demonstrated that *Neat1* expression levels are significantly increased in the aging hippocampus and drives histone H3K9 dimethylation mediated transcriptional silencing of memory-related genes. Additionally, siRNA *Neat1* knockdown in area CA1 of the hippocampus is sufficient to improve hippocampus-dependent memory deficits in aged mice. Interestingly, *in silico* analysis of the single-cell RNA sequencing database by Grubman and colleagues revealed that *Neat1* is overexpressed in specific cell-types in the entorhinal cortex of human AD brains. Therefore, we sought to determine whether *Neat1* was overexpressed in a cell-type specific manner within hippocampal subfields and if these cell-type specific changes contribute to age-related memory impairments. Using fluorescent in situ hybridization, we found that the proportion of astrocytes expressing *Neat1* increases in all hippocampal subfields of aged (18-24 months) compared to young (3-6 months) C57BL/6 mice. The average *Neat1* expression of hippocampal astrocytes is also increased with age, supporting the hypothesis that astrocyte specific changes in *Neat1* expression is correlated with memory impairments. In the hAPP-J20 transgenic mouse model of AD, we observed elevated hippocampal *Neat1* expression and increased histone H3K9 dimethylation. Similar to aged animals, the proportion of astrocytes expressing *Neat1* was significantly increased in the hippocampus of hAPP-J20 mice. Pancellular siRNA *Neat1* knockdown in area CA1 of the hippocampus was sufficient to rescue hippocampus-dependent memory in the hAPP-J20 mouse (4-6 months). Overall, these findings suggest that A-beta excess may accelerate the aging-related transcriptional state of hippocampal astrocytes contributing to memory dysfunction in normal aging and AD.

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Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 195.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA RF1 Grant AG056114
NIH/NIMH BRAIN Initiative UO1 Grant MH117079

Title: Genetic sparse labeling to characterize the morphology and pathology of disease-relevant cell types in the 5xFAD Alzheimer's disease mouse model

Authors: *A. DE LA ROCHA[1], C. PARK[1], C. LEE[1], X. YANG[1,2];
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Abstract: Alzheimer's disease (AD) is an age-related neurodegenerative disorder with no disease-modifying therapies. Our current understanding of the neuronal morphological degeneration and the microgliosis is limited due to challenges visualizing and quantifying the complete, detailed morphology of individual neurons and plaque-associated microglia. The goal of this study is to apply a novel genetic sparse labeling method to characterize the morphology of relevant cell types in the 5xFAD mouse model of amyloid deposition. We have previously developed a novel BRAIN Initiative technology called Mononucleotide Repeat Frameshift (MORF) mice, which confer Cre-dependent, sparse and stochastic, and ultra-bright labeling of genetically-defined neurons and glia cells brainwide (Veldman et al., 2020, PMID: 32795398). Here we crossed our newest generation, MORF3, with cortical neuronal and microglial CreER lines in 5xFAD to characterize the morphological changes of these disease-relevant cell types at single-cell resolution. We developed imaging pipelines, including light-sheet imaging of tissue-cleared brain hemispheres, to enable the analysis of MORF3-labeled neurons and microglia in the intact brains. We demonstrated the morphological differences between homeostatic and plaque-associated microglia in the context of amyloid deposition. Our ongoing analyses include digital reconstruction of MORF-labeled brain cells in wildtype and 5xFAD background to further characterize the morphological defects of cortical neurons and microglia at single-cell resolution, and examination of the relationships between such cellular defects to other known AD-like pathology (e.g. amyloid plaques and dystrophic neurites). Our study provides a proof-of-concept that MORF3 mice are a powerful new tool to illuminate the neuronal and glial cell pathology at single-cell resolution and brain-wide scale in mouse models of neurodegenerative disorders.

Disclosures: **A. De La Rocha:** None. **C. Park:** None. **C. Lee:** None. **X. Yang:** None.

Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 195.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RF1 AG061872
RF1 AG061729
P30 AG044271
P30 AG013319

Title: Effects of eliminating microglia in the context of Alzheimer's disease through the lens of lipidomics

Authors: *Z. XU, J. P. PALAVICINI, S. HE, A. BHATTACHARJEE, X. HAN;
Barshop Inst. - UT Hlth. San Antonio, San Antonio, TX

Abstract: Microglia act as immune cells in the brain and help clear cellular debris, its activation leads to neuroinflammation, which if chronic, could be detrimental in Alzheimer's disease (AD).

Yet, whether eliminating microglia can be beneficial for AD is still controversial. In this study, we first conducted shotgun lipidomics analysis using human postmodern brains (control, n=10; AD, n=10), and discovered an abnormal accumulation of specific phospholipid cleavage products known as lyso-phospholipids (LPLs) in Brodmann area 38 (BA38) in AD cases compared to cognitively normal controls. Traditionally, LPLs are considered key markers for neurodegeneration diseases since they have pro-inflammatory roles. The production of LPLs is through the cleavage of membrane phospholipids, including the two most abundant ones: phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Both PC and PE contain a hydrophilic head group and two hydrophobic acyl chains at sn-1 and sn-2 positions, which can be cleaved upon phospholipases activation or reactive oxygen species (ROS) attack. Given the role of microglia in generating ROS and mediating neuroinflammation, we initially hypothesized that the abnormal LPLs accumulation is driven by activated microglia in AD. To test the hypothesis, we tried to eliminate microglia using PLX5622 in the 5xFAD mouse model (5xFAD, n=8; 5xFAD+PLX5622, n=8), and test if this would rescue the abnormal lipids metabolism in the brains of AD mice. To our surprise, eliminating microglia did not attenuate LPLs accumulation, in contrast it actually exacerbated LPE accumulation in a lipid species-specific manner, leading to (1) an overt accumulation of neuronal-enriched sn-2 LPE species generated by oxidative stress, and (2) an accumulation of sn-1 LPE species generated by increased phospholipase A2 cleavage, presumably within neurons. These results strongly suggest the microglia have protective roles in suppressing neuronal oxidative stress and provide evidence that eliminating microglia may aggravate some aspects of AD pathology. This study provides novel insights into the role of microglia in regulating brain homeostasis, particularly in regards to preventing neuronal oxidative stress.

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Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 195.07

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH Grant 1RF1AG061774-01
Alzheimer's Association Grant AARG-18-529336
BrightFocus Foundation Postdoctoral Fellowship A2022028F

Title: Amelioration of sleep disruptions by targeting GABAergic neurons reprograms microglia and ameliorates pathological phenotypes in an Alzheimer's Disease model

Authors: *Q. ZHAO[1], M. MACI[1], M. MILLER[1], H. ZHOU[1], M. ALGAMAL[1], Y. LEE[1], S. HOU[1], F. ZHANG[2], H. LE[1], A. RUSS[1], E. LO[2], D. GERASHCHENKO[3], S. GOMPERTS[1], B. BACSKAI[1], K. KASTANENKA[1];

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Abstract: Alzheimer's patients exhibit memory disruptions and profound sleep disturbances, including disruption of deep non-rapid eye movement (NREM) sleep. Slow-wave activity (SWA) is a major restorative feature of NREM sleep and is important for memory consolidation. We examined the sleep architecture in APP mice, an animal model of Alzheimer's disease (AD). APP mice exhibited impairments in sleep architecture including decreased time spent in NREM sleep, decreased delta power, and increased sleep fragmentation compared to nontransgenic controls. Optogenetic stimulation of cortical GABAergic interneurons restored SWA and rescued sleep impairments. Furthermore, it slowed Alzheimer's progression by reducing amyloid deposition, normalizing neuronal calcium homeostasis, and improving memory function. These changes were accompanied by morphological transformation of microglia, elevated phagocytic marker expression, and enhanced Aβ phagocytosis. In summary, our study shows that optogenetic targeting of GABAergic interneurons rescues sleep disturbances, reprograms microglia responsivity, and ameliorates neuropathological as well as behavioral deficits in an AD mouse model.

Disclosures: Q. Zhao: None. **M. Maci:** None. **M. Miller:** None. **H. Zhou:** None. **M. Algamal:** None. **Y. Lee:** None. **S. Hou:** None. **F. Zhang:** None. **H. Le:** None. **A. Russ:** None. **E. Lo:** None. **D. Gerashchenko:** None. **S. Gomperts:** None. **B. Bacskai:** None. **K. Kastanenka:** None.

Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 195.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Joint Canada-Israel Health Research Program

Title: Astrocytes induce cognitive enhancement in Alzheimer mouse model

Authors: *T. KREISEL MERZEL, I. GOSHEN;
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Abstract: Long-lasting memories define who we are and how we experience the world. Inability to form and access these memories, as in neurodegenerative conditions such as Alzheimer's disease (AD), degrade the quality of life and impose a major burden on healthcare systems and society. Unfortunately, medical treatments that can prevent, slowdown, or reverse memory deficits are almost non-existent. To develop new strategies for targeting diseases associated with memory impairments, a better understanding of how the brain acquires and encodes memories is urgently needed, and novel mechanisms must be effectively harnessed for therapeutic value.

Interestingly, several studies reported abnormalities in both the number and function of *astrocytes* in human AD patients and in animal models of this disease. Recently, work from our lab and others has revealed unique abilities of astrocytes to communicate with, and affect, neurons in the brain. Specifically, we have found that in normal mice direct astrocytic activation using chemogenetic or optogenetic tools is sufficient to induce hippocampal long-term synaptic potentiation and activation of astrocytes during learning improves memory allocation, resulting in enhanced recall. To test whether astrocytes can increase cognitive function not just in normal mice but also in impaired memory in Alzheimer's model mice, we expressed the Gq-coupled designer receptor hM3Dq which allowed their time-restricted manipulation by the application of the designer drug clozapine-N-oxide (CNO), in the CA1 of 5XFAD mice. Astrocytic activation during the learning sessions in a radial arm water maze task (RAWM) resulted in a significant improvement in memory in 5XFAD mice. In addition- astrocytic activation caused a decrease in beta-amyloid (Aβ) plaques in the CA1. Moreover, chronic CNO administration in the drinking water caused an even higher improvement in memory and a decrease in Aβ plaques, that was accompanied by a decrease in microglia number and an increase in cfos in neurons. The 5XFAD mice not just showed enhanced memory but reached the same level as the controls, that have improved themselves. In summary, astrocytic activation can rescue memory performance after it already deteriorated, and partially clear existing Aβ plaques.

Disclosures: **T. Kreisel Merzel:** None. **I. Goshen:** None.

Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 195.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant NS085171
NIH grant AG065290
Neurodegeneration Consortium at MDAnderson

Title: A role for astrocytes in thalamocortical network dysfunction in Alzheimer's disease

Authors: ***J. CAMPBELL**[1], R. JAGIRDAR[1], N. RIVERA[2], M. BEIERLEIN[2], J. CHIN[1]; [1]Baylor Col. of Med., HOUSTON, TX; [2]Univ. of Texas Hlth. Sci. Ctr. at Houston, HOUSTON, TX

Abstract: As the aging population increases, the prevalence of age-related diseases such as Alzheimer's disease (AD) also rises. However, effective strategies to mitigate the devastating effects of AD are still lacking. This may be in part because by the time individuals are diagnosed with mild cognitive impairment or AD and begin treatment, significant pathological alterations have already taken place in the brain. However, new insights into the early phases of AD progression indicate that there are preclinical manifestations of the disease, sleep disruptions in

particular, that can go unnoticed but may critically contribute to disease progression. Therefore, a better understanding of how AD-associated sleep disturbances arise can provide insights into earlier treatment options that improve AD outcomes. Glial cells have been implicated as major players in both healthy brain function and in disease. Many investigations into the AD-related roles of glial cells have focused on the impact they have on hippocampal circuits or their interactions with pathological hallmarks of AD such as amyloid plaques and neurofibrillary tangles. However, their potential role in the development of sleep disturbances that arise early in disease progression has received less attention. Previously our lab demonstrated that hypo-functioning of the thalamic reticular nucleus (TRN) is linked to disruptions in sleep maintenance and slow wave sleep in transgenic mice expressing mutant human amyloid precursor protein (APP). Sleep disruptions in APP mice were observed by 2 months of age, just prior to the onset of cognitive deficits and months before the deposition of amyloid plaques. However, to date there is still no consensus regarding the mechanisms causing disruption of TRN function and related thalamocortical circuits. Given the prominent roles of astrocytes in regulating neuronal function, and their known importance for sleep, we examined whether astrocytes were altered in the TRN of APP mice. Immunohistochemical experiments demonstrated that although the overall numbers of astrocytes remained largely stable in the TRN of APP mice, the numbers of GFAP-expressing astrocytes were increased, particularly in the somatosensory segment of the TRN. Moreover, GFAP-expressing astrocytes exhibited alterations in morphology. These data suggest that the number of reactive astrocytes is increased in APP animals, indicating that their ability to influence neuronal function may be altered. Taken together, interactions between neurons and astrocytes may play key roles in shaping neuronal activity in the TRN, with implications for sleep and sleep-associated disruptions in AD.

Disclosures: **J. Campbell:** None. **R. Jagirdar:** None. **N. Rivera:** None. **M. Beierlein:** None. **J. Chin:** None.

Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

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Program #/Poster #: 195.10

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH Grant RF1 AG054223
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NIH Grant R01 AG063175

Title: Bin1 regulates pathology-specific microglial activation in two models of Alzheimer’s disease.

Authors: *A. SUDWARTS[1], M. HANSEN[1], S. WANG[1], S. SMIRNOU[1], G. THINAKARAN[2]; [2]USF Morsani Col. of Med., [1]USF Byrd Alzheimer's Inst., Tampa, FL

Abstract: Recent GWAS studies have identified key late-onset Alzheimer's disease (LOAD) risk genes as being highly or specifically expressed in microglia. This has sparked a deluge of investigations into microglial phenotypes in post-mortem Alzheimer's disease (AD) brains, and mouse models of AD and inflammation. Bridging Integrator 1 (*BIN1*) contains the second-most significant risk locus for LOAD. BIN1 transcript and protein levels are high in microglia, although it is expressed in oligodendrocytes and neurons as well.
Recently, we demonstrated that BIN1 is essential for microglial inflammatory responses and Alzheimer's 'disease-associated microglial' (DAM) phenotype transition. Our current work investigates the role of microglial BIN1 in modulating the pathogenesis of two AD models. Using a *Cx3cr1*^{CreER} driven Cre-Lox system, we deleted *Bin1* from microglia of PS19 and 5xFAD mice (tau and amyloid pathology, respectively). The loss of microglial *Bin1* decreased levels of phosphorylated tau in PS19 mice, suggesting that microglial BIN1 function is detrimental in tau pathology. However in 5xFAD mice, protein levels of amyloid plaque-induced DAM markers (e.g. surface CD11c, TREM2) were blunted by the loss of *Bin1*, demonstrating BIN1's key involvement in microglial transition into neuroprotective phenotypes during amyloid pathology. RNA sequencing identified crucial microglial mechanisms which are dependent on BIN1. Importantly, BIN1 regulated different pathways in each pathology, demonstrating the role of this key AD risk gene in orchestrating the insult-specific responses of these environmentally sensitive cells. The involvement of microglial phentoype-specific surface receptors in these pathways provides exciting targets for lead identification and therapeutic development.

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Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 195.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Defining hippocampal astrocyte dysfunction during progressive tauopathy

Authors: *R. MCREYNOLDS, III, Y. KOMURO, A. GLEICHMAN, T. CARMICHAEL, J. HINMAN;
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Abstract: Age-associated, region-specific changes in glial-cell gene transcription and activation are common symptoms during the prodromal stage of Alzheimer's disease (AD). A growing body of research suggests hippocampal astrocytes exhibit atrophy-associated changes evidenced by alterations to cytoskeletal glial fibrillary acidic protein (GFAP) staining. While these atrophy-associated changes have been observed in amyloid-based models of AD, whether astrocytes exhibit a similar phenotype in tau-mediated neurodegeneration remains unexplored. **The aim of this study is to investigate cytoskeletal, morphological, and transcriptional changes**

occurring in hippocampal astrocytes during progressive tauopathy as modeled by the P301S mouse model. We hypothesize that distinct morphological changes in hippocampal astrocytes precede the development of tauopathy and are driven by defined molecular pathways active at the transcription level. Using an adeno-associated virus, that encodes a membrane associated and cytosolic modified GFP protein containing V5 epitope tags (smV5), we sparsely labeled the full morphology of astrocytes. Visualization of finer, non-GFAP-filled processes enables greater insight into morphological changes associated with progressive tau accumulation. Surface area and volume of cytoskeletal and morphological astrocytic features were measured by co-registered GFAP immunoreactivity and viral smV5 expression. We find regional variation in GFAP surface area and volume as well as primary process number within the subregions of the hippocampus in wild-type and P301S mice. To profile the transcriptional changes in astrocytes, we injected pCDH-GFAP-Rpl10a-HA into the hippocampus resulting in HA-tagged ribosomes within astrocytes enabling the identification of actively transcribed genes and gene modules associated with astrocyte function and morphology during tauopathy. In wild-type mice, astrocyte vTRAP labeling results in the enrichment of astrocytic marker genes (GFAP, ALDH1L1) compared to input hippocampus samples. The application of vTRAP together with comprehensive morphologic labeling of astrocytes in P301S mice will identify molecular pathways driving structural changes in astrocytes. Early and progression-dependent astrocytic morphological and cytoskeletal atrophy could drive AD pathology by astrocytes retracting their perisynaptic astrocytic processes, endfeet, or disruption of intercellular channels implicating a new mechanism of vulnerability to AD.

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Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

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Program #/Poster #: 195.12

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH Grant R01DK031036
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NIH Grant R01MH125903

Title: Brain Cell type-specific insulin resistance exacerbates Alzheimer’s disease-like phenotypes in mice

Authors: *W. CHEN[1], W. CAI[2,1], Q. HUANG[2], M. WONG[2], K. LAZDON[3], D. FRENKEL[3,1], C. KAHN[1,4];
[1]Joslin Diabetes Center, Harvard Med. Sch., Boston, MA; [2]New York Inst. of Technol., Old Westbury, NY; [3]George S. Wise Fac. of Life Sci., Tel Aviv Univ., Tel Aviv, Israel; [4]Dept. of Med., Harvard Med. Sch., Boston, MA

Abstract: Brain insulin signaling controls peripheral energy metabolism and also plays a key role in the regulation of mood and cognition. Dysregulation of this signaling has been implicated in several brain disorders, including neurodegenerative diseases such as Alzheimer's disease (AD). Epidemiological studies have indicated a strong connection between type 2 diabetes (T2D) and AD and suggested that insulin resistance is a major risk factor for AD and other forms of dementia. Litter is known, however, of exactly how insulin resistance in the brain contributes to the comorbidity of T2D and AD. Here, we aim to understand the roles of insulin signaling in AD progression, with a particular focus on astrocyte and microglia, two major disease-associated brain cell types that are heavily implicated in AD pathology. To this end, we established several new mouse models, by crossing 5xFAD transgenic mice, a well-recognized AD mouse model that expresses 5 familial AD mutations, with mice that have selective insulin receptor (IR) deficiency in neurons (NIRKO), astrocytes (iGIRKO), or microglia (MG-IRKO). We hypothesize that insulin resistance in selective brain cell types drives comorbidity of T2D and AD. We performed behavioral and biochemical characterizations at several key time points (3- and 6-month age) that allow for the understanding of the dynamic role of insulin signaling in AD progression. Our results show that iGIRKO/5xFAD mice exhibited greater cognitive impairment at 6-month age than 5xFAD mice in the Y maze. These mice also exhibit abnormal contextual fear conditioning. This was associated with an elevated expression of AD risk genes and key blood-brain barrier genes in brain extracts of these mice. By mapping Aβ deposits in the whole brain using a tissue CLARITY approach, we generated a brain atlas that allows for the identification of selective brain areas that show the highest level of vulnerability to Aβ toxicity. To understand the mechanisms that contribute to these AD-like phenotypes, we infected primary astrocytes with adenovirus to knockout IR in vitro. This resulted in the loss of astrocytic insulin signaling, impaired Aβ uptake, and reduced ATP production and glycolic capacity. In conclusion, we find that insulin signaling in the brain, especially in astrocytes, contributes to AD pathology, highlighting the importance of a deeper understanding of insulin signaling in different brain cell types in brain disorders. This may also lead to the development of new cell-specific therapeutics for patients with T2D and AD.

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Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

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Program #/Poster #: 195.13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant NS085171 (JC)
NIH grant AG065290 (JC and MB)
Neurodegeneration Consortium at MD Anderson (JC and MB)

Title: Dysregulation of non-neuronal cells in the thalamic reticular nucleus of human amyloid precursor protein transgenic mice.

Authors: *R. JAGIRDAR[1], J. R. CAMPBELL[1], M. BEIERLEIN[2], J. CHIN[1];
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Abstract: Sleep-related issues such as disrupted sleep patterns and decreased amount of sleep are commonly observed in patients with Alzheimer's disease (AD). However, the network mechanisms underlying sleep disruptions are not well understood. The thalamic reticular nucleus (TRN), a major control nucleus in the thalamocortical network, is implicated in the generation of spindles during slow wave sleep (SWS) and plays a vital role in the maintenance of NREM sleep. We previously found that activity in the TRN is reduced in transgenic mice that express mutant human amyloid precursor protein (APP), and that chemogenetic activation of TRN neurons improved sleep maintenance and enhanced SWS sleep. Moreover, chronic activation of TRN led to sustained improvement of sleep architecture, and reduced amyloid-beta (Aβ) plaque accumulation. Reduced expression of an activity-dependent marker in human post-mortem samples indicated that similar changes in the activity of TRN neurons may also occur in patients with AD. To gain insight into the molecular mechanisms that influence the activity of neurons in the TRN, we performed RNA-sequencing of microdissected TRN from APP mice and nontransgenic (NTG) littermate controls, followed by ClueGO analysis of gene function. In addition to several neuronal genes that could influence neuronal activity, we found striking alterations in genes enriched in non-neuronal cell types, including oligodendrocytes, microglia, and astrocytes. The proportion of genes found to be altered that are involved in proliferation and differentiation of oligodendrocytes precursor cells (OPC), active myelination, and myelin development were particularly notable. These results suggest that alterations in myelin could contribute to reduced activity in the TRN. In addition, several differential genes we identified in the TRN of APP mice encode components of the perineuronal nets that surround parvalbumin-expressing inhibitory neurons including those in the TRN. Such components are secreted not only by neurons but also by astrocytes and oligodendrocytes. Together, these results suggest that altered states or functions of non-neuronal cell types may contribute to the reduction of neuronal activity in the TRN, with consequences for the maintenance of normal sleep patterns.

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Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

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Program #/Poster #: 195.14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1R01AG066489-01A1

Title: Proteomic analysis of inflammatory markers in the TgF344 rat model of Alzheimer's disease.

Authors: *M. L. GARCIA[1,3], C. M. HERNANDEZ[2], N. L. JACKSON[3], L. L. MCMAHON[3];
[1]Cell. Developmental Integrative Biol., [2]Neurobio., Univ. of Alabama, Birmingham, Birmingham, AL; [3]Neurosci. Dept., Med. Univ. of South Carolina, Charleston, SC

Abstract: Alzheimer's disease (AD) is the sixth leading cause of death in the United States. The greatest risk factor for developing AD is aging, and as our aging population is expected to rise, so too will the incidences of AD. Unfortunately, there are few advances in treatment options, and this is further complicated by limitations in rodent models that recapitulate human AD both at the level of histopathology and behavioral phenotypes. The TgF344-AD model is the most comprehensive rodent model of AD to date. This model exhibits AD-like pathologies in an age-dependent manner, including amyloid beta pathology and hyperphosphorylated tau, synaptic dysfunction, neuronal loss, increased neuroinflammation, and impaired learning and memory, increased anxiety, and abnormal fear extinction. The TgF344-AD rats display increased brain inflammation as early as six months of age shown by IHC and protein blot analysis of major markers of inflammation including GFAP, Iba1, and several cytokine markers. However, a more comprehensive inflammatory analysis could aid in the understanding of pathological and cognitive dysfunction seen in later stage disease. Thus, we have begun to use proteomics analysis of cytokines, pro-inflammatory receptors, and immunomodulatory markers in hippocampal and serum samples from TgF344-AD rats and find modest changes in several markers that will direct future studies in an attempt to find new therapeutic targets.

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Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA grant U54 AG054345
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NIA grant K01 AG054753

Title: Inpp5d deficiency attenuates amyloid pathology in a mouse model of alzheimer's disease

Authors: *P. B.-C. LIN, A. P.-Y. TSAI, D. SONI, A. LEE-GOSSELIN, M. MOUTINHO, S. PUNTAMBEKAR, G. E. LANDRETH, B. T. LAMB, A. OBLAK;
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Abstract: Introduction: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by accumulated beta-amyloid (Aβ) deposits and robust microgliosis. Recent genome-wide association (GWA) studies identified genetic risk factors in late-onset AD (LOAD) which are related to innate immunity. Among them, an intronic variant of Inositol polyphosphate-5-phosphatase D (INPP5D) confers an increased risk of developing AD (rs35349669; OR=1.08, 95%CI=1.06-1.11). The increased INPP5D expression in LOAD is associated with increased plaque deposition. As a microglia-specific lipid phosphatase, INPP5D negatively regulates signaling via several microglial cell surface receptors, including TREM2; however, the impact of INPP5D inhibition on AD pathology remains unclear. Methods: We utilized the amyloidosis mouse model, 5xFAD, expressing *Inpp5d* haplodeficiency to assess how INPP5D inhibition regulates amyloid pathogenesis. The NanoString GeoMx whole transcriptome atlas assay was conducted to determine the spatial transcriptomic profiles modulated by Inpp5d deficiency during the plaque development. Results: *Inpp5d* deficiency perturbs microglial intracellular signaling pathways that regulate the immune response, including phagocytosis, microglia-plaque engagement, and Aβ clearance. Importantly, *Inpp5d* haploinsufficiency leads to the preservation of cognitive function in 5xFAD mice. Furthermore, a novel spatial transcriptomic analysis revealed genetic profiles altered by *Inpp5d* haploinsufficiency are related to microglial functions, synaptic regulation, and immune cell activation. Conclusion: These data demonstrate that Inpp5d deficiency enhances microglial functions by increasing plaque clearance and preserves cognitive abilities in 5xFAD mice. Inhibition of INPP5D may serve as a potential therapeutic strategy targeting microglia for AD.

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Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

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Program #/Poster #: 195.16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Institute of Neurological Disorders and Stroke R21NS105437
National Institute of Neurological Disorders and Stroke R01NS101156
National Institute on Aging R01-AG077692

Title: Seizures in Alzheimer's disease are associated with increased microglial activation

Authors: *X. LI, S. GOURMAUD, E. SHRAYER, A. J. BARBOUR, D. J. IRWIN, F. E. JENSEN, D. M. TALOS;
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Abstract: About 45 percent of Alzheimer's disease (AD) patients experience epileptic activity throughout the course of the disease, and seizures can accelerate clinical progression to dementia, as some patients experience sudden worsening after seizure onset. Consistent with clinical observations, we and others have found that neuronal hyperexcitability contributes to AD pathology progression in both AD patients and mice (*Gourmaud et al., Brain 2022*). Besides amyloid beta and tau, AD is characterized by changes in microglial function that occur early in disease, suggesting that this cell type may play a critical role in driving AD pathology. As there is ample evidence that seizures can increase brain inflammation, we hypothesized that seizures and chronic hyperexcitability may promote microglial activation which may underlie seizure exacerbation of AD neuropathology. 5XFAD and wild-type (WT) mice were subjected to an established kindling protocol at the age of 3-3.5 months using pentylenetetrazol (PTZ) and were sacrificed three months later for brain tissue analysis (n=9-19/ group). Post-mortem temporal and frontal cortex samples from 10 control subjects and 19 AD patients (9 with seizure history, 10 without known seizures) were obtained from the Penn Center for Neurodegenerative Disease Research (CNDR). Quantitative Iba-1 immunohistochemistry was employed to examine regional microglial densities. We found increased Iba-1 coverage in the hippocampus of 5XFAD mice relative to WT mice (p < 0.0001), with kindling further elevating Iba-1 expression within the 5XFAD group (p < 0.01). However, kindling did not affect Iba-1 expression in WT mice. Cortex tissue analysis from the same cohorts revealed a similar significant elevation of Iba-1 coverage in the 5XFAD mice (p < 0.05), which was further exacerbated by kindling (p < 0.001). Again, Iba-1 coverage did not change after kindling in WT mice. Increased microglial densities were also observed in brain tissue from AD patients, both in the temporal lobe (p < 0.0001) and frontal lobe (p < 0.01) cortex, and to a greater extent in tissue from patients with a clinical history of epilepsy and AD (p < 0.05 and p < 0.001, respectively). Our data suggest that in AD, microglia proliferation and activation are augmented by seizures, providing novel insight into the underlying pathophysiology of AD with comorbid epilepsy. Identification of novel mechanisms and signaling pathways of microglial dysfunction that are engaged by seizures may reveal novel targets with high therapeutic potential for disease modification in AD.

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Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 195.17

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: Development of novel rabbit monoclonal antibodies to characterize microglial activation states in murine models of Alzheimer's disease

Authors: *A. AIELLO, R. GRAY, V. E. BAIN, A. SINGH, G. INNOCENTI, S. SINGH, T. C. WIEDERHOLD, R. W. CHO;
Cell Signaling Technology, Inc., Danvers, MA

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common form of dementia worldwide. Neuroinflammation is an important feature of AD pathology, but the precise contribution of neuroinflammation on disease progression is poorly understood. Microglia, the brain's resident macrophages, are likely to play an important role in initiating and maintaining neuroinflammatory responses that contribute directly or indirectly to AD etiology. Several genome-wide association studies in human AD patients identified genes enriched or uniquely expressed in microglia. Moreover, single-cell RNA sequencing (scRNA-seq) studies identified multiple microglia-enriched genes that are upregulated in the context of disease, both in human AD tissue as well as mouse models of AD. Development of tools to these specific genes or gene products can be used to identify disease-associated microglial states and further our understanding of the specific neuroinflammatory responses that contribute to disease. We have developed and validated a cohort of rabbit monoclonal antibodies that can be used to detect these microglial gene products. Here we used multiplexing techniques to establish microglial enrichment of these targets, including ASC/TMS1, GPNMB, TMEM119, and Galectin-3, in both mouse brain tissue and mouse models of AD. Within this cohort, we highlight Cathepsin D, a lysosomal aspartyl protease involved in protein degradation that is enriched in microglia, particularly in the context of disease. Here we report co-localization with Iba1+ microglia surrounding amyloid-beta plaques within a mouse model of AD. We continue to develop a comprehensive portfolio of monoclonal antibodies to further characterize microglia cellular processes and activation states to understand the role of microglia in neurodegenerative diseases.

Disclosures: **A. Aiello:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc. **R. Gray:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc. **V.E. Bain:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc. **A. Singh:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc. **G. Innocenti:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc. **S. Singh:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc. **T.C. Wiederhold:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc. **R.W. Cho:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc..

Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 195.18

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Development of novel TREM2 rabbit monoclonal antibodies that activate downstream, Syk-associated cell signaling pathways

Authors: *J. W. HIRSCHFELD, K. LEAO, A. LAMARCA, S. ROBERTS, T. C. WIEDERHOLD, R. W. CHO;
Cell Signaling Technology, Inc., Danvers, MA

Abstract: Triggering receptor expressed on myeloid cells 2 (TREM2), a protein expressed on the surface of microglia, is genetically linked to Alzheimer's disease (AD) and a potential therapeutic target for the disease. As a myeloid cell receptor, TREM2 binds a variety of ligands, including AD-related proteins Apolipoprotein E (APOE) and amyloid-beta. Upon stimulation, TREM2 activates various cell signaling pathways that drive several microglial functions that can either ameliorate or accelerate AD progression (Gratuze et al., 2018; Leyns et al., 2017; Price et al., 2020; Takahashi et al., 2007; Takahashi et al., 2005; Wang et al., 2015; Wang et al., 2016; Zhao et al., 2018). Characterization of TREM2 signaling pathways mediating these functions, however, is incomplete. Further investigation of these signaling cascades is required to understand the function of TREM2 in AD beyond its genetic component. In order to examine the events downstream of TREM2 activation, we aimed to develop research tools to specifically stimulate TREM2. Given that antibodies can activate receptor protein pathways to drive cell signaling events, we generated a library of rabbit and mouse host monoclonal antibodies that were specific to either human or mouse TREM2. We initially screened this library by western blot to identify clones that were specific to TREM2. We then identified several clones that could activate cell signaling pathways downstream of TREM2. One of these pathways includes spleen tyrosine kinase (Syk), which is suggested to be downstream of TREM2/Dap12 microglial activation (Gratuze et al., 2018; Jay et al., 2017; McQuade et al., 2020; Schlepckow et al., 2020; Zhao et al., 2018). Syk activation was characterized using a phospho-specific Syk antibody. Having established tools to activate TREM2, we screened other downstream proteins related to signaling pathways that may be engaged by TREM2 activation. Based on our findings, we suggest that Syk phosphorylation may be a reliable readout for TREM2-dependent microglia activation. The antibodies generated and validated in this study can be leveraged to further characterize additional signaling cascades and cellular responses (phagocytosis, inflammation, proliferation, etc.) downstream of TREM2. Full characterization of TREM2-signaling cascades can be applied to AD therapeutic research, targeting the benefits of upregulating or downregulating TREM2-dependent microglial activation to attenuate AD pathology.

Disclosures: **J.W. Hirschfeld:** A. Employment/Salary (full or part-time):; Employed by Cell Signaling Technology, Inc. **K. Leao:** A. Employment/Salary (full or part-time):; Employed by Cell Signaling Technology, Inc. **A. LaMarca:** A. Employment/Salary (full or part-time):; Employed by Cell Signaling Technology, Inc. **S. Roberts:** A. Employment/Salary (full or part-time):; Employed by Cell Signaling Technology, Inc. **T.C. Wiederhold:** A. Employment/Salary (full or part-time):; Employed by Cell Signaling Technology, Inc. **R.W. Cho:** A. Employment/Salary (full or part-time):; Employed by Cell Signaling Technology, Inc..

Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 195.19

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant RF1AG068175
NIH Grant P01AG026572-Project 1
NIH Grant P01AG026572-Analytic Core
Arizona Alzheimer's Consortium Pilot Project
Packer-Wenz research endowment

Title: Astrocytic Mitochondrial Dysfunction Induces Neurodegeneration that Resembles Alzheimer's Disease

Authors: Y. MI, G. QI, F. VITALI, Y. SHANG, A. C. RAIKES, T. WANG, R. D. BRINTON, ***F. YIN**;
Univ. of Arizona, Tucson, AZ

Abstract: Mitochondria are the major cellular sources of ATP *via* oxidative catabolism of glucose or alternative fuels. A bioenergetic deficit, encompassing a decline in mitochondrial bioenergetic function and glucose hypometabolism, is associated with brain aging and emerges in early stages of neurodegenerative diseases including Alzheimer's disease (AD). However, the metabolic profile of brain cells is highly diverse with different cell types manifesting differential fuel preference and susceptibility to mitochondrial phenotypic changes. Across cell types, mitochondrial dysfunction in neurodegeneration has been best documented in neuron, and more recently in microglia, yet the pathological role of mitochondria in astrocyte, the most abundant cell type in the brain, remains to be defined. Here we report that astrocytic mitochondrial dysfunction, triggered by the conditional knockout of transcription factor A mitochondrial (Tfam), is sufficient to induce neurodegeneration resembling AD. In the astrocyte specific Tfam knockout ($Tfam^{AKO}$) mice at 6-month-of-age, deficits in recognition memory and exploratory behavior were accompanied by a decline in hippocampal long-term potentiation and reduced synaptic density and dendrite complexity. These mice were also characterized by strong reactive astrogliosis, microglial activation, and elevated levels of pro-inflammatory cytokines in the hippocampus and cortex. Moreover, $Tfam^{AKO}$ mice exhibited significantly decreased indices of white matter microstructure and myelin integrity, including lower fractional anisotropy and lower axial- and mean diffusivity. Collectively, our data show that astrocytes with dysfunctional mitochondria induce neurodegeneration that recapitulates critical features of AD and thus suggest astrocytic mitochondria as a previously underappreciated contributor to the metabolic and functional changes implicated in neurodegenerative diseases. This work has been supported by the National Institute on Aging (NIA) grants RF1AG068175 to FY, P01AG026572 (Project 1 and Analytic Core to FY), Arizona Alzheimer's Consortium Pilot Project grants to FY, and the Packer-Wenz research endowment to FY.

Disclosures: Y. Mi: None. **G. Qi:** None. **F. Vitali:** None. **Y. Shang:** None. **A.C. Raikes:** None. **T. Wang:** None. **R.D. Brinton:** None. **F. Yin:** None.

Poster

195. Alzheimer's Disease and Other Dementias: Microglia and Astrocytes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 195.20

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant RF1AG068175
NIH Grant P01AG026572-Project 1
NIH Grant P01AG026572-Analytic Core
Arizona Alzheimer's Consortium Pilot Project

Title: Aberrant Fatty Acid Degradation by Astrocytic Mitochondria as a Mechanism of Brain Lipid Droplet Accumulation and Lipid Dyshomeostasis

Authors: *G. QI[1], Y. MI[2], Y. JIN[3], H. GU[4], F. YIN[5];
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Abstract: Abundant clinical evidence has demonstrated disrupted lipid homeostasis -including the accumulation of lipid droplets (LDs)- in early stages of Alzheimer's disease (AD), and a variety of lipid metabolism genes have been identified as top risk factors of the disease. Nevertheless, how lipid dyshomeostasis and LD accumulation emerge in the degenerating brain remain elusive. Fatty acids (FAs) are the essential building blocks for nearly all lipid classes. We reported previously that APOE-ε4 (ApoE4), the greatest genetic AD risk factor, induces a metabolic shift in astrocytes towards diminished FA degradation and elevated LD accumulation. Further, FA degradation enzymes are highly enriched in astrocytic mitochondria relative to neuronal mitochondria, indicating a role of astrocytic mitochondria in brain lipid metabolism. Here, by using a mouse model of astrocyte specific suppression of oxidative phosphorylation (OxPhos), we show that while mitochondrial OxPhos is dispensable for the astrocytic bioenergetics and survival, it is indispensable for the degradation of FA and protects the brain from lipotoxicity. Astrocytic deletion of transcription factor A mitochondrial ($Tfam^{AKO}$) induced accumulations of free FAs and neutral lipids including triacylglycerol and cholesteryl esters, which were paralleled with abundant astrocyte-located LDs in the hippocampus, and to a lesser extent, the cortex. Astrocytic mitochondrion-initiated perturbation to brain lipid homeostasis was further characterized by a targeted lipidomic panel, with 101 of the 153 detected lipid species differentially expressed in $Tfam^{AKO}$ brains relative to wildtype controls, including increased levels of ceramide species and decreased levels of phosphatidylserine and phosphatidylinositol species. Our findings suggest that although astrocytic mitochondria are functionally less active (lower OxPhos activity) and bioenergetically less significant (less ATP production) than their neuronal counterparts, a modest level of OxPhos activity is required for the degradation of FA and hence the homeostasis of all lipid classes in the brain. These data provide new insights into the unique role of astrocytes in maintaining brain lipid homeostasis, and potentially, in protecting the brain from lipid-involving neurodegenerative disorders. This work has been supported by the National Institute on Aging (NIA) grants RF1AG068175 to FY, P01AG026572 (Project 1 and Analytic Core to FY), and Arizona Alzheimer's Consortium Pilot Project grants to FY.

Disclosures: **G. Qi:** None. **Y. Mi:** None. **Y. Jin:** None. **H. Gu:** None. **F. Yin:** None.

Poster

196. Models of Alzheimer's Disease II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 196.01

Title: WITHDRAWN

Poster

196. Models of Alzheimer's Disease II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 196.02

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH/NIA AG072883

Title: Cognitive integrity is associated with preservation of dendritic spines in Non-Demented individuals with Alzheimer's Disease Neuropathology

Authors: *J. GUPTARAK, A. FRACASSI, G. TAGLIALATELA;
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Abstract: Synaptic plasticity involves elimination of preexisting spines, forming and reshaping new spines as well as their stabilization. These processes refine synaptic strength, neuronal networks and learning process. The integrity of synaptic plasticity is crucial for the proper maintenance of synaptic functions and formation of neuronal networks. Disruption in synaptic plasticity can led to synaptic disfunction, improper neuronal network connection, and cognitive impair as seen in subpopulations of neurodegenerative disease including Alzheimer’s Disease (AD). AD patients exhibit high accumulation of extracellular amyloid ß plaques and neurofibrillary tangles, neuronal loss, and cognitive decline. Loss of synapses occurs prior to neuronal loss and it is highly correlated with cognitive decline in AD. Nonetheless, certain individuals, here referred to as Non-Demented with Alzheimer’s Neuropathology (NDAN), exhibits full AD-liked neuropathology however remaining cognitively intact. NDAN and AD have similar distribution pattern of Aβ plaques but the former have less degree of synaptic loss than AD patients. We previously reported that NDAN synapses are resistant to the detrimental binding of Aβ and Tau oligomers. Deposition of Aβ plaques is associated with abnormalities of dendritic spines leading to synaptic disfunction and eventually to synaptic loss. To gain better understanding of the relationship between dendritic spines and Aβ plaques, we investigated

dendritic spines morphology in frontal cortex of NDAN in comparison to AD, and aged-matched individuals using the DiI dye staining technique. Aβ plaques were labeled with either Aβ 17-24 (4G8) or Thioflavin S. Synaptic health and postsynaptic density were assessed using pCREB and PSD95 antibodies, respectively. The spine morphology, spine density as well as dendritic diameter around Aβ plaques were quantified using Imaris analysis software. Less degree of dendritic abnormalities in NDAN was observed, consistent with these individuals having less degree of synaptic loss and being cognitively intact. On the other hand, we found that the number of spines and dendritic diameters were greatly reduced when the dendrite passed through or in closed proximity of Aβ plaque in AD individuals. These results suggest that synaptic structural integrity in the face of AD pathology is maintained in NDAN individual as a possible determinant of their cognitive resilience.

Disclosures: J. Guptarak: None. **A. Fracassi:** None. **G. Taglialatela:** None.

Poster

196. Models of Alzheimer's Disease II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 196.03

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: National Institute on Aging (NIA) through the Early Adversity & Later Life Reversibility Pilot Grant to CN under Award Number R24AG06517

Title: A translational approach for novel mitochondrial mechanisms of cognitive regulation in subjects with amnestic mild cognitive impairments and early Alzheimer’s disease

Authors: *B. BIGIO[1], R. A. LIMA-FILHO[3], O. BARNHILL[4], F. SUDO[6], C. DRUMMOND[6], N. ASSUNÇÃO[6], B. VANDERBORGHT[6], F. TOVAR-MOLL[6], P. MATTOS[6,7], S. T. FERREIRA[8], B. S. MCEWEN[5], F. G. DE FELICE[9], M. V. LOURENCO[10], C. NASCA[2]; [1]Psychiatry, [2]Psychiatry, Neurosci. & Physiol., NYU Sch. of Med., New York, NY; [3]Inst. of Med. Biochem., Federal Univ. of Rio De Janeiro, Rio de Janeiro, Brazil; [5]Lab. of Neuroendocrinology, [4]Rockefeller Univ., New York, NY; [6]D’Or Inst. for Res. and Educ. (IDOR), Rio de Janeiro, Brazil; [7]Inst. of Psychiatry, Rio de Janeiro, Brazil; [8]Fed. Univ. Rio de Janeiro, Rio de Janeiro, Brazil; [9]Fed Univ. Rio De Janeiro, Rio de Janeiro, Brazil; [10]Inst. of Med. Biochem. Leopoldo de Meis, Federal Univ. of Rio de Janeiro, Rio de Janeiro, Brazil

Abstract: The current, collaborative translational work is an outgrowth of a mechanistic framework in rodents characterized by decreased levels of the pivotal mitochondrial metabolite acetyl-L-carnitine (LAC) with the corresponding cognitive deficits and depressive-like behavior (Neuron 2017, 10.1016/j.neuron.2017.09.020, PNAS 2013, 10.1073/pnas.1216100110). Here, we used computational approaches and ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) to ascertain the role of this mitochondrial signaling pathway in subjects with cognitive impairments (CI), and potential sex differences in these mechanisms. We

used available plasma samples from a well-characterized cohort of 71 subjects, including subjects with CI (i.e.: subjects with Alzheimer's disease AD and amnestic mild cognitive impairments aMCI) and in age- and sex-matched cognitively healthy controls (HC) as we described in our prior reports (Nature Medicine 2019, 10.1038/s41591-018-0275-4). Our new findings showed decreased levels of LAC in subjects with CI as compared to age- and sex-matched HC, with sex differences in carnitine levels. The degree of carnitine deficiency reflected the severity of cognitive dysfunction as assessed by using the Mini Mental Status Exam (MMSE). Using computational approaches, we found that the integration of these mitochondrial measures with canonical biomarkers (CSF levels of Ab42 and total Tau) improves diagnostic accuracy. The current findings of sex differences in carnitine deficiency in subjects with aMCI and AD suggest a possible sex-specific mitochondrial phenotype of vulnerability to AD characterized by greater severity of cognitive dysfunction. These findings compel further research on the potential role of LAC-related mitochondrial metabolism as an innovative target to identify sex-specific clinical phenotypes of AD risk and pathophysiology for more effective treatment of cognitive dysfunction.

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Poster

196. Models of Alzheimer's Disease II

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 196.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01NS094597
NIH R01NS109075
NIH P30 AG062429
NIH T32GM007752

Title: Dysregulation of neuropeptide and tau peptide signatures in human Alzheimer's disease brain

Authors: *V. HOOK[1], S. PODVIN[2], Z. JIANG[2], B. BOYARKO[2], L.-A. ROSSITO[2], A. J. O'DONOGHUE[2], R. A. RISSMAN[3];
[1]Skaggs Sch. of Pharm. and Pharmaceut. Sciences, Dept. of Neurosciences, Dept. of Pharmacol., [2]Skaggs Sch. of Pharm. and Pharmaceut. Sci., [3]Dept. of Neurosciences, UC San Diego, La Jolla, CA

Abstract: Synaptic dysfunction and loss occur in Alzheimer's disease (AD) brains which results in cognitive deficits and brain neurodegeneration. Neuropeptides comprise the major group of

synaptic neurotransmitters in the nervous system. This study evaluated neuropeptide signatures that are hypothesized to differ in human Alzheimer's disease brain compared to age-matched controls, achieved by global neuropeptidomics analysis of human brain cortex synaptosomes. Neuropeptidomics demonstrated distinct profiles of neuropeptides in AD compared to controls consisting of neuropeptides derived from chromogranin A (CHGA) and granins, VGF (nerve growth factor inducible), CCK, and others. The AD group displayed a larger number of neuropeptides derived from CHGA compared to controls. CHGA accumulates at amyloid plaques and also functions to active microglia in inflammatory responses. These findings define neuropeptide sequences whereas immunoassay data cannot define peptide sequences. The differential neuropeptide signatures indicated differences in proteolytic processing of their proneuropeptides, occurring primarily at dibasic residue sites. Notably, tau peptide signatures differed in the AD compared to age-matched control human brain cortex synaptosomes. Unique tau peptides were derived from the tau protein through proteolysis using similar and differential cleavage sites in the AD brain cortex compared to control. Protease profiles differed in the AD compared to control, indicated by proteomics data. Overall, these results demonstrate that dysregulation of neuropeptides and tau peptides occurs in AD brain cortex synaptosomes compared to age-matched controls, involving differential cleavage sites for proteolytic processing of precursor proteins. These dynamic changes in neuropeptides and tau peptide signatures may be associated with the severe cognitive deficits of AD.

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Poster

196. Models of Alzheimer's Disease II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 196.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer Society Canada
Canadian Institutes of Health Research FC-MOP 102532
Canada Foundation for Innovation FC-10307
National Institute of Aging grants RF1AG15819
National Institute of Aging grants P30AG10161
Fonds de recherche du Quebec Sante

Title: Increased concentration of insoluble Filamin A in prodromal AD in the post-mortem brain

Authors: *E. AUMONT[1], C. TREMBLAY[2], S. LEVERT[4], D. A. BENNETT[5], F. CALON[3], N. LECLERC[6];
[1]Psychology, L'Université du Québec à Montréal, Montréal, QC, Canada; [2]Pharm., Univ. Laval, Québec, QC, Canada; [3]Fac Pharm. and CRCHUQ, Univ. Laval, Quebec, QC, Canada;

[4]Neurosci., Univ. de Montréal, Montréal, QC, Canada; [5]Rush Alzheimer's Dis. Ctr., Rush Univ. Med. Ctr., Chicago, IL; [6]Neurosci., Univ. De Montreal, Montreal, QC, Canada

Abstract: The neuropathological diagnosis of Alzheimer's disease (AD) is based on the characterization of Amyloid β (Aβ) and tau aggregates through the brain parenchyma. Other proteins interact with Aβ and tau to bring about the disease processes. One such protein, Filamin A (FLNA), colocalizes with tau fibrils and mediates induction of neuroinflammation and tau hyperphosphorylation by Aβ. Our goal was to investigate the association between FLNA accumulation and the progression of AD pathology. Using parietal cortex samples from 57 subjects from the Religious Order Study, we quantified insoluble $A\beta_{42}$ ($iA\beta_{42}$) by ELISA, insoluble total tau (iTau), insoluble phosphorylated tau (ipTau) and insoluble FLNA (iFLNA) normalized on sample weight by Western blot, and neuritic plaque (NP) count with Bielschowsky silver impregnation. We also quantified synaptophysin, vesicular acetylcholine transporters (VAChT) and choline acetyltransferase (ChAT) from 35 subjects by Western blot. AD staging was based on the ABC scoring method combining Thal, Braak and the CERAD staging. Subjects with "intermediate" or "high" scores on the ABC scale were then subcategorized based on clinical diagnosis: Preclinical AD (cognitively healthy), Prodromal AD (mild cognitively impaired (MCI)) and AD dementia (ADD). The remaining subjects were classified as non-AD. We used receiver-operating characteristic for the detection of prodromal AD among MCI subjects by FLNA. We found significant positive linear correlations between iFLNA concentrations and $iA\beta_{42}$ and NP, as well as clinical, ABC, Thal and CERAD stages ($p<.05$ False discovery rate-corrected). No correlation between iFLNA and iTau, ipTau, Braak stages and synaptophysin was noted. We found a non-significant negative correlation between iFLNA and both VAChT ($p=.097$) and ChAT ($p=.058$) concentrations. iFLNA concentrations were significantly higher in the Prodromal AD and ADD groups when compared to non-AD subjects. iFLNA concentration was effective at identifying subjects with prodromal AD among the MCI (sensitivity: .818, specificity: .875, AUC: .898, $p=.003$). Our analysis revealed a correlative trend between iFLNA and cholinergic markers, which could indicate an association between iFLNA and disruptions of the cholinergic system. We observed increased iFLNA concentrations in AD-wrought post-mortem brains at an intermediate stage that coincides with the appearance of cognitive symptoms. As such, it may be a key event in the transition from preclinical to prodromal AD. In our brain samples, iFLNA was able to identify prodromal AD among the MCI subjects indicating that it might be a hallmark of prodromal AD.

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Poster

196. Models of Alzheimer's Disease II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 196.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG069433

Title: Intact autophagy and reduced tauopathy in non-demented individuals with Alzheimer neuropathology

Authors: *B. TUMURBAATAR, A. FRACASSI, W.-R. ZHANG, G. TAGLIALATELA; Univ. of Texas Med. Br., Univ. of Texas Med. Br., Galveston, TX

Abstract: Alzheimer's disease (AD) is a terminal neurodegenerative disease and the most common cause of dementia. Notably, some individuals, here referred to as Non-Demented individuals with Alzheimer Neuropathology (NDAN) are cognitively intact despite the presence of neuropathology consistent with fully symptomatic AD and have decreased tau oligomers at synapses. Accumulating evidence proposes that autophagy, the major cell mechanism responsible for removing protein aggregates and reportedly a primary route of clearance for tau in healthy neurons but disturbed in AD, is required for synaptic functions including neurotransmission and synaptic plasticity. Thus, the understanding of the neuroprotective mechanisms to evade cognitive decline to preserve synaptic functional integrity is important for the development of therapeutic approaches to prevent dementia. Here we evaluated autophagy as a key protective mechanism for maintenance of cognitive integrity in NDAN subjects by efficient removal of tau oligomers. Using postmortem brain samples from age matched NDAN (n=7), AD (n=7) and healthy controls (n=6), we performed immunofluorescence and comparative western blot analyses for regulatory molecules and ATGs (autophagy-related genes) involved in autophagy. Tauopathy was accessed by the expression and phosphorylation levels at Ser202/Thr205 (AT8), Thr231 (AT180) and Ser396 (PHF13.6) of tau oligomers. We found that NDAN subjects have significantly increased regulatory mTor (p&It0.05), Raptor (p&It0.03), Beclin-1 (p&It0.02) and Atg16L (p&It0.02), Atg12(p&It0.01), Atg3 (p&It0.01), Atg5 (p&It0.008), Atg7(p&It0.02), LC3AB (p&It0.01, p&It0.005) and proteasomal PSMA5 (p&It0.01), but not lysosomal LAMP1 (p&It0.1) as compared to AD. Expression levels were comparable with healthy controls. Autophagic proteins, except p62, were negatively (Atg16L, 3, 5 and LC3AB with significance) correlated to tau oligomer (&gt65 kDa), not to total tau (&gt45 kDa), indicating autophagy being specific to tau oligomer clearance. Tau oligomer expression and levels of phosphorylation at Ser202/Thr205 (p&It0.03), Thr231 (p&It0.03) and Ser396 (p&It0.02) were significantly reduced in hippocampal tissue and synaptosomes from NDAN, and absent in frontal cortex as compared to AD. Our results indicate, for the first time, intact autophagy and associated reduced tauopathy as one of protective mechanism in cognitive intact NDAN. This novel observation supports the potential of autophagy-induced strategies in AD therapeutics.

Disclosures: B. Tumurbaatar: None. **A. Fracassi:** None. **W. Zhang:** None. **G. Taglialatela:** None.

Poster

196. Models of Alzheimer's Disease II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 196.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1 R15 NS101608-01A1
Southern Illinois University Edwardsville Graduate School Competitive Graduate Award

Title: The Tetraspanin, Tsp42Eg, suppresses amyloid beta accumulation at the Drosophila neuromuscular junction

Authors: *E. L. HENDRICKS[1], F. L. LIEBL[2];
[1]Southern Illinois Univ. Edwardsville, [2]Southern Illinois Univ. Edwardsville, Edwardsville, IL

Abstract: A common feature of neuronal dysfunction in Alzheimer's disease is the accumulation of amyloid beta (Abeta) fragments at the synapse. Abeta fragments are generated from the amyloidogenic processing of a transmembrane protein, amyloid precursor protein (APP), by beta- and gamma-secretase enzymes. For APP cleavage to occur, beta- and gamma-secretases must be localized to sites where APP is found. Thus, proteins that regulate the dynamics of beta- and gamma-secretases may also regulate the cleavage of APP. One of these gamma-secretase-interacting proteins is the Tetraspanin (Tsp), CD63, which has recently been identified as a biomarker for decreasing cognitive function in Alzheimer's disease. CD63, therefore, represents a potential regulator of Abeta production and/or accumulation. To explore the potential implications of CD63 in Alzheimer's disease, we use the *Drosophila* neuromuscular junction (NMJ) as a model glutamatergic synapse. We find that the *Drosophila* CD63 ortholog, Tsp42Eg, suppresses synaptic accumulation of Abeta indicating that *tsp42Eg* mutants either produce more Abeta or are deficient in clearing Abeta aggregates. In *tsp42Eg* mutants, this increase in Abeta is coupled to a decrease in the synaptic localization of the *Drosophila* APP ortholog, amyloid precursor protein-like (APPL). Furthermore, we find that Tsp42Eg negatively regulates endocytosis. Together, these findings implicate Tsp42Eg as a regulator of key synaptic processes. However, it is unclear whether Tsp42Eg mitigates Abeta production by directly repressing endocytosis or whether Tsp42Eg is involved other aspects of synaptic function that regulate Abeta production and/or clearance. We have identified three potential mechanisms by which CD63-related Tsp42Eg may regulate Abeta dynamics. One possibility is that Tsp42Eg controls the activity of beta-secretase-regulating proteins, like Synapsin, to prevent Abeta production as *tsp42Eg* mutants have an increase in synapse-localized Synapsin. Another possibility is that Tsp42Eg restricts gamma-secretase from cleaving APPL and producing Abeta fragments. Both scenarios reflect high Abeta production without comparable Abeta clearance. Finally, the increase in Abeta at *tsp42Eg* mutant synapses may occur because the derepression of endocytosis leads to high endosomal Abeta production and synaptic deposition. Investigating these mechanisms will help characterize the role of CD63 in Alzheimer's disease-associated Abeta accumulation. Additionally, these findings will contribute to the overall understanding of Alzheimer's disease pathology and provide evidence for potential pharmacologic treatment targets.

Disclosures: E.L. Hendricks: None. **F.L. Liebl:** None.

Poster

196. Models of Alzheimer's Disease II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 196.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS074256

Title: Beta-secretase-1 (BACE1) modulates cognition and neuronal excitability in the hippocampal CA1 region in a cell-autonomous manner

Authors: *A. YAO, R. YAN;
Neurosci., Univ. of Connecticut Hlth. Ctr., FARMINGTON, CT

Abstract: Alzheimer's Disease (AD), the most common cause of dementia, is a debilitating disease that leads to progressive memory loss, cognitive impairment, and ultimately death. Pathological hallmarks of AD include extracellular amyloid beta (Aβ) plaques. β-secretase-1 (β-site APP cleaving enzyme 1, BACE1) is the rate-limiting enzyme of toxic Aβ generation. BACE1 KO mouse models of AD led to suppression of AD pathology, which suggests that inhibiting BACE1 may be a rational strategy for AD treatment. However, human clinical trials have shown that BACE1 inhibitors are inefficacious, even worsening cognitive function, among AD patients. This benchtop-to-bedside translational failure is due to our incomplete understanding of BACE1's physiological function. In particular, the mechanisms underlying neuronal and synaptic impairments in BACE1 deficiency or inhibition is poorly understood. To investigate how BACE1 regulates excitability in excitatory pyramidal neurons (PNs) without the potential compensatory or compounding effects of BACE1 deletion in inhibitory neurons, we have adopted a mouse model (Nex1-cre$^{+/wt}$;Bace$^{fl/fl}$) that induces BACE1 deletion specifically in forebrain excitatory neurons after early developmental stages. Whole-cell patch-clamp recordings of hippocampal CA1 PNs in brain slices prepared from 1.5-month-old mutant mice display increased excitability in both active and passive membrane properties, including a more depolarized resting membrane potential (RMP), increased evoked action potential (AP) spiking, and reduction in spike frequency adaptation. These findings were recapitulated in acute brain slices prepared from 4.5-month-old Nex1-cre$^{+/wt}$;Bace$^{fl/fl}$ compared to control. A battery of behavioral testing demonstrated that BACE1 deletion in excitatory neurons is sufficient to produce cognitive impairment on open field and fear conditioning tests that indicate a hyperactive phenotype and impaired associative learning. These findings provide evidence that selective BACE1 deletion in excitatory neurons leads to neuronal hyperexcitability, suggesting that BACE1 deletion disrupts intrinsic neuronal function in a cell-autonomous manner in the hippocampus, a major substrate of memory storage derailed by AD. Ultimately, these findings will provide insight into BACE1's function in regulating the activity of excitatory neurons and synapses in the hippocampus, which will provide critical insight into our fundamental understanding of learning and memory, synaptic transmission and plasticity, and the development of AD therapeutics.

Disclosures: **A. Yao:** None. **R. Yan:** None.

Poster

196. Models of Alzheimer's Disease II

Location: SDCC Halls B-H

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Program #/Poster #: 196.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NIA R21 and NIA R01

Title: An Amygdala Story: How chronic Phospholipase D1 inhibition leads to slowing neurodegeneration in 3xTg-AD neuropathology model of Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is a multifaceted neurodegenerative disorder that makes up a predicted 80% of all dementia diagnoses with an estimated 45 million people affected worldwide. Despite its high prevalence and extensive research, treatment of AD is difficult with no cures currently available. Current treatment research is often focused on slowing neuropathologies that degrade memory function in their early stages; this lab has reported that aberrantly increased neuronal Phospholipase D isoform 1 (PLD1), a lipolytic enzyme designed to break down membrane phospholipids, leads to the synaptic dysfunction and memory deficits seen in AD. Our lab has also supported many aspects of how aberrantly elevated PLD1 contributes to AD, including behavioral and memory changes, synaptic dysfunction, and decreased neurological spine integrity. Our lab previously showed that chronic inhibition of PLD1 preserved dendritic spine integrity in the 3xTg-AD mouse model in the hippocampal CA1 region. Here, we report significant evidence of chronic PLD1 inhibition leading to preservation of dendritic spines in aged 3xTg-AD mouse models in multiple regions of the amygdala, a region of the brain that is known to contribute memory, particularly strong, emotionally driven memory that is impaired in many neuropsychiatric conditions and also an early event in the progression of different forms of dementia, including AD. Based on this data, we hypothesize the mechanism by which PLD1 inhibition slows the progression of AD: inhibition leads to preservation of dendritic spines in multiple brain circuits that promotes the resilience that we observe towards progression of cognitive decline. Our research is poised to inform how dendritic spine preservation is key to preventing neuropathological states including AD.

Disclosures: K. Ramaswamy: None. **C. Natarajan:** None. **B. Krishnan:** None.

Poster

196. Models of Alzheimer's Disease II

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Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ)

Title: Mtorc1 signaling in human brain and ab-based murine models of alzheimer's disease

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Abstract: The mammalian target of rapamycin (mTOR) plays a fundamental role in cell proliferation, survival and autophagy. In particular, mTOR complex 1 (mTORC1) has a key role in the regulation of protein synthesis activated by stimuli such as amino acids, insulin and other growth factors. Both insulin signaling and protein synthesis are impaired in Alzheimer's disease (AD), the most common cause of elderly dementia. Because of that, the mTORC1 signaling pathway has received much attention in AD research in the last years. However, results from such studies remain controversial. While most groups have shown an upregulation of the mTORC1 pathway in experimental models of AD, others have reported an opposite effect. We are currently studying the mTORC1 pathway in *post-mortem* AD brains and in two murine models: wild-type *Swiss* mice receiving intracerebroventricular (i.c.v.) infusions of amyloid-β oligomers (AβO), toxins that build up in AD brain and are thought to cause synapse failure and memory loss, and in transgenic APPswe/PS1ΔE9 mice, engineered to overproduce the amyloid-β peptide. Results so far indicate that, while no clear change was observed in AD brains, mTORC1 pathway was inhibited in murine hippocampi 7 days after AβO infusion. Surprisingly, we did not find any changes in mTORC1 signaling in the hippocampi or frontal cortex of APPswe/PS1ΔE9 mice, at either 4 or 8 months of age, when AD-related neuropathology and memory impairment are detected. In addition, we injected AβO in transgenic heterozygotic mice for *4E-BP2* and *Fmr1*, genes known to encode translational repressors. Ultimately, results showed increased activity of the mTORC1 pathway by reducing *4E-BP2* or *Fmr1* prevented AβO-mediated memory loss. Altogether, results may point to potentially critical differences between acute and chronic models of AD, and to disease stage-dependent regulation of mTORC1 signaling. Resolving these apparently conflicting results may be an important step towards proper understanding of mTORC1 signaling in AD, and to the development of therapies focused on reestablishing proteostasis.

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Poster

196. Models of Alzheimer's Disease II

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant U01AA025932
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Title: Cognitive Flexibility Impairment of Instrumental Learning in a Mouse Model of Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is characterized by reduced executive function including cognitive flexibility in instrumental behavior. Learning motor skills requires the implementation of action sequences to promote work efficiency while minimizing cognitive burden. This process is mediated by cortico-basal ganglia circuits, which are impacted by normal aging. In this study, we employed the 5xFAD mouse model of AD to investigate cognitive and behavioral flexibility deficits at different ages through operant-conditioning tasks. First, we examined 3-month-old (early stage) and 6-month-old (middle stage) 5xFAD animals in a lever-press instrumental learning procedure. We found that both 3-month-old and 6-month-old 5xFAD animals failed to adjust the action-outcome contingencies after the contingencies were reversed. 3-month-old 5xFAD animals showed deficits in extinction learning. 6-month-old 5xFAD animals showed deficits in lever-press rate during initial and reversal learning procedures. Using ArcTRAP;Ai140;5xFAD mice, we captured activated neurons during initial or reversal learning stages in 6-month-old animals. Histological studies found that 5xFAD mice contained a lower density of active neuron populations in the dorsomedial striatum (DMS) during initial training but exhibited a greater increased density of captured neurons in DMS during reversal learning. Secondly, 12-month-old (late-stage) 5xFAD animals showed excessive nose pokes and increased latency of reward collection during the reversal learning phase AD is also characterized by reduced cholinergic function, which is required for reversal learning. We thus used a genetically encoded sensor to measure acetylcholine (ACh) release in the striatum of 6-month-old 5xFAD mice. We found that a GABAA receptor antagonist, picrotoxin, increased ACh release at a similar extent in slices from 5xFAD and WT mice. However, NMDA in the presence of

picrotoxin significantly increased more ACh release in 5xFAD than WT control. Taken together, our results suggest that cognitive and behavioral flexibility deficits manifest in the early stages of the AD mouse model (3-month-old) and persist in the middle (6-month-old) and late (12-month-old) stages of AD. Our findings demonstrate that instrumental behavioral test is helpful for the early detection of AD and provide ensemble mechanisms underpinning reduced cognitive and behavioral flexibility.

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Poster

196. Models of Alzheimer's Disease II

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Excitation-inhibition imbalance disrupts visual familiarity in amyloid and non-pathology conditions

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Abstract: Neuronal hyperactivity induces memory deficits in Alzheimer's disease. However, how hyperactivity disrupts memory is unclear. By imaging ~20,000 synapses *in vivo* in the mouse visual cortex, we found a spatially restricted structural excitatory-inhibitory synapse imbalance favoring hyperactivity in early amyloidosis. Consistently, neurons displayed hyperexcitability and reduced stimulus specificity. Compensatory changes that maintained activity homeostasis disrupted functional connectivity and increased population sparseness such that very small fractions of neurons dominated population activity. These properties rendered visual recognition memory vulnerable to interference from non-specific visual experiences, which disrupted the neural representation of visual familiarity. Depriving non-specific visual experiences improved visual familiarity. In contrast, in non-pathological conditions, depriving non-specific visual experiences induced disinhibition, increased excitability, and disrupted visual familiarity despite normal plasticity. We show that familiarity is disrupted in amyloid and non-pathology conditions when high-responsive neurons and the neural representation's persistence to memory-associated stimulus are not constrained due to hyperexcitability.

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Poster

196. Models of Alzheimer's Disease II

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Kibra repairs synaptic plasticity and improves memory in mice with pathogenic tau accumulation

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Abstract: Alzheimer's disease (AD) is characterized by an accumulation of pathogenic tau in the brain that induces synapse dysfunction and progressive memory decline. Long-term potentiation (LTP) is inhibited in neurons containing pathogenic tau. LTP impairment caused by pathogenic tau coincides with AD-related memory loss, however, the mechanisms that disrupt LTP remain unclear. KIdney/BRAin (KIBRA) is a scaffolding protein containing several protein binding domains involved in multiple signaling pathways that regulate neuronal function. Lower protein levels of KIBRA are associated with dementia in human AD brains. We intend to uncover how dysregulation of KIBRA signaling contributes to the loss of synaptic plasticity in AD and whether KIBRA signaling can be targeted for therapeutic intervention to prevent memory loss. We generated an N-terminal fragment of KIBRA (NT-KIBRA) and C-terminal fragment of KIBRA (CT-KIBRA) that were expressed in dissociated rat hippocampal cultures to identify which protein subregion of KIBRA can restore synaptic plasticity in neurons expressing pathogenic tau. We found that only CT-KIBRA restores AMPA receptor trafficking during LTP in neurons expressing pathogenic tau. To study the effects of CT-KIBRA in vivo, we injected lentivirus for expression of CT-KIBRA into the hippocampus of transgenic mice that mimic the hyperacetylation of tau in AD ($tauKQ^{high}$). These mice have a reduction in postsynaptic KIBRA, impaired LTP, and hippocampal-dependent memory deficits. CT-KIBRA restored LTP and memory in $tauKQ^{high}$ mice and exerted this effect without altering the levels of phospho-tau or reversing tau-mediated synapse loss in the hippocampus of $tauKQ^{high}$ mice. We showed that CT-KIBRA interacts with PKMζ, a constitutively active kinase that is upregulated in the maintenance of LTP and memory. PKMζ levels are reduced in $tauKQ^{high}$ mice and higher levels

of PKMζ in the presence of CT-KIBRA associate with better memory. The C-terminus region of KIBRA can restore LTP and memory in mice with high levels of pathogenic tau related to AD. Our work suggests CT-KIBRA repairs synaptic plasticity and improves memory at least in part through an interaction with PKMζ.

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Poster

196. Models of Alzheimer's Disease II

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Title: Loss of LAMP5 interneurons is associated with neuronal network dysfunction in Alzheimer's disease

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Abstract: In Alzheimer's disease (AD), where amyloid-β (Aβ) and tau deposits in the brain, hyperexcitation of neuronal networks is an underlying disease mechanism, but its causes remain largely unknown. Here, we used the Collaborative Cross forward genetics platform to identify modifier genes of neuronal hyperexcitation in mice. We found LAMP5 as a novel regulator of hyperexcitation in mice. Functionally, neuronal *LAMP5* was largely uncharacterised but more recently has been identified as a maker of a distinct subpopulation of inhibitory interneurons. We next examined the LAMP5 expression in different regions of brain sections from human AD, tau-only form of Frontotemporal lobar degeneration (FTLD-tau) patients, as well as neurologically healthy controls (CTR). We found a marked reduction of LAMP5+ neurons and their neuronal projections/synaptic boutons in the frontal cortex and other brain regions compared to CTR. Similarly, LAMP5+ neurons were reduced in numbers in a range of AD mouse models with either Aβ or tau expression and pathology, including the APP23, APP/PS1 and TAU58 lines. We furthermore showed that genetic reduction of LAMP5 levels led to the degeneration of LAMP5 interneurons in cortex and dentate gyrus in 3 months old Lamp5-

deficient (Lamp5$^{\Delta/\Delta}$) mice and augmented functional deficits and neuronal network hypersynchronisity in Aβ- or tau-driven AD mouse models. To this end, our work defines the first specific function of LAMP5 interneurons in neuronal network hyperexcitation in AD and related forms of dementia with tau pathology.

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Poster

196. Models of Alzheimer's Disease II

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Title: 27-hydroxycholesterol modulates brain's acetylcholinesterase homeostasis: a preclinical evidence

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Abstract: Alterations in cholesterol metabolism within the brain have a significant role in Alzheimer's Disease (AD) pathophysiology. Previous studies demonstrated that Swiss mice fed a high cholesterol diet displayed short-term spatial memory impairment, and it was correlated with an increase in the acetylcholinesterase (AChE) catalytic activity in the prefrontal cortex and hippocampus (HP) brain areas. Even though cholesterol cannot transpass the blood-brain barrier (BBB), its oxidized metabolite, 27-hydroxycholesterol (27OH), can freely cross the BBB and it has been observed that its high concentrations mediate the disruption of several systems within the brain, promoting cognitive decline and neurodegeneration. However, the relation between high levels of 27OH and AChE remains unknown. Therefore, this study aimed at elucidating the involvement of 27OH in the homeostasis of brain's AChE. We evaluated the gene expression, protein density, and catalytic activity of AChE in HP and cortico-hippocampal neurons in culture [treated with 27OH (0.5 or 1μM) or dimethyl sulfoxide (DMSO) (1 μM) for 6 hours], as well as in cortical and HP cells of 3-month-old male (n = 4) and female (n = 6) CYP27A1

overexpressing mice (with 5-6 times higher concentrations of 27OH in their brains) in comparison to age-matched male (n = 5) and female (n = 4) wild-type C57Bl/6 mice. $P \leq 0.05$ was considered significant. Dunnett's *post-hoc* test was performed after significant interaction effects. Protocol nº 4884-2019. We observed that the treatment with 27OH (0.5µM) increased AChE's protein density in HP neurons [$F(2,14) = 9.11$, $p = 0.002$, $p \leq 0.001$]. Moreover, both concentrations of 27OH increased AChE's catalytic activity in cortico-hippocampal cultured-neurons [$F(2,79) = 6.60$, $p = 0.002$, $p \leq 0.05$]. Cyp27Tg mice showed constitutive alterations in AChE's gene expression (Female: presented an increase in cortical ($t = 2.22$, $df = 8$, $p \leq 0.05$) and a decrease in HP cells ($t = 2.87$, $df = 8$, $p \leq 0.05$); Male: showed increase in HP cells ($t = 3.18$, $df = 7$, $p \leq 0.01$) in comparison to sex-matched wild-type mice. Regarding to AChE's protein density, male Cyp27Tg mice showed a tendency of an increase in the HP cells ($t = 2.25$, $df = 6$, $p = 0.06$). AChE's protein density in the cortex as well as the catalytic activity of this enzyme in both structures still needs to be assessed. Notably, high levels of 27OH have been found in the brains of early-onset and sporadic AD patients (Heverin *et al*., 2004). Hence, these results might help us understand why high levels of this oxysterol are associated with mild cognitive impairment seen in AD patients. Overall, the present findings suggest 27OH as a modulator of the AChE homeostasis within the brain.

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Poster

196. Models of Alzheimer's Disease II

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Program #/Poster #: 196.16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA R21
NIA R01

Title: Targeted phospholipase D1 reduction in 12-month-old 3xTg-AD male and female mice prevents dendritic spine dystrophy associated cognitive decline

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Abstract: Background:Phospholipase D (PLD), primarily functions as a lipolytic enzyme breaking down membrane phospholipids. Abnormal elevation of PLD activity implicating both the isoforms have been well documented. We documented preservation of memory following treatment with PLD1 inhibitor in 6-month-old 3XTg mice relative to their control sibling administered with saline. We discovered preservation of dendritic spines following PLD1 specific inhibitor treatment explaining the mechanism of action. In this study, we are

hypothesizing a role of elevated PLD1 in advanced stages of AD with emphasis on tau. We also explore a new direction towards a novel pharmacological approach against cognitive decline and there is a need to understand sex differences. **Methods:**We studied the association of PLD1 with Aß and tau in human post-mortem AD brains. 12-month-old 3XTg AD mice cohorts were treated with small molecule inhibitor VU0155069 and 0.9% saline over a period of 30 days designed on a chronic treatment regimen. Following the treatment, the cohorts were subjected to behavioral studies specific to learning and memory, such as the NOR (novel object recognition) and fear conditioning (FC). Synaptic perturbations were then studied using high frequency stimulation long-term potentiation, HFS-LTP as well as low frequency stimulation long-term depression, LFS-LTD. **Result:**We observed significant differences in association of Aß and tau with PLD1 in AD patients relative to control subjects in post-mortem brains. Aberrantly elevated levels of PLD1 affected hippocampal dependent synaptic functions (excitatory and inhibitory) in the Schaffer Collateral in saline treated cohort. In contrast, the PLD1 inhibitor treated cohort showed rescued LTP and decreased LTD in conjunction with their performance in NOR and FC. Interestingly, the sex specific differences were observed in amygdala-dependent cued memory between males and females in FC study for saline treated group. However, there were no differences between males and females in the inhibitor treated group suggesting the need to understand the effect at the endocrine level in regulating synaptic function. We report preservation of dendritic spines in PLD1 inhibitor treated females.**Conclusion:**In the light of these observations, we conclude progressively elevated PLD1 expression drives cognitive decline via synaptic dysfunction by affecting dendritic spine integrity. This preclinical study documents dendritic spine preservation and hence the resilience against cognitive decline in AD. The outcomes here in turn demonstrates a complementary strategy to immunoquenching approaches and slowing the progression of AD.

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Poster

196. Models of Alzheimer's Disease II

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Program #/Poster #: 196.17

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: Early-stage increase in dorsal CA1 synaptic excitability is accompanied by a reduction in CA3 parvalbumin density in the TgF344-AD transgenic model for Alzheimer’s disease in vivo

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Abstract: Hippocampal synaptic hyperexcitability and disinhibition associate with early-stage memory impairment in animal models for Alzheimer’s disease (AD). Using the TgF344-AD rat

model that expresses age-dependent beta-amyloid (Aß) aggregation, tauopathy and neuroinflammation, we sought to track the model's AD-related prodromal changes in hippocampal synaptic strength and plasticity as well as changes in GABAergic inhibition and neuroinflammation.To determine the induction properties for short- and long-term synaptic plasticity, as well as baseline connectivity, *in vivo* evoked field excitatory post-synaptic potentials (fEPSPs) were recorded in the hippocampal CA3 to CA1 projection of 6- and 9-month-old TgF344-AD (TG) and wild-type (WT) rats (males; 6mo: n=10, 9mo: n=8). For GABAergic inhibition, levels of parvalbumin (PV) were measured in the dorsal hippocampus by Simple Western analysis (Wes) and immunohistochemistry of age-matched rats. Synaptic markers, SNAP25 and PSD95, were also measured by Wes. Density and activation of microglia with the production of the cytokine interleukin-1ß were determined with immunostaining methods.TG rats at 9 months old showed significantly lower paired-pulse facilitation (PPF) and an upwardly-shifted baseline input/output (I/O) curve, changes which were not seen in 6-month-old TG rats. Long-term potentiation (LTP) induced by Schaffer-collateral high-frequency stimulation (HFS) was significantly reduced in CA1 of 6- and 9-month-old TG rats. In 6-month-old animals, low-frequency stimulation (LFS; 900 pulses at 1Hz) was applied after LTP to determine the capacity for downregulation of synaptic plasticity. Consequently, post-LFS response magnitude of TG rats was markedly smaller compared to WT and depressed below pre-HFS baseline. Immunohistochemical analyses revealed that PV levels were significantly lower in hippocampal CA2/3 (but not CA1 or dentate gyrus) in 9-month-old TG rats, while no PV difference was found in 6-month-old rats. Wes analysis showed 6-month-old TG rats had lower SNAP25, but higher PSD95 levels compared to WT. Ongoing measures of neuroinflammation in TG and WT at these ages will be presented.Collectively, synaptic dysfunction was found in the Schaffer collateral pathway of 6- and 9-month TgF344-AD rats. The reduced inhibition upstream of this CA3->CA1 synapse could account for abnormal changes in synaptic plasticity at 9 months of age. Since the model animals do not show an abundance of Aß plaques at the ages tested here (Cohen et al., 2013), these results may represent mechanisms for disease progression as Aß oligomer production rises in the early stages of AD.

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Poster

196. Models of Alzheimer's Disease II

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Title: Cytoplasmic expression of TDP-43 in aged mice display hippocampal sclerosis-like degeneration and neuronal loss with reduced lifespan.

Authors: *A. ANDERSON[1], M. DOPLER[1], D. OSEI-KANKAM[1], S. DAVIS[1], J. DOWELL[3], M. A. GITCHO[2];
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Abstract: TDP-43 functions as a heterogeneous nuclear ribonucleoprotein involved in mRNA transport, mRNA stability, transcription, mitochondrial metabolism, and forms stress granules in the cytosol. TDP-43 is the major pathological protein in frontotemporal dementia and ALS. Previously, TDP-43 pathology has been described in up to 50% of those with Alzheimer's disease. Recent evaluation of this cohort revealed a distinct pathological staging of TDP-43 proteinopathy in an aged population, which overlaps frontotemporal lobar degeneration (FTLD-TDP) and Alzheimer's disease. This overlapping pathological cohort is named limbic-predominant age-related TDP-43 encephalopathy (LATE). Both human TDP-43 and TDP-43 nuclear localization signal defective (ΔNLS) driven it the hippocampus in an APP/PSEN1 background show severe neuronal loss in the hippocampus, a change in plaque deposition, and a decrease in survival. Further, we have seen concomitance in AD with the underreported yet common pathology of hippocampal sclerosis (HS) of aging. Utilizing quantitative shotgun proteomics on hippocampal brain tissue, we show changes in novel pathways that are directly involved in inflammation, APP regulation, phosphorylation, neurofilaments, mitochondrial metabolism, synaptic density, calcium flux, protein quality control, and mitophagy. This new pathological model hopefully will provide a greater understanding of the pathogenesis of HS with TDP-43 proteinopathy.

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Poster

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Title: Restoring chloride neuronal extrusion reverses cognitive decline linked to Alzheimer's disease mutations

Authors: *I. KERAMIDIS[1,2], B. B. MCALLISTER[3], J. BOURBONNAIS[1], F. WANG[1], D. ISABEL[1], R. SANSONETTI[1], P. DEGAGNE[3], A. G. GODIN[1,2], M. H. MOHAJERANI[3], Y. DE KONINCK[1,2];
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Abstract: Network dysfunction and hyperexcitability due to disruption of $GABA_A$-mediated transmission during early stages of Alzheimer's disease (AD) are postulated to underlie AD-associated cognitive deficits. While disrupted $GABA_A$ signaling may result from several mechanisms, recent evidence points to deficits in the potassium-chloride cotransporter KCC2, responsible for maintaining low intracellular chloride in neurons and the robustness of inhibition. Thus, we tested whether impaired KCC2 function and disrupted chloride transport underlie these cognitive deficits. In both the multi-mutation transgenic 5xFAD mice and the amyloid precursor protein knock-in APP^{NL-G-F} mice we found a decrease in membrane protein levels of KCC2 in layer II/III of the prefrontal cortex and the CA1 region of the hippocampus as compared to their non-transgenic (NonTg) age-matched littermates. In addition, *ex vivo* chloride imaging revealed weakened chloride transport in the 5xFAD mice while *in vivo* chloride imaging revealed higher steady-state $[Cl^-]_i$ in 5xFAD cortical pyramidal neurons as compared to NonTg neurons. Finally, short-term administration of CLP290, a KCC2 enhancer developed by our laboratory, in the 5xFAD mice improved spatial memory retention in the Morris Water Task (MWT) and social interaction deficits as compared to vehicle-treated 5xFAD mice, while long-term treatment with CLP290 augmented learning performance in the MWT. All together, these results indicate that KCC2 may be a viable target for reversing deficits in $GABA_A$-mediated inhibition in AD and attenuating cognitive deficits in mice carrying AD-linked mutations.

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Poster

196. Models of Alzheimer's Disease II

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 196.20

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: TL1

Title: Long-term physiological changes in an AD mouse model

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Abstract: Current diagnostic methods for Alzheimer's disease (AD) include neurological examination, blood tests, brain imaging techniques and spinal fluid analysis all of which are incapable of detecting earlier stages of AD where treatment is most effective. Functional electrophysiological markers in AD diagnosis and treatment are rare. Although gamma oscillations have been shown to be abnormal in AD, it is still poorly understood how the various neuronal oscillations are disrupted, especially whether they can be used as a diagnostic predictor in the earlier stages of AD and/or monitoring tool for the progression of the disease. Mouse AD models are widely used to study and document the pathological progression throughout life. We performed chronic (over 1 year) electrophysiological recordings in the hippocampus in the same mice, behavioral, and pathological analysis during the development of AD-like syndrome in the APPSI/PSEN1dE9 mouse model. We found alteration of sharp wave ripple amplitude in the hippocampus and alteration in sleep architecture which reliably predicted behavior deficits in the T-maze, Barnes maze and metabolic chamber. In addition, theta depth profile, theta/gamma coherence, dentate spikes and place-specific firing map stability features are currently being investigated to document that longitudinal changes and for comparison with other physiological markers.

Disclosures: M. Soula: None. **Z. Zheng:** None. **G. Buzsaki:** None.

Poster

196. Models of Alzheimer's Disease II

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: (NINDS) NS72406
NIH DC004154

Title: Cerebellar memory deficits in a mouse model of alzheimer's disease

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Abstract: CEREBELLAR MEMORY DEFICITS IN A MOUSE MODEL OF ALZHEIMER'S DISEASEMacarena Martinez-Rey[1], Sriram Jayabal[1] and Jennifer L. Raymond[1][1]Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA, U.S.A.

Abstract

Studies in both human Alzheimer's patients and murine models of the disease have reported anatomical and physiological phenotypes in the cerebellar cortex that were correlated with

behavioral deficits. Nevertheless, the role of the cerebellum remains a highly understudied aspect of Alzheimer's disease. We evaluated whether the experimental and analytical tractability of oculomotor learning tasks could be leveraged to advance our understanding of the cerebellar contribution to Alzheimer's disease, and, more specifically, to distinguish deficits in cerebellum-dependent learning versus cerebellum-dependent memory. Cerebellum-dependent oculomotor learning and memory retention were assessed in transgenic mice that overexpresses three familial Alzheimer's disease (FAD) mutations in human amyloid beta (A4) precursor protein 695 and two FAD mutations in human presenilin 1 (5xFAD mice). In 5xFAD mice aged 6-7 months, the acquisition of learning was normal on three different oculomotor learning tasks: 5xFAD mice were indistinguishable from WT on learning to increase the gain of the vestibular-ocular reflex (VOR), learning to decrease the gain of the VOR, and adaptation of the optokinetic reflex (OKR), as assessed at the end of a 30-min (VOR) and 60-min (OKR) training session. Whereas oculomotor learning was normal, the 5xFAD mice exhibited a deficit in oculomotor memory retention. In particular, the 5xFAD mice exhibited significant forgetting of a learned increase in the gain of the VOR when tested 24 hr after training, compared to WT mice. Notably, although the other two oculomotor learning tasks are also cerebellum-dependent, retention of a learned decrease in the gain of the VOR and retention of OKR adaptation were similar in the 5xFAD and WT mice. Thus, the 5xFAD mice are selectively impaired on the retention of certain cerebellar memories, and not others. These results establish oculomotor learning and memory as an experimental system for deeper analysis of the specific cerebellar pathophysiology in Alzheimer's disease and its contribution to memory deficits.
Character count (no spaces): Current 2158 Limit: 2300

Disclosures: **M. Martinez Rey:** None. **S. Jayabal:** None. **J.L. Raymond:** None.

Poster

196. Models of Alzheimer's Disease II

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA R21 Grant 1R21AG071884
NIA T32 Grant T32AG050061
ADRC Pilot Grant UC Davis P30AG010129

Title: Major Histocompatibility Complex I protMajor Histocompatibility Complex I proteins are modulated by AB and contribute to behavioral and synaptic deficits in Alzheimer's disease models

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease characterized by amyloid plaques, neurofibrillary tangles, neuronal atrophy, and cognitive deficits. Although excitatory synaptic dysfunction and neuroinflammation strongly correlate with phenotype severity in AD and AD models, it remains unclear how these two pathways converge to produce AD. In addition to well-documented microglia-mediated synaptic pruning, it is also possible that classical immune molecules expressed in the synapse play a role in AD. Major histocompatibility complex I (MHCI) molecules are expressed in neurons and found in synapses, where they negatively regulate both excitatory and inhibitory synapse density and function (Glynn et al., 2010). MHCI has also been implicated in aging (Smith et al., 2015; Lazarczyk et al., 2016) and linked to AD (Candore et al., 2004). Further, recent work indicates that MHCI contributes to amyloid plaque deposition in an APOE-dependent manner (Zalocusky et al., 2021). However, the contribution of MHCI to synaptic and behavioral deficits in AD is unknown. Here, we found that MHCI expression is upregulated in cultured hippocampal neurons in response to exogenous Aβ and that removal of sMHCI or classical MHCI molecules prevents Aβ-induced excitatory synapse loss. To determine if MHCI is required for AD phenotypes in vivo, we generated a novel mouse model by crossing the APP/PS1 mouse model of AD and a ß2-microglubulin knockout mouse, which has no classical MHCI on the surface of its cells (sMHCI). Removal of sMHCI rescues deficits in novel object recognition and partially rescues deficits in context-dependent freezing during fear conditioning in the APP/PS1 background. Finally, we also found that Aβ may cause synapse loss through a novel interaction between MHCI and neuroligin-1 (NL1). NL1 is a synaptogenic cell adhesion molecule (Sudhof 2008) that is downregulated in AD (Sindi et al., 2014). Rescuing NL1 levels has been shown to be sufficient to reverse AD-associated synaptic and behavioral deficits (Bie et al., 2014). Here, we show that MHCI directly binds NL1 in HEK293 cells *in vitro* and in neurons and brain *in vivo*. Further, MHCI bidirectionally and negatively modulates NL1 protein levels, and removal of MHCI in APP/PS1 animals restores NL1 protein levels. Overall, our data provide evidence for a novel mechanism linking inflammation and synapse loss in AD that involves Aβ elevating MHCI levels, which decrease NL1 and cause synapse loss and cognitive deficits.

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Poster

196. Models of Alzheimer's Disease II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 196.23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR PJT-156054

Title: Shank3: Relationship between cognitive impairment and synaptic density in a mouse model of Alzheimer's Disease

Authors: *M.-F. OYE MINTSA MI-MBA[1,2,3], O. LANDRY[1,2,3], A. FRANCOIS[2], M.-T. TRAVERSY[1], C. TREMBLAY[2], V. ÉMOND[2], D. A. BENNETT[4], J. D. BUXBAUM[5], F. CALON[1,2,3];
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Abstract: Alzheimer's disease (AD) is an age-related pathology characterized not only by an accumulation of Aβ plaques and hyperphosphorylation of tau protein, but also by synaptic loss, indexed by a reduction of pre and post-synaptic proteins. However, the consequences of lowering the expression of synaptic protein remain elusive. The postsynaptic protein Shank3 (SH3 and multiple ankyrin repeat domains) may be an exception because the loss of a single allele of the Shank3 gene is sufficient to cause profound cognitive symptoms in children. We therefore hypothesized that Shank3 deficiency contribute to the development or worsening of cognitive symptoms and neuropathology in AD. We first confirmed a significant postmortem reduction of Shank3 in the parietal cortex of AD patients (Braak stage 4-5) as well as a correlation between Shank3 levels and antemortem cognitive scores in these patients. To investigate the cause-effect relationship in AD, we developed an animal model by crossing Shank3-deficient ($Shank3^{\Delta ex4-9}$) and non-Shank3-deficient mice with a 3xTg-AD mouse model showing AD-associated neuropathology. The model was validated by in situ hybridization on sections and in Western blot on PSD extracts from the parietotemporal cortex. Results showed that Shank3 protein and mRNA expression were significantly reduced by 30-50% in Shank3-deficient mice compared to non-deficient animals, consistent with the results found in AD patients. We observed synergistic deleterious effects of Shank3 deficiency and AD neuropathology on object recognition memory at 9, 12 and 18 months of age and on anxious behavior at 9 and 12 months of age in hemizygous $Shank3^{\Delta ex4-9}$-3xTg-AD mice. Western blot analyses of PSD extracts from the parietotemporal cortex showed that levels of synaptic proteins such as PSD-95, drebrin, homer1 remained unchanged in hemizygous Shank3 mice. ELISA and Western blot analyses showed that Shank3 deficiency increased the levels of soluble $A\beta_{42}$ and human tau at 18 months of age compared to 3xTg-AD mice with normal Shank3 expression. The results of this study in human brain samples and in transgenic mice are consistent with the hypothesis that SHANK3 deficiency plays a key role in the apparition of cognitive impairment in AD. Further investigations using dietary docosahexaenoic acid (DHA) treatment to increase Shank3 levels in the brain and lead to an improvement of cognitive symptoms are underway.

Disclosures: M. Oye Mintsa Mi-mba: None. **O. Landry:** None. **A. Francois:** None. **M. Traversy:** None. **C. Tremblay:** None. **V. Émond:** None. **D.A. Bennett:** None. **J.D. Buxbaum:** None. **F. Calon:** None.

Poster

196. Models of Alzheimer's Disease II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 196.24

Title: WITHDRAWN

Poster

196. Models of Alzheimer's Disease II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 196.25

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: VA BLR&D 5IL2BX004105

Title: Retrograde tracing of BDNF from the entorhinal cortex to the locus coeruleus in Alzheimer's disease

Authors: F. S. MOJABI[1], A. M. HEATH[1,2], J. MEZA[1], A. CASEY[3], D. MURRAY[1,2], B. D. HEIFETS[3], J. YESAVAGE[1,2], *M. W. MCNERNEY[1,2];
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Abstract: Background. Alzheimer's disease (AD) is one of the most common and debilitating neurodegenerative diseases. It is characterized by a loss of brain tissue in key areas for memory and cognition. One such area is the locus coeruleus (LC), which degenerates early in the progression of the disease, and is the main supplier of norepinephrine (NE) to the rest of the brain. Little is known about the cause, but one possibility is the loss of neurotrophic factor transport from the cortex. We therefore set out to investigate the transport of BDNF from the cortex to the LC in 3xTgAD mice.
Method. Atto-labeled BDNF was injected into the entorhinal cortex of 3xTgAD and B6 mice, aged one year (N = 19). After 16hrs, mice were perfused, their brains were extracted, and fixed with 4% paraformaldehyde. One hemi brain was sliced (30um) and stained for tyrosine hydroxylase (TH) and parvalbumin (PV), while the other underwent iDISCO clearing (dichloromethane, dibenzyl ether) and 3D light sheet microscopy (6um slices). Images were registered to a common atlas and then analyzed via *Illastik* for fluorescence quantification. A further subset of mice were lightly perfused for staining with TrkB.
Results. Immunostaining for TH and PV for LC neurons revealed overall minimal transport to the LC, but a higher amount of transport to PV neurons adjacent to the LC. Additionally, there was a small deficit in the transport of BDNF to both PV and LC neurons in 3xTgAD mice as compared to B6. Staining for TrkB revealed similar findings, such that TrkB was localized primarily in PV neurons adjacent to the LC. 3D imaging analysis was used to explore other novel possible regions of BDNF transport. This information can thus hopefully be utilized to discover new ways to improve the transport of BDNF across the brain and reduce the loss of cognitive function in AD.

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Poster

197. Alzheimer's Disease: Preclinical Insights

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Program #/Poster #: 197.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA/1RF1AG069425-01
NIH RF1AG051495

Title: A novel treatment paradigm with an RXR-agonist : Can acute, early treatment have sustained neuroprotective effects in Alzheimer's disease?

Authors: *S. PUNTAMBEKAR[1], M. MOUTINHO[2], P. B.-C. LIN[2], M. A. BENITO[3], G. XU[1], G. E. LANDRETH[4], B. T. LAMB[2];
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Abstract: Nuclear Receptors (NR) such as retinoic X receptor (RXR), have known functions in regulating multiple biological pathways/mechanisms implicated in Alzheimer's disease (AD), including Aβ homeostasis, anti-inflammatory signaling and neuronal/synaptic functions. The FDA approved RXR agonist, bexarotene (Bex) has been tested in animal models and clinical trials in AD. These studies show that while chronic Bex treatment reduces brain Aβ loads with a concomitant increase in serum levels of Aβ and improved cognition, Bex treated patients had significant hepatocytotoxicity and high levels of serum triglycerides. Thus, although bexarotene may be a promising drug candidate in facilitating Aβ clearance, chronic treatment paradigms pose a significant risk of cardiovascular disease, stroke and liver dysfunction. To circumvent the issue of peripheral toxicity, this study aims to test whether a novel paradigm of a short-term, acute bexarotene treatment confers sustained neuroprotection in the 5xFAD mouse model of AD. 2 month-old 5xFAD mice were treated with 100mg/kg Bexarotene for 7 days. Bex vs. Vehicle treated 5xFAD mice were aged to 6 months and evaluated for AD pathology. Acute Bex treatment resulted in elevated mRNA expression of canonical target genes namely, *Apoe* and *Abca1* in 6 month-old 5xFAD mice indicating sustained long-term effects in the brain. Increased expression of genes associated with Aβ phagocytosis/metabolism, namely *Trem2*, *Hexokinase-2*, *Lpl*, *Axl*, *Mertk*, *Becn1*, *Atg7* and *Rage* correlated with reduced plaque loads in the hippocampus and subiculum, along with reduced synaptic loss and neurodegeneration in the hippocampus, subiculum and cortex in 6 month-old 5xFAD mice treated with Bex. Nanostring based transcriptomics revealed increased expression of genes associated with protective microglial activation in Bex-treated mice. These data correlated with increased microglial plaque coverage and efficient plaque compaction following Bex treatment. Acutely treated 5xFAD mice also

displayed increased expression of genes associated with neuronal differentiation and neuron projection, indicating that overall neuronal health is preserved in Bex-treated 5xFAD mice. This study is the first to design a novel, therapeutic paradigm to show that early, acute treatment with Bex confers significant long-term neuroprotection in aged 5xFAD animals. Our data represents initial steps towards revised clinical trials designed to minimize drug exposure, thereby mitigating any subsequent off-target, peripheral drug toxicity.

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Poster

197. Alzheimer's Disease: Preclinical Insights

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Program #/Poster #: 197.02

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: Piano di incentivi per la ricerca (PIA.CE.RI.) 2020–2022, Linea di intervento 2 (Projects: 3N-ORACLE)
Piano di incentivi per la ricerca (PIA.CE.RI.) 2020–2022, Linea di intervento 2 (Projects: 3N-ORACLE)
PRIN grant no. 2017YH3SXK from the Italian Ministry of Research

Title: Intranasal administration of a TRAIL neutralizing monoclonal antibody adsorbed in PLGA nanoparticles and NLC nanosystems: an in vivo study on a mouse model of Alzheimer’s disease

Authors: *G. DI BENEDETTO[1], T. MUSUMECI[2], C. CARBONE[2], A. BONACCORSO[2], G. AMATO[2], M. J. LO FARO[3], C. BURGALETTO[1], G. PUGLISI[2], R. BERNARDINI[1], G. CANTARELLA[1];
[1]Dept. of Biomed. and Biotechnological Sci., [2]Dept. of Drug and Hlth. Sci., [3]Dept. of Physics and Astronomy "E. Majorana", Univ. of Catania, Catania, Italy

Abstract: Alzheimer’s disease (AD) is a neurodegenerative disorder that progressively compromises cognitive functions. Tumor necrosis factor (TNF)-Related Apoptosis Inducing Ligand (TRAIL), a proinflammatory cytokine belonging to the TNF superfamily, appears to be a key player in the inflammatory/immune orchestra of the AD brain. Despite the ability of an anti-TRAIL monoclonal antibody to reach the brain producing beneficial effects in AD mice, we attempted to develop such a TRAIL-neutralizing monoclonal antibody adsorbed on lipid and polymeric nanocarriers, for intranasal administration, in a valid approach to overcome issues related to both high dose and drug transport across the blood-brain barrier. The two types of nanomedicines produced showed physico-chemical characteristics appropriate for intranasal administration. As confirmed by enzyme-linked immunosorbent assay (ELISA), both nanomedicines were able to form a complex with the antibody with an encapsulation efficiency

of ≈99%. After testing in vitro the immunoneutralizing properties of the nanomedicines, the latter were intranasally administered in AD mice. The antibody-nanocarrier complexes were detectable in the brain in substantial amounts at concentrations significantly higher compared to the free form of the anti-TRAIL antibody. These data support the use of nanomedicine as an optimal method for the delivery of the TRAIL neutralizing antibody to the brain through the nose-to-brain route, aiming to improve the biological attributes of anti-TRAIL-based therapy for AD treatment.

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Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.03

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: Neuroimaging markers, behavioral phenotype and biomarkers in 5xFAD mouse model of Alzheimer’s disease

Authors: T. HEIKKINEN, P. KARHUNEN, T.-K. STENIUS, H. KOSUNEN, K. LEHTIMÄKI, J. ROKKA, J. RYTKÖNEN, A. SHATILLO, ***D. MISZCZUK**;
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Abstract: 5xFAD mice (B6SJL-Tg(APPSwFlLon,PSEN1*M146L*L286V)6799Vas/Mmjax)) express five AD linked mutations in human APP (K670N/M671L, I716V and V717I) and PSEN1 (M146L and L286V) transgenes. APP and PSEN1 mutations cause human like AD pathology by overproducing Aβ42, leading to plaque accumulation in the brain. The objective of this study was to characterize the behavioral changes, neuroimaging markers by brain volumetry, FDG-PET, functional ultrasound and metabolic profile by MR spectroscopy, beta amyloid accumulation and other biomarkers in 5xFAD mice. 14 female and 15 male transgenic mice and 11 female and 10 male wild-type mice were used for the study starting from 4 months of age. The mice were subjected to behavioral tests including open field (OF), elevated plus maze (EPM), Y-maze tests (YM), nest building (NB) and radial arm water maze (RAWM). Brain volumes and hippocampal metabolic profile were measured using MRI and MRS. In addition, the FDG PET and functional ultrasound (fUS) imaging were performed. In vivo measurements were performed at 4-10 months and brain samples were collected at 6 and 11 months for analysis of beta amyloid levels, brain inflammatory markers and neurofilament light chain (NfL) levels in plasma and CSF. RAWM and NB showed a significant deficit in 5xFAD mice. OF, YM and EPM did not reveal significant phenotype. FDG uptake was significantly reduced in 5xFAD, and hippocampal metabolic profile was altered at 6 months. Functional ultrasound demonstrated vascular reactivity changes in transgenic mice at 10 months - which could reflect the

translational aspect for vascular pathology in human AD. Astroglia (GFAP), microglia (Iba1), amyloid plaques (WO2 staining) and CSF NfL showed remarkably high levels in transgenic 5xFAD mice.These data suggest that the 5xFAD mice exhibit cognitive and metabolic changes resembling those observed in AD. These deficits were evident starting from 6 months of age. The 5xFAD mice, with observed in vivo and biomarker phenotype, offer a tool to investigate potential treatment effects in a model of beta amyloid over expression.

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Poster

197. Alzheimer's Disease: Preclinical Insights

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Program #/Poster #: 197.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ECU Startup Funds
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Title: Cellular and molecular basis of neuronal resilience by reduced Rab10 level in the brain

Authors: ***W. P. BUNNER**[1], J. WANG[3], D. BASHTOVYY[1], S. COHEN[4], L. HARRIS[1], T. LANDRY[2], R. STACKMAN[4], T. TRAN[1], R. YASUDA[3], E. M. SZATMARI[1];
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Abstract: A central question on the field of aging research is identifying the cellular and molecular basis of neuroresilience. One of such factors is the small GTPase, Rab10. Reduced level and activity of Rab10 lead to retaining of normal cognitive function in the face of dementia ("cognitive resilience"). Here we used Rab10+/- mice to identify novel molecular mechanisms by which reduced Rab10 level guards the aging brain. We found that physical attributes and brain morphology are normal in Rab10+/- mice compared to their Rab10+/+ littermates. Brain expression analysis of 880 genes involved in neurodegeneration showed that Rab10+/- mice have higher activation scores of pathways associated with neuronal metabolism; structural integrity; neurotransmission and neuroplasticity compared to their Rab10+/+ littermates. Lower activation scores were observed for pathways involved in neuroinflammation and aging. We identified several differentially expressed genes (DEG) including Stx2, Stx1b, Vegfa, Lrrc25 (downregulated); and Prkaa2, Syt4 and Grind2d (upregulated). Transcriptome profiling was validated at DNA level by qPCR and at protein level using Western Blotting. Finally, behavioral characterization showed that Rab10+/- mice perform better in a hippocampus-dependent spatial task (Object in Place Test), while their performance in a classical conditioning task (eye blink conditioning; EBC) was significantly impaired. Therefore, our findings indicate that Rab10

differentially controls the brain circuitry of hippocampus-dependent spatial memory and higher level behavior, that requires intact cortex-hippocampal circuitry. Transcriptomic and biochemical characterization of these mice strongly suggest that Glutamate Ionotropic Receptor NMDA Type Subunit 2D is a potential mediator of Rab10+/- behavioral phenotypes. We conclude that Rab10+/- mice described here can be a valuable tool to study the mechanisms of resilience in AD model mice on reduced Rab10 background and to identify novel therapeutical targets to prevent cognitive decline associated with normal and pathological aging.

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Poster

197. Alzheimer's Disease: Preclinical Insights

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Topic: C.02. Alzheimer’s Disease and Other Dementias

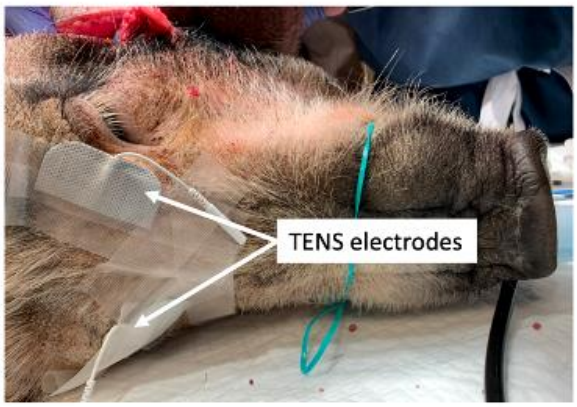
Support: NIH 1-U18-EB029251-01

Title: Alzheimer's Therapy: Cranial Nerve Stimulation to Promote Glymphatic Function

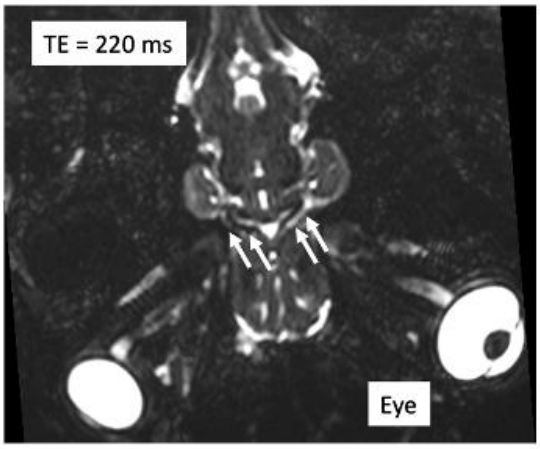
Authors: *N. VERMA[1], M.-L. JEN[2], R. MOSKWA[2], M. LALUZERNE[1], J. FRANK[2], J. A. WELLS[3], S. HURLEY[2], J. WILLIAMS[1], W. BLOCK[2], K. JOHNSON[2], K. LUDWIG[1]; [1]Biomed. Engin., [2]Univ. of Wisconsin - Madison, Madison, WI; [3]Ctr. For Advanced Biomed. Imaging UCL, London, United Kingdom

Abstract: Background: The brain’s waste clearance systems have been acclaimed as a potential therapeutic target for Alzheimer’s Disease (AD). In the glymphatic system, the interchange of cerebrospinal fluid (CSF) and interstitial fluid (ISF), facilitated by the membrane water channel aquaporin-4 (AQP4), leads to the removal of waste and metabolic byproducts from the brain parenchyma. CSF/ISF exchange is driven in large part by cerebral hemodynamics and there is a strong correlation between slow-wave oscillations and elevated clearance activity. We have previously shown that stimulation of the vagus nerve with a specific temporal pattern can increase the penetrance of a dye injected in the CSF into the brain parenchyma in rodents. **Methods:** We build on this work in a large-animal model using clinically feasible methods to assess cerebral hemodynamics and CSF movement in the brain during electrical stimulation of the trigeminal nerves. Firstly, 4DFlow, an MRI method, is used to measure blood flow in the middle cerebral artery (MCA). Secondly, a diffusion tensor imagining (DTI) method is used to assess CSF flow in the paravascular spaces (PVS) around the MCA. CSF flow in these larger PVS spaces is thought to serve the role of the pump in the glymphatic waste clearance system. Lastly, a PET method with a non-specific tracer is used to assess CSF penetrance and clearance from the brain parenchyma. Mature mini-pigs are used as the large-animal model and their

trigeminal nerves are stimulated non-invasively at the V1 and V3 level using temporally patterned waveforms. **Result:** This study is in progress. Preliminary results show modulation in cerebral blood flow during electrical stimulation of the trigeminal nerve suggesting successful neural target engagement. Four subjects should be complete by the conference.



Non-invasive stimulation of trigeminal nerve (V1)



CSF in PVS of MCA (CSF in white, MCA in black)

Discussion: Clinical imaging modalities were used in a large animal model to evaluate increase in ISF and CSF clearance in the brain during cranial nerve stimulation. This study will set the groundwork for an early feasibility study in humans.

Disclosures: **N. Verma:** A. Employment/Salary (full or part-time):; Abbott Neuromodulation, BioCircuit Technologies. **M. Jen:** None. **R. Moskwa:** None. **M. Laluzerne:** None. **J. Frank:** None. **J.A. Wells:** None. **S. Hurley:** None. **J. Williams:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuraWorx. **W. Block:** None. **K. Johnson:** None. **K. Ludwig:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuraWorx, NeuroOne. F. Consulting Fees (e.g., advisory boards); Cala Health, Abbott Neuromodulation.

Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.06

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: DFG Grant RA 689/12-1

Title: Impact of translocator protein activation via XBD173 on Aβ-induced synaptotoxic effects - focus on learning and memory-related processes

Authors: *A. K. PRADHAN[1], T. NEUMÜLLER[1], C. T. WOTJAK[2], P. LIERE[3], M. SCHUMACHER[3], G. RAMMES[1];
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Abstract: Alzheimer's disease (AD) is characterized by accumulation of β-amyloid peptide (Aβ). There is an increasing evidence that depression is a common antecedent of AD and may be an early manifestation of dementia, suggesting partly overlapping biological mechanisms of these diseases. The mitochondrial translocator protein (18 kDa) (TSPO) promotes neurosteroidogenesis and ligands targeting TSPO are shown to be neuroprotective. Since the TSPO ligand XBD173 induces rapid anxiolysis, we hypothesize that XBD173 exerts early (neuro) protective effects in the AD pathophysiology. We observed that $A\beta_{1-42}$ (50 nM), when applied for 90 min to murine hippocampal slices, prevented the development of CA1-LTP after tetanic stimulation of the Schaffer collaterals and reduced the total spine density of CA1 pyramidal neurons (n=6). XBD173 (300 nM) restored LTP deficit as well as spine density in the presence of $A\beta_{1-42}$. Rendering the spines by Imaris reveals that XBD173 incubation recovered mushroom and thin spines (n=5). Interestingly, XBD173 incubation could not restore the LTP deficit caused by $A\beta_{1-42}$ in a global TSPO knockout (KO) (n=6) mouse model indicating a TSPO-mediated action of XBD173. Chronic administration of XBD173 (1mg/kg every second day for 3 months) ameliorates the cognitive deficits in both male and female 9 months ArcAβ (transgenic AD) mice accessed by the water cross maze (n=10, Kruskal-Wallis test). In ArcAβ mice, XBD173 reduced plaque load (Methoxy-04 staining) and total $A\beta_{1-42}$ levels (ELISA) in the cortex (n=5). Measurement of neurosteroid levels by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) indicates an increase in the 3β5α-Tetrahydrodeoxycorticosterone (3β5α-THDOC) levels in the hippocampus of XBD173-treated mice. Interestingly, THDOC (100 nM), like XBD173 (300 nM), restored the LTP deficit in $A\beta_{1-42}$ treated slices (n=6). However, both XBD (300 nM) and THDOC (100 nM), could not restore these LTP deficits in GABA delta KO mice (n=7). These findings suggest that XBD173 promotes a TSPO-mediated synthesis of THDOC which, upon release, elevates GABAA receptor activity containing GABA delta subunit. Additionally, our immunohistochemical study using GFAP and postsynaptic markers suggests chronic XBD173 treatment reduces astrocytic synaptic pruning in hippocampus, which was exacerbated in AD mice (n=3). In summary, the present results indicate beneficial effects of XBD173 against Aβ-derived pathology. Chronic XBD173 treatment ameliorates cognition and suggests a disease-modifying effect when applied at early stages of AD. This hypothesis is supported by reduction of plaque, soluble Aβ levels and synaptic pruning by XBD173.

Disclosures: A.K. Pradhan: None. **T. Neumüller:** None. **C.T. Wotjak:** None. **P. Liere:** None. **M. Schumacher:** None. **G. Rammes:** None.

Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KHIDI grant HU20C0206
Ministry of SMEs and Startups grant S3146264

Title: Hydroxylated forms of pterosin upregulate BDNF through direct activation of PKA in cultured cortical neurons, and improve cognitive functions in 5X FAD mice.

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Abstract: Alzheimer's disease (AD) pathology includes amyloid plaques, tau neurofibrillary tangles, synaptic dysfunction, and loss of cholinergic and other neurons. Recently, we reported that pterosins, components of *Pteridium aquilinum*, may have beneficial effects in AD by inhibiting both b-secretase and cholinesterase. In the present study, we present evidence that some of pterosins may provide additional beneficial effects by directly activating PKA, and thus enhancing CREB/BDNF/TrkB signaling. Cultured cortical neurons were treated for 1 hr with 1 μM pterosins (A, B, C, D). Western blots for p-CREB and BDNF showed that c3-hydroxylated pterosins C and D markedly increased their expression levels. Since CREB-phosphorylation is downstream of PKA, we examined the possibility that these pterosins activated PKA. Western blots showed that pterosins increased levels of phosphor-(Ser/Thr) substrates of PKA. Addition of H-89 dihydrochloride hydrate, a PKA inhibitor, completely blocked the effects of pterosins. PKA is activated physiologically by cAMP. However, as confirmed by cAMP assay, pterosins neither increase cAMP levels, nor they inhibited phosphodiesterases (PDEs). To examine the neurotrophic effect of upregulated BDNF, young cultured cortical neurons (DIV 12) were treated for 1 days with 1 μM pterosin D. Both neurite lengths and neuronal cell number were increased by the pterosin treatment in a PKA-dependent manner. Lastly, we found that pterosins D alleviated cognitive impairment in 5XFAD mouse model. Present results indicated that hydroxylated forms of pterosins are potent direct activators of PKA. Thusly, hydroxylated pterosins may enhance synaptic functions and attenuate neuronal apoptosis through PKA/CREB-mediated BDNF upregulation, both of which may also be beneficial in AD. Hydroxylated pterosins may belong to a novel class of chemicals that may bind to the cAMP binding sites and directly activate PKA. Considering the safety and high potential BBB permeability, these pterosins seem to have a great potential as multimodal therapeutic agents in AD.

Disclosures: H. Kim: None. **G. Park:** None. **Y. Yoon:** None. **Y. Han:** None. **J. Koh:** None.

Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

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Program #/Poster #: 197.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RF1AG069378
NIH P20GM113123
NIH U54GM128729
NIH P20GM103442

Title: Anti-$\alpha_4\beta_7$ integrin therapy attenuates gliosis in a mouse model of alzheimer's disease

Authors: *S. CHANDRASEKARAN, A. M. MCINTEE, S. NOOKALA, B. SAHU, A. M. FLODEN, C. K. COMBS;
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Abstract: Using mouse models of Alzheimer's disease (AD) we previously demonstrated changes in intestinal function and inflammatory state that paralleled disease-associated changes in the brain. This association suggests a communication of disease between the intestine and brain. Lymphocyte homing into the gut mucosa is facilitated by the binding of $\alpha_4\beta_7$-integrin to mucosal addressin cell adhesion molecule 1 (MAdCAM-1), expressed on endothelial venules of Peyer's patches and the intestinal lamina propria. We hypothesized that preventing infiltration of lymphocytes with a gut-selective immunomodulatory therapy might be an effective strategy to alleviate immune-related brain changes in AD mice. To test this idea, male and female wild-type (WT) littermate control and age-matched *App*$^{NL\text{-}G\text{-}F}$ knock-in mice (5-7 months old) were left untreated or treated with either anti-$\alpha_4\beta_7$ or an isotype control antibody (IgG2a) at 10 mg/kg/week for 10 weeks, intraperitoneally. One week before euthanizing, the novel object recognition test was used to assess memory. Brains were used to quantify cytokines, Aβ levels, and gliosis. The anti-$\alpha_4\beta_7$ antibody treatment had no dramatic effects on memory, Aβ levels, or plaque load. However, antibody treatment significantly reduced both astrogliosis and microgliosis assessed by astrocyte-specific glial fibrillary acidic protein (GFAP) and microglial marker ionized calcium-binding adaptor protein-1 (Iba-1) immunoreactivity in male and female *App*$^{NL\text{-}G\text{-}F}$ mice compared to controls. Interestingly, both anti-α4β7 and isotype control antibody similarly reduced brain levels of numerous cytokines in both sexes of *App*$^{NL\text{-}G\text{-}F}$ mice compared to controls. Male *App*$^{NL\text{-}G\text{-}F}$ mice demonstrated selective increases in IL-17 and IL-22 levels following anti-α4β7 injection. Our data demonstrate a clear association between intestinal immune cell behavior and disease-associated gliosis in male and female *App*$^{NL\text{-}G\text{-}F}$ mice. In addition, sex-selective changes in brain cytokines are also modulated by the intestine. These data verify a gut-brain communication relevant to AD.

Disclosures: S. Chandrasekaran: None. **A.M. McIntee:** None. **S. Nookala:** None. **B. Sahu:** None. **A.M. Floden:** None. **C.K. Combs:** None.

Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Validation of Tg2576 mice as a preclinical Alzheimer's disease model of neuronal hyperexcitability and evaluation of a selective SSTR4 agonist

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Abstract: Accumulating evidence indicates that neuronal hyperexcitability contributes to Alzheimer's Disease (AD). Further, anti-epileptic therapies such as levetiracetam are known to improve memory deficits in both human patients and mouse models of B-amyloid neuropathology. Somatostatin (SST) and SST receptors (SSTRs) have been found to be reduced in the brain of AD patients, which may contribute to a shift in excitatory/inhibitory (E/I) balance; however, the role of SSTRs in AD hyperexcitability remains elusive. To address whether selective modulation of the hippocampus would reduce aberrant network activity, we leveraged the finding that SSTR4 is enriched in the hippocampus and used hippocampal electrophysiological sleep recordings of interictal spikes (IIS), a marker of epileptic hyperexcitability, in conjunction with pharmacological manipulation of either a selective SSTR4 agonist or glutamatergic transmission (the anticonvulsant levetiracetam) in a Tg2576 mouse model of AD. The dose of levetiracetam was back-translated from human efficacy studies. Consistent with previous findings, we show that hippocampal interictal spikes in Tg2576 mice occur at a higher frequency during rapid eye movement (REM) sleep compared to slow wave sleep (SWS) and wake states. Per-os administration of the SSTR4 agonist at any dose tested did not change interictal spike frequencies during REM, SWS or Wake states compared to Vehicle despite reaching significant levels of estimated SSTR4 receptor occupancy. In contrast, levetiracetam induced a significant reduction in interictal spike frequencies compared to Vehicle-treated animals during both REM, SWS and Wake states. This study further validates the Tg2576 mouse AD model as model of neuronal hyperexcitability and the use of levetiracetam as a translational tool molecule. Our results, however, indicate that selective SSTR4 agonism may not have sufficient inhibitory drive to alleviate aberrant hippocampal activity in the Tg2576 mouse model.

Disclosures: C. Herry: None. **C. Dejean:** None. **G. Hater:** A. Employment/Salary (full or part-time):; TTakeda Development Center Americas. **D.L. Buhl:** A. Employment/Salary (full or part-time):; Takeda Development Center Americas. **J. Selimkhanov:** A. Employment/Salary (full or part-time):; Takeda Development Center Americas. **K. Schleicher:** A. Employment/Salary (full or part-time):; Takeda Development Center Americas. **N. English:** A. Employment/Salary (full or part-time):; Takeda Development Center Americas. **M. Zientek:** None. **O. Toury:** None. **B.**

Buisson: None. **R.E. Petroski:** None. **N.J. Broadbent:** A. Employment/Salary (full or part-time):; Takeda Development Center Americas.

Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Bright Focus Foundation A2019112S
UKRI Future Leaders Fellowship MR/S017003/1
UK Dementia Research Institute, which receives its funding from DRI Ltd., funded by the Medical Research Council, Alzheimer's Society and Alzheimer Research UK

Title: Combination treatment targeting amyloid-β and tau rescues cortical circuit dysfunction in a mouse model of Alzheimer's Disease in vivo

Authors: *R. A. ELLINGFORD[1], S. S. HARRIS[1], M. KEHRING[1], C. SALA FRIGERIO[1], L. A. WELIKOVITCH[2], A. HESLEGRAVE[1], H. ZETTERBERG[1,3], B. T. HYMAN[2], M. A. BUSCHE[1];
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Abstract: There is currently no effective treatment to restore cognitive function in Alzheimer's Disease (AD) patients. Neuropathological diagnosis of AD is dependent on the aggregation of two proteins within the cortex: amyloid-β (Aβ) and tau. Our recent work has shown that co-accumulation of these proteins results in the widespread silencing of neuronal activity within the mouse neocortex. This effect is synergistic, such that the dysfunction cannot be rescued by suppressing tau expression alone. We therefore hypothesised that a combination treatment strategy designed to suppress both Aβ and tau will be necessary to rescue cortical circuit dysfunction in the context of AD. In this study, we utilised an AD mouse model jointly expressing pathogenic forms of human Aβ and tau. We employed in vivo two-photon Ca2+ imaging of neocortical neurons and high-density extracellular recordings of neuronal activity (i.e. Neuropixels) along the neocortical-hippocampal axis to assess the impact of a 6-week combination treatment designed to suppress the production of both Aβ and tau and compared the effects with control treatments suppressing Aβ or tau alone. We demonstrate that the combination treatment significantly reduced the fraction of abnormally silent neurons within the neocortex. In striking contrast, treatments targeted to individual proteins alone had no impact on dysfunctional neocortical activity. Additionally, by employing a combination of immunohistochemistry and blood plasma analysis, we show that while combination treatment prevented the accumulation of soluble AD-associated biomarkers, neuropathological plaques and

tangles were still evident in cortical tissue posttreatment. This result suggests that reducing the levels of soluble pathogenic proteins is necessary for functional improvement rather than the clearance of protein aggregates. Together, these results provide experimental evidence, at the single-cell level, that combination treatment simultaneously targeting Aβ and tau improves neuronal function in a pathological context relevant to AD and suggest that similar treatments may provide clinical benefits to the cognitive function of AD patients.

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Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.11

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: Musashi-1 (MSI1) knockdown reduces tau accumulation in aged brains of P301L tauopathy mouse models

Authors: *L. FUNG, M. MONTALBANO, R. KAYED;
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Abstract: Tauopathies, such as frontal temporal dementia (FTD) and Alzheimer’s Disease (AD), are debilitating neurodegenerative diseases that impact cognitive functioning in a growing number of the elder population. These diseases are characterized by the buildup of toxic tau species in the brain, which is thought to play a significant role in disease progression. While our current understanding of the mechanisms underlying tau aggregation is limited, growing evidence indicates that RNA-binding proteins (RBPs) may play a major role in toxic protein aggregation. In amyotrophic lateral sclerosis (ALS) and FTD, the dysregulation of the RBP Transactive Response DNA binding protein (TDP-43) leads to its aggregation and subsequent toxicity. Similarly, our recent studies have found that another RBP, Musashi-1 (MSI1), forms oligomers and co-aggregates with toxic tau oligomers in several tauopathies. In this study we sought to determine the effects of MSI1 knockdown on tau aggregation and accumulation in a tauopathy mouse model. We performed intracerebroventricular injections of MSI1 antisense LNA oligonucleotides into the hippocampi of 18-month-old P301L mice. Two sets of controls were used, one injected with scramble oligonucleotides and the other receiving no treatment. The levels and aggregation profiles of MSI1 and tau proteins from the hippocampus, cortex, and cerebellum were analyzed and quantified using immunofluorescence microscopy and biochemical assays. Our results show a significant decrease in MSI1 expression in the hippocampus and cerebellum of the MSI1-knockdown mice, confirming the efficacy of our treatment. Furthermore, we found that pathological tau was significantly decreased in the cerebellum and hippocampus of the MSI1-knockdown mice. These results suggest that MSI1

expression plays a role in tau aggregation, revealing it as a possible therapeutic target for tauopathies.

Disclosures: L. Fung: None. **M. Montalbano:** None. **R. Kayed:** None.

Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR Grant MOP-84480
APRI- ASANT Grant
SynAD postdoctoral fellowships grant

Title: Therapeutic potential of native PLGA nanoparticles in the treatment of Alzheimer's disease pathology

Authors: *S. KAR[1], B. G. ANAND[2], Q. WU[2], M. NAKHAEI-NEJAD[3], K. GOVINDARAJAN[2], L. DOROSH[4], S. AMIDIAN[5], A. DAHAL[6], M. STEPANOVA[7], H. WILLE[8], F. GIULIANI[9];
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Abstract: Alzheimer's disease (AD), the prevalent cause of dementia affecting the elderly, is believed to be triggered by increased levels/aggregation of β-amyloid (Aβ) peptides. At present, there is no effective treatment for AD. In this study, we evaluated the therapeutic potential of FDA-approved unconjugated poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles on Aβ aggregation as well as in cellular and animal models of AD. Our results showed that unconjugated PLGA can not only suppress the spontaneous aggregation but can also trigger the disassembly of matured Aβ fibers. Spectroscopic studies, molecular dynamics simulations, and biochemical analyses revealed that PLGA, by interacting with the hydrophobic domain of $A\beta_{1-42}$, prevents a conformational shift towards the β-sheet structure, thus precluding the formation and/or triggering disassembly of Aβ aggregates. Aβ samples collected following PLGA treatment can significantly enhance neuronal viability by restoring impaired lysosomal pH, reducing phosphorylation of tau protein, and the activation of the caspase cascade. Additionally, we showed that PLGA administration into the 5xFAD mouse model of AD can attenuate memory deficits as well as Aβ levels/deposits in the affected cortical regions of the brain. Most

importantly, our data also revealed that unconjugated PLGA can protect iPSC-derived neurons from AD patients against Aβ toxicity by decreasing tau phosphorylation and its associated signaling mechanism. These findings provide unambiguous evidence that unconjugated PLGA, by targeting different facets of the Aβ axis, can have beneficial effects in mouse neurons/animal models as well as iPSC-derived AD neurons - thus signifying its unique therapeutic potential in the treatment of AD pathology.

Disclosures: S. Kar: None. **B.G. Anand:** None. **Q. Wu:** None. **M. Nakhaei-Nejad:** None. **K. Govindarajan:** None. **L. Dorosh:** None. **S. Amidian:** None. **A. Dahal:** None. **M. Stepanova:** None. **H. Wille:** None. **F. Giuliani:** None.

Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.13

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: 45/ 59/2018/PHA/BMS

Title: Alpha 7nAChR activation protects against oxidative stress, neuroinflammation and central insulin resistance in icv-stz induced sporadic Alzheimer’s Disease

Authors: *Y. PERUMAL[1], R. R S[2], S. YADAV[3], J. DHALIWAL[1], M. YADAV[3], K. KONDEPUDI[4], K. CHOPRA[1];
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Abstract: Central insulin resistance is considered as one of the pathological hallmarks of Alzheimer's disease (AD), similar to formation of amyloid plaques and neurofibrillary tangles (NFT). Activation of α7nAChR by GTS-21 has been indicated to reverse peripheral insulin resistance and exert neuroprotection. Therefore, the aim of the present study was to determine the effect of α7nAChR agonist (GTS-21) on intracerebroventricular administration of streptozotocin (ICV-STZ)-induced oxidative stress, neuroinflammation, cholinergic dysfunction, central insulin resistance and cognitive deficits. GTS-21 (1, 4 and 8 mg/kg; *i.p.*) was administered for 21 days following bilateral ICV-STZ administration (3 mg/kg) in C57BL/6 mice. Neurobehavioral assessments were performed using Morris water maze (MWM) and novel object recognition (NOR). Inflammatory markers (TNF-α, IL-6 and IL-1β) were determined using ELISA. Oxido-nitrosative stress (GSH, MDA and nitrite) and cholinergic activity (acetylcholine esterase and choline acetyltransferase) were estimated in the cortex and hippocampus through biochemical methods. Gene expression of insulin receptor (IR), IRS1, IRS2, BACE1, APP, PI3-K, AKT and GSK3β were determined by q-RT-PCR. ICV-STZ administration induced memory impairment, increased oxidative stress and neuroinflammation,

and caused cholinergic dysfunction. Our results demonstrated that activation of α7nAChR by GTS-21 treatment improved memory in MWM and NOR test. Moreover, GTS-21 treatment significantly decreased oxido-nitrosative stress, inflammatory markers and cholinergic dysfunction in cortex and hippocampus. Finally, GTS-21 treatment restored ICV-STZ induced downregulation of IR, IRS1, IRS2, PI3-k, Akt and attenuated GSK3β, APP and BACE-1 indicating improved insulin signalling. Therefore, activation of α7nAChR through GTS-21 might be the potential target for the amelioration of central insulin resistance induced AD.

Disclosures: Y. Perumal: None. **R. R s:** None. **S. Yadav:** None. **J. Dhaliwal:** None. **M. Yadav:** None. **K. Kondepudi:** None. **K. Chopra:** None.

Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.14

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The aged dog model of Alzheimer's Disease progression: changes in biomarkers with age and with RD2, a novel AD-therapeutic

Authors: *C. DE RIVERA[1], J. PRENDERVILLE[2], J. KUTZSCHE[3], D. JURGENS[3], L. ASHDOWN[1], J. BRANDOW[1], K. CROSS[1], R. WINTERS[2], D. WILLBOLD[3], J. ARAUJO[1]; [1]Intervivo Solutions Inc., Fergus, ON, Canada; [2]Transpharmation Ltd., Dublin, Ireland; [3]Forschungszentrum Jülich Gmbh, Jülich, Germany

Abstract: Canine aging is associated with cognitive decline linked to several neuropathological changes that parallel those seen in Alzheimer's disease (AD), such as deposition of amyloid-β. Domain specific cognitive decline is also observed, with short-term working memory and executive function impacted early in canine aging. One contributing factor of AD is neuroinflammation, including activation of microglia and astrocytes, which are also seen in the aged canine brain. The first study sought to investigate whether concentrations of TNFα, IL-2, IL-6 and IL-8 vary in cerebrospinal fluid (CSF) along with levels of Aβ40 and Aβ42. In the second study we investigated whether a novel AD-therapeutic, RD2, would slow down cognitive impairments in aged dogs and whether such changes would be reflected in the following biomarkers: Aβ42, total tau, GFAP, NfL and β-synuclein in CSF samples. In the first study, concentrations of the biomarkers in CSF from three age groups of dogs were quantified using a commercially available canine multiplex cytokine kit and analyzed by the MESO QuickPlex SQ. In the second study, 36 aged beagle dogs (age 9.8 ± 2.5 years) were treated orally for three months with low (3mg/kg/day) or high doses (30mg/kg/day) of RD2 or placebo. Behavioral assessments were conducted longitudinally, and CSF samples were collected at baseline and every month during the treatment period and during an additional two months after treatment discontinuation.
In the first study, there was a significant effect of age group on concentrations of IL-2 [$p < 0.05$]

and IL-6 [$p < 0.05$] and on Aβ40 [$F(2,37) = 12.1587$, $p < 0.0005$] and Aβ42 [$F(2,37) = 6.3943$, $p < 0.005$]. There were no significant differences in TNF-α and IL-8 levels. In study 2, RD2 showed efficacy on cognition in the DNMP and selective attention test, and the changes were sustained even after treatment. There was also a significant treatment-dependent CSF tau oligomer decrease at 30 mg/kg RD2 compared to the other treatment groups ($p < 0.05$). There were no significant changes in the other biomarkers. Collectively, these studies further support the use of aged dogs for examining disease-modifying AD therapeutics. AD relevant biomarkers may be used to select target subject groups representing various stages of AD progression, which can then be examined longitudinally for establishing preclinical efficacy**.**

Disclosures: C. de Rivera: A. Employment/Salary (full or part-time):; Intervivo Solutions Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intervivo Solutions Inc. **J. Prenderville:** A. Employment/Salary (full or part-time):; Transpharmation Ltd. **J. Kutzsche:** A. Employment/Salary (full or part-time):; Forschungszentrum Jülich Gmbh. **D. Jurgens:** A. Employment/Salary (full or part-time):; Forschungszentrum Jülich Gmbh. **L. Ashdown:** A. Employment/Salary (full or part-time):; Intervivo Solutions Inc. **J. Brandow:** A. Employment/Salary (full or part-time):; Intervivo Solutions Inc. **K. Cross:** A. Employment/Salary (full or part-time):; Intervivo Solutions Inc. **R. Winters:** A. Employment/Salary (full or part-time):; Transpharmation Ltd. **D. Willbold:** A. Employment/Salary (full or part-time):; Forschungszentrum Jülich Gmbh. **J. Araujo:** A. Employment/Salary (full or part-time):; InterVivo Solutions Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); InterVivo Solutions Inc..

Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.15

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH Grant AG067049

Title: Blocking PSD-95 depalmitoylation rescues memory deficits in female APP/PS1 mice but not in males

Authors: A. Q. PHAM, M. MANIKKOTH, K. PHILHOWER, K. A. MERRITT, S. WAHABZADA, ***K. B. DORE**;
UCSD, UCSD, La Jolla, CA

Abstract: Several studies have shown that PSD-95 is significantly depleted in brain tissue of Alzheimer’s patients as well as in neurons exposed to Aβ. We recently discovered that elevated levels of PSD-95 can prevent synaptic weakening by Aβ (Dore et al, Cell Reports, 2021). The

amount of synaptic PSD-95 is controlled by a process called ‘palmitoylation’ which mediate the insertion of PSD-95 in post-synaptic membranes. Using an intrabody that specifically binds to palmitoylated PSD-95, PF11, we saw that both Aβ (C99) and mutant Tau (TauP301L) led to significant reductions in PF11 signal and minimal effect on total PSD-95 amounts in hippocampal cultures. In the hippocampus of 6-month old APP/PS1 mice, we observed a 50% reduction in palmitoylated PSD-95 while total PSD-95 amounts were reduced by only 10%. This suggests that loss of PSD-95 palmitoylation would happen before reductions in total PSD-95 during Alzheimer's. Moreover, we characterized PSD-95 palmitoylation in male and female mice of different ages to assess the time course of this decrease and evaluate if there is a sex difference. Interestingly, reductions in palmitoylated PSD-95 were more important in female mice. The specific enzyme responsible for PSD-95 depalmitoylation, ABHD17, removes PSD-95 from synapses. In vitro experiments showed that a commercially available inhibitor of that enzyme (Palmostatin B) could rescue Aβ-induced synaptic depression and Aβ-mediated effects on dendritic spines. Notably, using immunohistochemistry, we saw that Palmostatin B injections in the intraperitoneal cavity rapidly rescued palmitoylated PSD-95 in a dose dependent manner in APP/PS1 mice, which indicates that this drug can access brain synapses in vivo. Moreover, Palmostatin B injections in 9 months-old female APP/PS1 mice rescued memory deficits observed using the Morris Water Maze test. Interestingly, Palmostatin B did not improve the performance of male mice. Since deficits start to appear as early as 3 months in this mouse model, these results suggest that our approach can rescue deficits in animals with severe AD phenotype. Our data show that proteins implicated in Alzheimer's disease pathogenesis significantly reduce PSD-95 palmitoylation and that it is also reduced in female APP/PS1 mice, which might indicate a previously unknown step in the pathophysiology of Alzheimer’s disease. Furthermore, pharmacological blockade of PSD-95 depalmitoylating enzyme rescued memory deficits in vivo in 9 months-old female APP/PS1 mice, likely by increasing PSD-95 palmitoylation levels in the hippocampus. Consequently, PSD-95 depalmitoylating enzyme could be an exciting new drug target against Alzheimer’s disease.

Disclosures: **A.Q. Pham:** None. **M. Manikkoth:** None. **K. Philhower:** None. **K.A. Merritt:** None. **S. Wahabzada:** None. **K.B. Dore:** None.

Poster

197. Alzheimer's Disease: Preclinical Insights

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Program #/Poster #: 197.16

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIA, RF1AG068292

Title: Evidence for G-quadruplexes as pathogenic drivers in Alzheimer’s disease

Authors: *V. M J[1], R. DIAZ ESCARCEGA[1], A. A PATIL[1], C. TAN[1], J. MORUNO MANCHON[1], A. URAYAMA[1], S. P. MARRELLI[1], D. MONCHAUD[2], L. D.

MCCULLOUGH[1], A. S. TSVETKOV[1];
[1]Neurol., Univ. of Texas Hlth. Sci. Center, Houston, McGovern Med. Sch., Houston, TX; [2]Inst. de Chimie Moléculaire de l'Université de Bourgogne, Dijon, France

Abstract: Guanine (G)-rich nucleic acid sequences in the human genome and transcriptome can fold into non-canonical secondary structures known as G-quadruplexes (G4s or G4-DNA/RNA). G4s play significant roles in many cellular processes such as DNA recombination, replication, telomere maintenance and repair, along with many regulatory roles in RNA functions. Stabilizing G4-DNA structures leads to enhanced genomic instability in neurons triggering accelerated aging. Many G4-binding transcription factors, G4-binding proteins (G4BPs), and G4 helicases bind to the G4 structures and modulate their landscapes in cells. Whether and how G4BPs and G4 helicases contribute to aging and age-associated neurodegeneration is not clear. In our studies, we found that stabilizing G4s lead to genomic instability in cultured neurons. We observed a significant increase in the expression of one of the G4-helicase in old AD brains compared to younger ones. Importantly, in Tg2576 Alzheimer's disease (AD) mice, we demonstrated that G4s are abnormally stabilized in the brains, well before the deposition of amyloid occurs. We also discovered that PSEN1 M146L neurons contain more G4 structures than wild-type neurons, likely contributing to genomic instability and altered transcription in AD neurons. Our data demonstrate that G4s are dysregulated in AD models. This area of research provides a foundation for investigating G4s, G4BPs and helicases as important molecular mechanisms in AD that warrant detailed investigation.

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Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.17

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: Effects of CRF_1antagonism on progression of Alzheimer's disease in Tg344-AD male and female rats

Authors: *N. C. REYNA[1], J. T. MADDEN[2], B. J. CLARK[3], S. R. ALLES[5], N. S. PENTKOWSKI[4];
[1]The Univ. of New Mexico, Albuquerque, NM; [2]Psychology, [3]Psychology Dept., [4]Univ. of New Mexico, Albuquerque, NM; [5]Anesthesiol., Univ. of New Mexico Sch. of Med., Albuquerque, NM

Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative brain disorder that leads to various neurological changes, such as cognitive decline and functional impairment. Many

patients diagnosed with AD also exhibit neuropsychiatric symptoms including anxiety and depression. Chronic stress is a common biological mechanism that may exacerbate both anxiety and AD pathogenesis. Corticotropin releasing factor type 1 receptors (CRF_1) are known to play a critical role in the proper functioning of the stress system. Previous studies have shown that AD patients have an upregulation of CRF_1 receptors and that activation of CRF_1 in transgenic mice drives AD pathology. The present study examined the effects of systemic CRF_1 antagonist (antalarmin) injections on anxiety-like behavior in the rat TgF344-AD model that displays full spectrum AD pathology. Tg344-AD and wild type (WT) male and female rats (n=18) 5-5.5 months in age received daily injections of antalarmin (10 mg/kg) or saline for 28 days and then were tested in the elevated plus maze (EPM) to assess anxiety. Rats were then rapidly decapitated, and brains were processed using Western blot hybridization to examine the effects of antalarmin on standard neural markers of AD pathology. This study aims to determine if antagonizing CRF_1 receptors in a transgenic rat model affects neurobiological markers of AD progression through analysis of hippocampal amyloid and tau burden. Additionally, this study aims to determine if chronic administration of antalarmin impacts anxiety-like behaviors of TG344-AD and WT rats during the EPM task.

Disclosures: **N.C. Reyna:** None. **J.T. Madden:** None. **B.J. Clark:** None. **S.R. Alles:** None. **N.S. Pentkowski:** None.

Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.18

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIA Grant R01AG057555
DSRG, Biochemistry Program, Graduate Center CUNY

Title: Agomelatine a melatonin receptor agonist and serotonin receptor antagonist ameliorates pathology and improves cognition in a transgenic rat model of Alzheimer’s Disease

Authors: *G. TERRY[1,4], L. XIE[2], P. ROCKWELL[1], P. SERRANO[3], M. FIGUEIREDO-PEREIRA[1];
[1]Biol. Sci., [2]Computer Sci., [3]Psychology, Hunter College, CUNY, New York, NY; [4]Biochem., The Grad. Center, CUNY, New York, NY

Abstract: Alzheimer's Disease (AD) is the most common neurodegenerative disease in humans. Currently, there are limited treatments for AD. Thus, the need to explore novel drug targets for AD is crucial. Our lab identified agomelatine (AGO) through a high-throughput drug screening algorithm that identifies possible treatments for AD by analyzing off-target effects of FDA approved pharmaceuticals. AGO, also under the brand name Valdoxan, is used to treat clinical depression. It has potential to treat AD due to its MT1/MT2 melatonin receptors agonistic action

coupled to its $5HT_{2C}$ serotonin receptor antagonistic action. This synergistic mechanism of action of AGO could benefit AD as it is a multifactorial disease. To test the efficacy of AGO we used the Fisher transgenic 344-AD rat model of AD, which expresses human mutant "Swedish" amyloid-precursor protein (APPsw) and a Δ exon 9 presenilin 1 (PS1ΔE9). TgF-344AD (Tg) rats exhibit age-dependent progressive AD pathology more closely than other model systems. Male and female TgF344-AD and their wild type littermates were included in our studies. Oral treatment with AGO (dose ~10 mg/kg/day), started at 5 months of age (pre-pathology) and continued for 6 months until the endpoint at 11 months of age. The effect of AGO on cognitive decline was assessed using the hippocampal-dependent active place avoidance task at 9 and 11 months of age. At 11 months of age immunohistochemistry was used to analyze the AGO effects on the hallmarks of AD. So far, our results show that untreated Tg rats exhibit cognitive deficits and amyloid-beta plaques compared to their wildtype (WT) littermates. Notably, AGO-treated Tg rats exhibited lower levels of cognitive decline and amyloid-plaque burden than Tg non-treated rats. We also analyzed the levels of SOX-2, a transcription factor postulated to regulate some AD relevant genes, such as alpha secretase. We found that SOX-2 levels were lower in Tg than WT rats, and AGO-treatment partially reversed the SOX-2 decrease observed in the Tg rats. This rescue effect on SOX-2 levels suggests a potential mechanism for AGO to alleviate amyloid-beta plaque burden in the Tg-treated rats, by increasing non-amyloidogenic processing of APP by alpha-secretase. Based on our data, we propose that AGO, through its role as both a MT1/MT2 agonist and $5\text{-}HT_{2c}$ antagonist, is a drug that can be repurposed to treat AD.

Disclosures: G. Terry: None. **L. Xie:** None. **P. Rockwell:** None. **P. Serrano:** None. **M. Figueiredo-Pereira:** None.

Poster

197. Alzheimer's Disease: Preclinical Insights

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Program #/Poster #: 197.19

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01NS083704
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Title: Virus-like particle (VLP)-based vaccines targeting the pSer396/pSer404-tau (PHF-1 site) phosphorylation site outperform pS199/pS202-tau (AT8 site) at reducing tau pathology in the rTg4510 mouse model of tauopathy

Authors: *J. HULSE, N. MAPHIS, J. PEABODY, B. CHAKERIAN, K. BHASKAR;
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Abstract: Alzheimer's disease (AD) is characterized by the accumulation of tau neurofibrillary tangles (NFTs) and amyloid-β plaques in the brain with accompanied neurodegeneration. Pathological alterations of tau and NFT accumulation correlate with disease progression. Among

many post-translational modifications, phosphorylation of tau on specific epitopes, including the S199/S202 (AT8 antibody site) and the S396/S404 site (PHF-1 antibody site), occur early in the disease process and have been shown to drive tau pathology. Many approaches have been developed to target hyperphosphorylated tau and promote its clearance. Previously, we have utilized Qβ bacteriophage virus-like particle (VLP) platforms to display phosphorylated Thr181 tau peptide and reported robust immune response, tau clearance, and improved memory. Here we report characterization and comparison of a Qβ-PHF-1-VLP and a Qβ-AT8-VLP vaccine. 2-month-old non-transgenic and transgenic rTg4510 mice were administered three bi-weekly intramuscular injections of Qβ control or Qβ conjugated to 25-mer peptides corresponding to the tau PHF-1 site or 21-mer peptides corresponding with the tau AT8 site. Serum antibody titers were assessed using ELISA after the second injection. One week after the third injection, cognitive function was assessed using Morris Water Maze (MWM) and Novel Object Recognition (NOR) behavioral tasks. Western blot analysis and immunohistochemical analyses were performed to assess the levels of phosphorylated and aggregated tau. Both Qβ-PHF-1 and Qβ-AT8 vaccination induced a robust antibody response compared to Qβ control. Qβ-PHF-1 significantly reduced phosphorylated and Sarkosyl-insoluble tau in the brain compared to Qβ control while Qβ-AT8 did not. Qβ-PHF-1 vaccination ameliorated delay-dependent memory deficits assessed by NOR but did not rescue spatial memory deficits assessed by MWM. Qβ-AT8 failed to rescue any deficits in delay-dependent or spatial memory. Qβ-PHF-1 vaccination decreases soluble phosphorylated tau, insoluble tau and exhibits some functional rescue in working memory but is insufficient to rescue deficits in spatial memory (unlike Qβ-pT181 that we previously reported - PMID: 31428463 - which also rescues spatial memory). In summary, Qβ-PHF-1 vaccine outperforms the Qβ-AT8 vaccine, but both are less efficacious than Qβ-pT181.

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Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

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Program #/Poster #: 197.20

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: ReST/UM 201446
SATT AxLR/UM 210324

Title: Neuroprotective effects of Fluoroethylnormemantine (FENM) after chronic infusion by Alzet pumps in the Aß$_{25-35}$mouse model of Alzheimer's disease.

Authors: *A. CARLES[1], A. FREYSSIN[1,2], S. GUEHAIRIA[1], T. REGUERO[1], G. RUBINSTENN[2], T. MAURICE[1];

[1]MMDN, Univ. Montpellier, EPHE, INSERM, Montpellier, France; [2]ReST Therapeut., Montpellier, France

Abstract: Alzheimer's disease (AD) is a devastating neurodegenerative disease and available treatments are mainly symptomatic. In particular, memantine (Ebixa) is a non-competitive NMDA receptor antagonist which mechanism of action suggested promising symptomatic and neuroprotective effects. Fluroethylnormemantine (FENM) is a structural analogue of memantine originally synthesized as a precursor for positron emission tomography radioligand. We previously reported that FENM, administered intraperitoneally, prevented the toxicity, neuroinflammation and learning deficits in Swiss mice intracerebroventricularly injected with oligomerized $A\beta_{25-35}$ peptide [Couly et al., Int J Neuropsychopharmacol 2021]. The drug showed a superior efficacy than Memantine and an absence of direct amnesic effect at higher doses. In the present study, we compared efficiency of two modes of administrations of FENM: chronic subcutaneous infusion with Alzet pump and repeated intraperitoneal injections. C57Bl/6J mice were treated intracerebroventricularly with oligomerized $A\beta_{25-35}$ peptide and after different time points behavioral, biochemical and physiological parameters were analyzed. Different memory tests were used including spontaneous alternation in the Y-maze and object recognition test. Then, toxicity induced in the mouse hippocampus or cortex was analyzed biochemically or morphologically, using markers of apoptosis, neuroinflammation and oxidative stress. (De)regulations of NMDA receptor subunits and its scaffold PSD-95 were also analyzed in hippocampus homogenate or synaptosome preparations by western blots. Data showed that infused FENM is effective after infusion with effective doses in the 0.03-0.3 mg/kg/day range. Learning and memory deficits, neuroinflammation (astrocytic and microglial reactions, cytokines release), oxidative stress and apoptotic markers were attenuated after infusion of FENM. Alteration of PSD-95 and GluN2A/GluN2B ratio will be discussed in the model. FENM therefore appeared as a potent neuroprotective drug using two administration modes in the pharmacological model of AD. These results open the possibility of using the compound either as a patch or tablets in human therapy.

Disclosures: **A. Carles:** None. **A. Freyssin:** A. Employment/Salary (full or part-time):; ReST employment. **S. Guehairia:** None. **T. Reguero:** None. **G. Rubinstenn:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock, patent holder, CEO ReST. **T. Maurice:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; SATT AxLR.

Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.21

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: Selective RNA targeting of mTORC 1 and 2 to ameliorate Alzheimer's disease pathogenesis

Authors: *J. STAHL, A. JOJI, C.-H. VOLMAR, O. KHORKOVA, C. R. WAHLESTEDT; Univ. of Miami Miller Sch. of Medicine, Ctr. for Therapeut. Innovation, Miami, FL

Abstract: Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by amyloid beta deposition and pathological tau protein, with aging being the highest risk factor. It has been well described that inhibition of the kinase, mechanistic target of rapamycin (mTOR), improves age-related pathologies including AD. However, mTOR exists as a complex and exerts differential functions depending on its binding partners. Regulatory-associated protein of mTOR (raptor) is a component of mTOR complex 1 (mTORC1) while rapamycin-insensitive companion of mTOR (rictor) is a component of mTORC2. Suppression of mTORC1 has yielded positive outcomes for AD-related measures (decreased amyloid beta and tau deposition, improved brain insulin sensitivity, and improved cognitive function). However, in contrast, increased mTORC2 activity also resulted in positive outcomes in AD models, suggesting that an increased ratio of mTORC2 to mTORC1 may be desirable for AD. Most inhibitors of mTOR, including rapamycin, do not discriminate sufficiently between mTORC1 and mTORC2, as mTOR is a component of both. Specific activators of mTORC2 have not been reported. Furthermore, most small molecules targeting unique binding partners of either complex lack specificity and tend to have pleiotropic effects. We demonstrate efficient inhibition of mTORC1 by siRNA constructs targeting raptor. We also describe mTORC2 upregulation strategies by targeting the natural antisense transcript (NAT) of rictor using our previously described AntagoNAT technology. By extensive use of AntagoNATs in multiple models, we have successfully upregulated representative target genes by some 2- to 20-fold. In sum, we show that increasing the mTORC2/1 ratio with highly selective RNA therapeutics which bind their targets based on sequence complementarity affects AD-related biomarkers and cellular functions in a cell type specific manner, with minimal off-target effects.

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Poster

197. Alzheimer's Disease: Preclinical Insights

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 2021 FISDU 00182
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2017 SGR 106

Title: Targeting N^6-methyladenosine (m6A) enzymes involved in Alzheimer's disease from two approaches: pharmacological intervention and gene editing in in vivo and in vitro studies

Authors: *A. BELLVER SANCHIS[1], A. IRISARRI-MARTÍNEZ[1], L. LABRADOR[2], M. PALLÀS[1], G. CASADESUS[2], C. GRIÑÁN-FERRÉ[1];
[1]Dept. of Pharmacol. and Therapeut. Chem., Inst. de Neurociències-Universitat de Barcelona, Barcelona, Spain; [2]Dept. of Pharmacol. and Therapeut., Univ. of Florida, Gainesville, FL

Abstract: Epigenetic mechanisms are involved in the functioning of the nervous system and participate in neurological disorders as well as in cognition, learning, and memory formation. Thus, interventions targeting epigenetic regulation might be effective in treating a range of age-related neurodegenerative disorders, and are therefore of considerable translational importance in the field of neuroprotection. One of the most abundant RNA modifications in the human brain is the N^6-methyladenosine (m6A) RNA, a key player in the posttranslational regulation of messenger RNA (mRNA) splicing, translation, and degradation. Therefore, we hypothesize that m6A altered deposition is involved in regulating the gene expression in AD, the levels of β-amyloid deposition as well as other AD-hallmarks, which are the causative factor in AD. Although a relatively high level of m6A RNA has been demonstrated in the brain, the study of m6A modification in the brain is a nascent field, and the importance of this epigenetic mark in brain development and neuronal function is beginning to be unraveled. Indeed, m6A is a biological marker of dynamic and reversible regulation, which relies on the combined action of methyltransferase and demethylase. These enzymes are involved in neurotransmitter delivery and processes related to learning and memory formation, among others. Here, we propose to combine pharmacological intervention and genetic modulation approach in *in vivo* and *in vitro* models to elucidate the role of m6A enzymes as new epigenetic targets for a beneficial CNS effect. Thus, our findings deepen the description of the role of m6A epigenetic mark-related enzymes as a potential target for neurodegenerative diseases.

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Poster

197. Alzheimer's Disease: Preclinical Insights

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Program #/Poster #: 197.23

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH Grant RF1AG056032

Title: Fingolimod treatment improves memory and reduces AD-associated pathology in the PSAPP mouse model of Alzheimer's disease

Authors: *C. M. TOGNONI[1,3], S. MEKNARIT[1,3], I. CARRERAS[1,3,2], A. DEDEOGLU[1,3,4]; [1]Neurol., [2]Biochem., Boston Univ. Sch. of Med., Boston, MA; [3]VA Boston Healthcare Syst., Boston, MA; [4]Radiology, Massachusetts Gen. Hosp. and Harvard Med. Sch., Boston, MA

Abstract: Alzheimer's disease (AD) patients' brains have abnormal lipid metabolism, including increased accumulation of sphingosine and reduced sphingosine-1-phosphate (S1P) levels that correlates with amyloid beta (Aβ). Fingolimod is a structural analog of S1P and an FDA-approved treatment for multiple sclerosis, in which its inhibition of lymphocyte egress from lymph nodes prevents autoimmune damage to the central nervous system (CNS). S1P receptors are also expressed in CNS cells and have been attributed to a variety of functions, including cell survival, angiogenesis, and anti-inflammatory responses - all of which may be beneficial to target in AD. We recently showed that fingolimod treatment administered in drinking water from 1-3 and 1-8 months of age in the aggressively-progressing 5xFAD mouse model of AD provides neuroprotection and reduces neuroinflammation, optimally at a low dose (0.03 mg/kg/day) that does not suppress circulating lymphocytes. Here, we tested if fingolimod treatment could provide protection in the more slowly-progressing PSAPP model. Fingolimod was administered from 1-8 or 1-14 months of age to evaluate long-term treatment periods that begin before the onset of AD-related pathology, and from 11-14 or 17-20 months to evaluate treatments that begin in advanced disease states. Treatment resulted in improved memory performance on the Morris water maze in PSAPP mice when administered from 1-8 months of age, but not from 1-14, 11-14, or 17-20 months. For instance, while untreated PSAPPs had impairments on a 24 hr memory probe at 8 months of age compared to wildtype (WT) mice, fingolimod-treated PSAPPs performed similarly to WTs in spending 31% percent more time in the target quadrant compared to untreated PSAPPs and were also no different from WTs in number of pass-throughs or latency to the target area. Treatment from 1-8 months of age also significantly decreased cortical peptide levels of insoluble Aβ40 (by 43%) and both soluble (by 38%) and insoluble (by 40%) Aβ42. Plaque burden determined from Aβ42 immunostaining was also reduced (by 38%) in this group. Fingolimod treatment from 1-14 months decreased soluble Aβ40 (by 40%), and treatment from 11-14 months decreased soluble Aβ42 (by 32%), while there was no effect on Aβ levels from the 17-20 month period. Fingolimod's strong effect on Aβ pathology in PSAPP mice from 1-8 months but less so from other treatment periods suggests that it is beneficial during the early stages of the development of AD-like pathology. Together, our studies are showing that the modulation of S1P signaling in the CNS with low-dose fingolimod may be a putative therapy to provide neuroprotection and improve cognition in AD.

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Poster

197. Alzheimer's Disease: Preclinical Insights

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Program #/Poster #: 197.24

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSERC

Title: Impaired object memory updating in male 3xTg-AD mice despite intact object recognition memory

Authors: *K. H. ABOUELNAGA, K. H. JARDINE, B. D. WINTERS;
Univ. of Guelph, Univ. of Guelph, Guelph, ON, Canada

Abstract: Reactivation-induced memory destabilization places consolidated memories into a labile state in which they are open to modification. Memories can then become reconsolidated with updated information. Previous evidence suggests that memory updating breaks down in patients with dementia, but there is limited research characterizing the mechanism that leads to such cognitive deficits. In this study, we utilized the post-reactivation object memory modification (PROMM) task to characterize object memory updating deficits in the 3xTg Alzheimer's Disease (AD) mouse model. The PROMM task is an exploration paradigm that exposes mice to novel contextual information following object memory reactivation. Based on previous evidence that the 3xTg mice exhibit cholinergic dysfunction at 6 months of age and evidence from our lab that the cholinergic system is implicated in object memory updating, we hypothesized that cholinergic system dysfunction associated with AD pathological progression contributes to object memory updating deficits. Findings indicated that male 3xTg mice exhibit object memory updating deficits at 4 months of age. Next, we investigated whether this effect was exclusive to object memory updating and not based on a general memory impairment. To test this, the spontaneous object recognition (SOR) task was conducted, and findings indicated that the 3xTg mice possess intact object recognition memory, suggesting that deficits exhibited in the PROMM task at this age are exclusive to memory updating. These findings point to the possibility that cholinergic dysfunction in AD is a main contributor to object memory deficits and targeting the cholinergic system could alleviate memory updating deficits in AD patients.

Disclosures: K.H. Abouelnaga: None. **K.H. Jardine:** None. **B.D. Winters:** None.

Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.25

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JSPS KAKENHI 19K16288

Title: Identification of molecular mechanisms for accurate axonal regeneration in Alzheimer's disease model mouse brains

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Abstract: Alzheimer's disease (AD) is a progressing neurodegenerative disorder developed by deposition of Aβ and disruption of neural networks in the brain. We consider that it is important to regenerate neural circuits for recovering memory function in AD. We previously found that diosgenin, a constituent of Dioscorea Rhizoma, restored Aβ-induced axonal atrophy in cultured neurons and improved memory deficits in a mouse model of AD, 5XFAD. In the present study, we investigated whether diosgenin promoted long-distance axonal regeneration toward their intrinsic target area in 5XFAD brains, and clarified molecular mechanisms for accurate pathfinding of injured axons. To investigate axonal regeneration effect of diosgenin, we focused on a long-distance neural circuit for memory formation; the hippocampus to the prefrontal cortex. Retrograde tracing revealed that 14-day administration of diosgenin promoted axonal regeneration from the hippocampus to the prefrontal cortex in 5XFAD mice. Subsequently, naïve neurons and axon-regenerated neurons in the brain slices were individually captured by laser microdissection to serve DNA microarray. The gene (the name is closed; gene A), whose expression was the most elevated in axon-regenerated neurons, was overexpressed in hippocampal neurons of 5XFAD mice. As a result, overexpression of protein A significantly promoted axonal regeneration in the brain and recovered memory deficits in 5XFAD mice. Next, we focused on a protein A-interacting molecule, collagen I, because we found that collagen I existed on extracellular space like axonal trails even after axons were degenerated by Aβ treatment. When collagen I was coated on direction-specific extracellular space, protein A-overexpressed neurons extend axons more preferable to collagen I-coated direction. Our study showed that axons in AD brains have capacities to regenerate toward long distance away target area by diosgenin administration. In addition, protein A-collagen I interaction is a key molecular pair for controlling accurate pathfinding of injured axons in AD brains. This finding proposes a novel therapeutic strategy to promote axonal regeneration for AD treatment.

Disclosures: **X. Yang:** None. **C. Tohda:** None.

Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.26

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Anti-pathological tau antibody raised against AD brain derived competent seeds reduced tau pathology in transgenic mice

Authors: *K. YANAMANDRA[1], N. VENKAT[1], H. PATEL[1], H. SANDERS[1], R. CHANG[1], A. DARCY[2], N. BROWN[2], H.-Y. WU[2], J. WU[1], T. KWON[1], J. MANOS[1], E. KARRAN[1], T. DELLOVADE[1], X. LANGLOIS[1];
[1]AbbVie - Foundational Neurosci. Ctr., Cambridge, MA; [2]Global Biologics, AbbVie - Bioresearch Ctr., Worcester, MA

Abstract: Anti-pathological tau antibody raised against AD brain derived competent seeds reduced tau pathology in transgenic mice Kiran Yanamandra[1], Nandini Venkat[1], Hiral Patel[1], Hollie Sanders[1], Rui Chang[1], Anhdao Darcy[2], Nathan Brown[2], Hai-Yan Wu[2], Jessica Wu[1], Taekyung Kwon[1], Justine Manos[1], Eric Karran[1], Tammy Dellovade[1], Xavier Langlois[11]AbbVie Foundational Neuroscience Center, Cambridge, MA, USA[2]AbbVie Bioresearch Center, Global Biologics, Worcester, MA, USA
Accumulation of intracellular hyperphosphorylated tau protein into aggregates called neurofibrillary tangles is a hallmark of Alzheimer's disease (AD) and other neurodegenerative diseases. In AD, tau species trans-synaptically propagate in a defined pattern and the load of aggregates in affected brain regions correlates with cognitive decline. We hypothesized that antibodies targeting the tau species responsible for the propagation of tau pathology could slow the disease progression. However, it is not clear which type of tau is responsible for propagation. The objective of our study was to isolate and characterize the bioactive tau species from AD brain and develop antibodies against them. Here we used sucrose gradient fractionation and observed that a strikingly small amount of tau enriched across 30% fraction were seeding competent while majority of tau observed in the 0% sucrose fractions was not bioactive on ultrasensitive HEK biosensor cells or hTau primary neurons. We injected 0%, 30% and 50% fractions into the hippocampus of 2mo old Tg4510 mice and confirmed that only high-density AD sucrose fractions carry seeding competent tau. Additionally, we separated AD brain-derived tau species by size-exclusive chromatography and confirmed increased seeding activity in high molecular weight fractions, but not in low molecular weight fractions. In parallel we developed various SIngle MOlecular Array (SIMOA) assays to evaluate the quantity of phosphorylated and aggregated tau species in the different fractions. We raised a panel of antibodies against 30-50% fractions and identified a confirmation-specific and phospho-specific tau antibody (E01-008). In a Tg4510 tau seeding model, we observed that E01-008 at the dose of 10 mpk reduced the level of tau pathology induced by hippocampal injection of AD brain lysate. Overall, our data suggest that only a small portion of tau species are involved in seeding activity and propagation and antibody could be raised against that rare material. However, although such antibody could inhibit tau seeding in vitro and in vivo, their capacity to block the trans-synaptic propagation of tau remains to be demonstrated.

Disclosures: K. Yanamandra: A. Employment/Salary (full or part-time):; AbbVie. **N. Venkat:** A. Employment/Salary (full or part-time):; AbbVie. **H. Patel:** A. Employment/Salary (full or part-time):; AbbVie. **H. Sanders:** A. Employment/Salary (full or part-time):; AbbVie. **R. Chang:** A. Employment/Salary (full or part-time):; AbbVie. **A. Darcy:** A. Employment/Salary (full or part-time):; AbbVie. **N. Brown:** A. Employment/Salary (full or part-time):; AbbVie. **H. Wu:** A. Employment/Salary (full or part-time):; AbbVie. **J. Wu:** A. Employment/Salary (full or part-time):; AbbVie. **T. Kwon:** A. Employment/Salary (full or part-time):; AbbVie. **J. Manos:** A. Employment/Salary (full or part-time):; AbbVie. **E. Karran:** A. Employment/Salary (full or part-time):; AbbVie. **T. Dellovade:** A. Employment/Salary (full or part-time):; AbbVie. **X. Langlois:** A. Employment/Salary (full or part-time):; AbbVie.

Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.27

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Identification of new drug that reduces BACE1 expression with cell-based screening system

Authors: *Y. LEE, J. PARK, H.-G. BAE, D.-G. JO;
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Abstract: Alzheimer's disease (AD) is a chronic neurodegenerative disease. AD exerts an enormous burden on managing costs though the cause of AD is still unclear. Amyloid hypothesis postulated that exceeding extracellular amyloid-beta (Aβ) deposits are the fundamental cause of the disease. Aβ is made by sequential proteolysis to amyloid-beta precursor protein (APP) by β-secretase (BACE1) and γ-secretase. Another important phenomenon in AD patients is increased BACE1 expression. However, direct and complete blocking of enzymatic activity of BACE1 can cause unpredictable side effects because of numerous physiological substrates of BACE1. Therefore, we tried to find specific drugs reducing BACE1 expression rather than direct inhibition of BACE1. Using USA FDA-approved drug library (Prestwick Chemical Library), we could discover putative therapeutic chemicals by cell-based assay. Using Bace1 promoter-reporter screening, we found an effective drug for reducing BACE1 gene expression. This compound effectively reduced the levels of BACE1 protein and mRNA in SH-SY5Y cells. In primary cultured neurons, BACE1 decreased with this compound treatment in a dose-dependent manner. We also confirmed that the BACE1 lowering compound could improve the cognitive functions of 3xTg-AD mice. In Morris water maze test, this compound treated 3xTg-AD mice showed restored performance compared to vehicle-treated 3xTg-AD mice. The compound-treated 3xTg-AD mice also exhibited similar latency of chamber entering in passive avoidance test compared to WT. The drug-treated AD mice showed a decreased level of Aβ deposition and BACE1 expression. Together, we suggest that the BACE1 expression lowering agent could be a novel drug that modulates the expression of the BACE1 level, providing a new direction for AD therapy.

Disclosures: Y. Lee: None. **J. Park:** None. **H. Bae:** None. **D. Jo:** None.

Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.28

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Undergraduate Research Grant from the Office of Sponsored Programs at Central Michigan University

John G. Kulhavi Professorship in Neuroscience
E. Malcolm Field and Gary Leo Dunbar Endowed Chair in Neuroscience

Title: Use of Progesterone for Reducing Learning Deficits in 3xTg Mouse Model of Alzheimer's Disease

Authors: *E. LAUZON[1,2,3,4], S. KONERU[1,2,3], N. WEDSTER[1,2,3], P. OTERO SEQUEIROS[1,2,3,5], E. IDZIOR[1,2,4], N. MOJARRADLANGROUDI[1,2,3], C. BUENO ALVAREZ[1,2,3], J. WASSELL[1,2,3], R. SCHALAU[1,2,3], B. KEMMERLING[1,2,3], J. PATEL[1,2,3], K. BELGYA[1,2,3,6], N. SHARMA[1,3,7], J. ROSSIGNOL[1,2,3,5], G. L. DUNBAR[1,2,3,8,9];
[1]Central Michigan Univ., Mount Pleasant, MI; [2]Field Neurosciences Inst. Labortory for Restorative Neurol., Saginaw, MI; [3]Program in Neuroscience, Central Michigan Univ., Mount Pleasant, MI; [4]Dept. of Biology, Central Michigan Univ., Mount Pleasant, MI; [5]Col. of Medicine, Central Michigan Univ., Mount Pleasant, MI; [6]Eckerd Col., St. Petersburg, FL; [7]Sch. of Hlth. Sciences, Central Michigan Univ., Mount Pleasant, MI; [8]Dept. of Psychology, Central Michigan Univ., Mount Pleasant, MI; [9]Field Neurosciences Inst. Ascension St. Mary's, Saginaw, MI

Abstract: Alzheimer's disease (AD) is a neurodegenerative disease that first affects the entorhinal cortex and hippocampus regions of the brain. It is characterized by the aggregation of amyloid-beta (Aβ) protein in the form of plaques in the extracellular space and inclusions in the blood vessels, and hyperphosphorylation of the protein tau in the form of intraneuronal aggregates of neurofibrillary tangles (NFTs). Clinical manifestations of AD include progressive memory loss, psychological and emotional disturbances, attention deficits, and visuospatial deterioration. Currently, no treatment has been shown to effectively modify the disease progression in AD. Most of the current treatments focus on specific aspects of AD and provide, at best, palliative treatments on the targeted symptoms. Progesterone was chosen to treat memory dysfunction in the 3xTg mouse model of AD because of its pleiotropic effects shown in past studies. Progesterone is a major gonadal and neurosteroid hormone that has been shown to exert neuroprotective effects in in vitro and in vivo studies of brain injury and disease, including some rodent models of AD. Progesterone influences neuronal function by modulating gene transcription and cellular activity by binding to intracellular receptors abundant in the central nervous system. The mouse model used in the current study, 3xTg-AD shows both Aβ plaques and NFTs, as well as the synaptic dysfunction that mimics what is observed in AD patients. The 3xTg-AD mice display behavioral impairments such as severe memory loss, as well as the emotionality and psychological changes that are representative to those seen in AD patients. As the primary goal of this project was to determine if progesterone is an effective treatment for reducing the memory deficits in the 3xTg mouse model, a spatial reversal task was utilized using a water- T-maze. Daily oral-gavage injections of progesterone (5 mg/kg) or equivalent volumes of the vehicle were administered for 40 days. The results indicated that progesterone-treated C57 wild-type male mice made fewer errors than vehicle-treated control and 3xTg mice, with the progesterone-treated 3xTg male group showing marginal improvement in their performance on the water-T-maze tests. The results of the behavior test are supported by the blood serum Luminex assay data which indicates that progesterone enhanced cognitive functioning in the wild-type mice. Although further data analysis is required of additional behavioral tests, the results suggest that progesterone may provide benefits in reducing AD-induced cognitive deficits.

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Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.29

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant RF1 AG020670
NIH Grant RF1 NS093652
NIH Grant P01 AG066606

Title: Oleoylethanolamide analog, KDS-5104, attenuates lipid dysregulation and amyloid beta pathology in an Alzheimer's disease mouse model

Authors: *M. M. COMEROTA[1], M. GEDAM[1], L. DENG[2], F. JIN[1], M. WANG[1], J. WANG[2], H. ZHENG[1];
[1]Huffington Ctr. on Aging, [2]Dept. of Pharmacol. & Chem. Biol., Baylor Col. of Med., Houston, TX

Abstract: Alzheimer's disease (AD) is characterized by the accumulation of amyloid beta (Aβ) plaques and extracellular tangles consisting of hyper-phosphorylated tau. Emerging evidence suggests that metabolic dysfunction, specifically lipid dysregulation, is linked to AD pathology and disease progression. Recently, oleoylethanolamide (OEA) was identified as one of the lipid signaling pathways impaired in AD patients. OEA, a lipid amine and a potent PPARα agonist, has previously been shown to promote longevity in *C. elegans* and increase lipid metabolism in high fat diet mouse models. The goal of our study was to determine the role of OEA in the regulation of AD related dyslipidemia and Aβ pathology accumulation. In addition to lipid signature changes in the cortex of the Aβ mouse model, 5xFAD, we also identified a reduction of PPARα expression in Aβ plaque associated microglia. This suggests that targeting OEA could enhance the PPARα pathway leading to improved microglia metabolic fitness and efficiency of Aβ clearance. We utilized the more stabilized OEA analog, KDS-5104, to study the properties of phagocytosis in primary microglia cultures from wild type and PPARα knockout mice. We found that microglia treated with KDS-5104 have increased phagocytosis via a PPARα dependent CD36 upregulation. KDS-5104 promotes Aβ and lipid uptake, as well as, accelerated degradation. This enhanced clearance contributes to decreased inflammatory response. We further utilized the acute LPS induced inflammation mouse model to demonstrate the role of KDS-5104 in the suppression of neuroinflammation. To determine the role of KDS-5104 in the mediation of AD pathology, we treated the 5xFAD mouse model with KDS-5104. We found that KDS-5104 treatment reduced neuroinflammation and altered the lipid profile of 5xFAD mice. In

addition, the KDS-5104 treated 5xFAD mice displayed reduced Aβ plaque accumulation and improved cognitive function. Together, our current experiments have provided valuable insight into the role of OEA in microglial phagocytosis and the mediation of AD pathology.

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Poster

197. Alzheimer's Disease: Preclinical Insights

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 197.30

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: FAU Bridge Grant-Brain Institute

Title: Granulocyte colony stimulating factor as a potential therapy for Alzheimer's disease

Authors: *S. BHANDARI[1], P. SARTIPI[1], H. SONTAG[1], H. PRENTICE[2], J.-Y. WU[3]; [2]Charles E. Schmidt Col. of Med., [3]Professor of Biomed. Sci., [1]Florida Atlantic Univ., Boca Raton, FL

Abstract: Alzheimer's disease (AD) is the most common cause of dementia in older adults. Despite best efforts from industry and academia, there is no effective treatment to slow down or halt its progression. Granulocyte-colony stimulating factor (G-CSF) is quite effective in preventing neuronal injuries and improving neuronal functions in the animal models of stroke, Parkinson's, and Alzheimer's disease. In order to understand the potential of G-CSF in the treatment of neurodegenerative diseases, we constructed Human G-CSF gene therapy (hG-CSF) using replicant deficient AAV serotype-2 vector carrier with cytomegalovirus (CMV) promoter. Mice(3xTgAD) were given approx. 3*10^9 pfu of this therapy on the left eye as a single dose treatment. To validate drug delivery, we measured the expression of hG-CSF protein and gene products in the mice's brains. Western blot analysis showed expression of human G-CSF protein in the mice brains, and RT-qPCR data re-confirmed this result. AD affects multiple cellular processes, including activating pro-death cascades resulting in oxidative, mitochondrial, ER stress, autophagy, and neuroinflammation. While neuroinflammation is a body's normal mechanism designed to protect the brain by removing or inhibiting different pathogens, the clearance ability of microglia and astrocytes in AD is overwhelmed, which leads to the release of pro-inflammatory inflammatory cytokines as well as the inhibition of anti-inflammatory response signals, resulting in exacerbating AD pathologies. Neuroinflammatory response in AD is mediated through the mTOR signaling pathway, and our study shows a significantly reduced level of mammalian target of rapamycin (mTOR) activation in AD mice, and hG-CSF gene therapy significantly upregulated its phosphorylated product.Next, we aimed to understand the neurogenic potential of hG-CSF gene therapy, including functional improvement. Our ongoing study shows the potential of hG-CSF to stimulate hippocampal neurogenesis as measured by the

upregulation of neuronal progenitor proliferation and their differentiation and its potential to promote integration into the Dentate Gyrus and CA3 hippocampus. Our behavioral data, namely water maze, shows improvement in the ability of mice to find the hidden platform after drug administration. Drug-treated mice also improved the latency to the target quadrant. To conclude, we believe that we have developed a simple yet effective non-invasive gene delivery system for the AAV-GCSF vector. We have demonstrated the efficacy of hG-CSF gene therapy by showing neuroprotection and neurogenic capacity, including improved functional outcomes in the mice model of AD.

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Poster

198. Alzheimer's Disease: Biomarkers III

Location: SDCC Halls B-H

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Program #/Poster #: 198.01

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: National Institute of Health Grant R01NS083704
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National Institute of Health Grant RF1NS083704
National Institute of Health Grant P20GM121176

Title: Apoptosis-associated speck-like protein containing a CARD (ASC) as a potential biomarker of dementia in cerebrospinal fluid

Authors: *K. E. SANCHEZ[1], S. JIANG[2], S. HOBSON[1], J. F. THOMPSON[1], S. P. DESAI[2], G. A. ROSENBERG[1], K. BHASKAR[2];
[1]Ctr. for Memory and Aging, [2]Dept. of Mol. Genet. and Microbiology, Univ. of New Mexico, Albuquerque, NM

Abstract: Dementia impacts about fifty-five million individuals world-wide, and this number is expected to double every twenty years according to the World Health Organization. Though amyloid beta plaques and neurofibrillary tangles comprised of the hyperphosphorylated microtubule-associated protein tau are established hallmarks of dementia, inflammation is critical to its pathology. In a pathological state, microglia, which are the innate immune cells of the brain, assemble a multiprotein complex called the inflammasome. The inflammasome is composed of the NACHT, LRR, and PYD domain-containing protein 3 (NLRP3); the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC); and inflammatory caspase 1 (cysteine-dependent aspartate-directed protease 1). The ASC and pro-caspase 1

components of the complex lead to the activation of caspase-1, which can facilitate the cleavage of IL-1β and IL-18. Upon labeling for ASC, the inflammasome complex appears as a speck. This visualized "ASC-speck" is approximately 1 μm in diameter, secreted into the extracellular space, and is capable of cross-seeding in a prionoid-manner. Our recent study in a small patient population (PMID: 34551296) detected ASC-specks in the cerebrospinal fluid (CSF) of patients with tauopathies. However, it is unclear if ASC-specks could serve as a potential biomarker in a larger patient cohort. To determine if ASC is a feasible biomarker of disease, flow cytometry was utilized to quantify ASC specks in CSF. Here, we report for the first time to our knowledge that levels of ASC specks are significantly increased (p= 0.0033) in the CSF samples of dementia patients (n=18;1.9x104 specks/μL ± 1918) in a group primarily comprised of AD patients compared to community member controls (n=11; 1x104 specks/μL ± 1756). Preliminary investigations suggest that the amount of ASC in the CSF correlates with phosphorylated threonine 181 tau (pT181+ tau). Together, these studies suggest that ASC-speck levels could serve as a valid inflammatory biomarker for dementia diagnosis and supplement pT181+ tau levels in the CSF.

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Poster

198. Alzheimer's Disease: Biomarkers III

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 198.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Fluid biomarkers and neuroinflammation panel for preclinical proof of concept studies in AD

Authors: S. CARMANS, W. DEJONCKHEERE, ***T. CORNELISSEN**;
reMYND, Leuven, Belgium

Abstract: The interest in CSF and blood-based biomarkers has significantly increased the last years. Not only in diagnosis and follow up of Alzheimer's disease (AD) patients but also in clinical studies as primary and secondary outcomes. In addition, the role of neuroinflammation and the potential of anti-inflammatory therapies have taken a center stage in the pathogenesis of AD. Since translation between preclinical models and clinical data is of the utmost importance, we set out to investigate the presence of fluid biomarkers in preclinical AD mouse models. For this reason we are following the A (Amyloid)-T (Tau)-N (Neurodegeneration) framework which is being used more and more in the clinic (Clifford R.J. et al; 2016). In this aspect, we found in our APP-London x TauP301S model that plasma NF-L levels (as a marker for ongoing neurodegeneration) are clearly increased, suggesting that persistent neurodegeneration is ongoing. Next to longitudinal markers for neurodegeneration we are also assessing Abeta and (p)Tau pathology in the plasma of AD mice. In addition GFAP plasma levels will be assessed

since it was postulated that GFAP plasma levels correlate very well with Abeta pathology in the brain (Verberk I.M.W. et al.; 2020). Due to the recent increasing demand in assays measuring neuroinflammation in AD preclinical models we sought to determine the level of several inflammatory markers in the mouse brain. Previously we were already able to show CD45 and GFAP levels to be increased in the mouse brain of aged APP-London x TauP301S mice, as assessed by IHC. In addition we also resided to qPCR and measured the mRNA levels of several cytokines and found that Il1b and Il33 mRNA levels were altered in our APP-London x TauP301S model. In the near future, we hope to expand this with other inflammasome proteins like NLRP3. Recapitulating intermittent fluid biomarkers and neuroinflammation assays in e.g. the APP-London x TauP301S mice could therefore be of great contribution to preclinical AD research. As a next step, we are extending the above set of models and concomitant biomarker and neuroinflammation read-outs to a second-generation APP knock-in model. Humanizing animal models at the level of microglia, to bridge the species barrier upfront, is another exciting avenue that we are exploring. This will open a completely new array of questions that can be more credibly posed already in preclinical stage.

Disclosures: **S. Carmans:** A. Employment/Salary (full or part-time):; reMYND. **W. Dejonckheere:** A. Employment/Salary (full or part-time):; reMYND. **T. Cornelissen:** A. Employment/Salary (full or part-time):; reMYND.

Poster

198. Alzheimer's Disease: Biomarkers III

Location: SDCC Halls B-H

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Program #/Poster #: 198.03

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIA R01 AG055449
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NIGMS S10 OD023573

Title: Plasma TDP-43 Concentration is Negatively Associated with Gray Matter Volume in Medial Temporal Lobe Structures

Authors: *B. T. GOLD[1], C. BAUER[1], V. ZACHARIOU[2], T. L. SUDDUTH LEE[3], P. T. NELSON[1], G. JICHA[1], D. M. WILCOCK[1];
[2]Neurosci., [1]Univ. of Kentucky, Lexington, KY; [3]Sanders-Brown Ctr. on Aging, Univ. of Kentucky Chandler Med. Ctr., Lexington, KY

Abstract: Tar DNA binding protein of 43kDa (TDP-43) proteinopathy is the hallmark pathological feature of limbic-predominant age-related TDP-43 encephalopathy (LATE). LATE affects more than one-third of individuals over the age 85 and is associated with medial temporal

lobe neurodegeneration with or without comorbid Alzheimer's disease. At present, the diagnosis of TDP-43 pathology can only be made at autopsy. There is thus an urgent need for a clinical biomarker for LATE and a blood-based method would be optimal for clinical and economical reasons. Here, we assessed the relationship between plasma TDP-43 levels with neuroimaging measures of gray matter (GM) volume. Forty-three older adults (age range = 60-85 years old) without dementia participated in this study. Plasma TDP-43 concentration (pg/ml) was assessed on the Quanterix HD-X instrument using the Simoa TDP-43 Advantage kit at a 1:10 dilution according based on optimization performed in our biomarker core. Participants underwent neuroimaging within one year of their plasma being collected. Neuroimaging was conducted at the University of Kentucky MRISC using a 3 Tesla MRI Siemens Prisma scanner with a 64-channel head coil. A T1-weighted MPRAGE sequence with 1 cubic mm voxels covering the entire brain was acquired. FreeSurfer was used to estimate intracranial volume (ICV), lobar volumes, and hippocampal subfield volumes. Multivariate linear regression models controlling for age, sex, and ICV were conducted in SPSS using plasma TDP-43 as the predictor and volumetric measurements as dependent variables. Results indicated a negative association between plasma TDP-43 concentration and GM in the temporal lobe ($p=0.013$), but not other cortical lobes. In addition, plasma TDP-43 levels were negatively associated with bilateral hippocampal subfield volumes in CA3 ($p=0.022$), CA4 ($p=0.027$) and the dentate gyrus ($p=0.037$). Our results indicate that high levels of plasma TDP-43 are associated with lower GM volume in regions known to be preferentially affected by TDP-43 pathology in LATE. These findings suggest that further exploration of plasma TDP-43 as a biomarker of TDP-43 pathology is warranted. This possibility should be tested in future studies with larger sample sizes that incorporate neuropathology results.

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Poster

198. Alzheimer's Disease: Biomarkers III

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Translational peripheral biomarkers for SHIP1 inhibitors in Alzheimer's disease

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Abstract: Alzheimer's disease (AD), the most common neurodegenerative disease, is characterized by cognitive decline, neuroinflammation and neuronal death. Several microglia specific genes are associated with AD risk and microglia are brain resident macrophages directly involved in neuroinflammation. Here we focus on a non-coding variant (rs35349669) in the inositol polyphosphate-5-phosphatase (INPP5D) locus, also known as SHIP1, that has been associated with increased risk of developing AD. SHIP1 is primarily expressed in macrophages including microglia and is thought to be involved in the signaling pathways influencing phagocytosis as well as cytokine release. There are very few blood-based biomarkers for neuroinflammatory targets. Therefore, we sought to identify markers related to modulation of SHIP1 in peripheral blood mononuclear cells (PBMCs), which would serve as measures of target engagement as these molecules advance to clinical trials. PBMCs were plated at 1 million cells per well for overnight incubation. Then, 10 µM SHIP1 inhibitor, or DMSO (control), was added to the cells. After 24h, the supernatant was collected, the cells were washed in PBS and frozen for RNA extraction. Cytokine release in the supernatant was measured using Ella and MSD kits, and gene expression in the PI3K pathway was measured with NanoString nCounter panel Vantage 3D RNA MAPK PI3K Pathways. We find that pro-inflammatory cytokines CCL2 and TNF-α are decreased with SHIP1 inhibitor. We observed an increase in the low-density lipoprotein receptor (LDLR) gene expression after SHIP1 inhibitor administration. LDLR is thought to play a role in phagocytosis of amyloid through interactions with APOE associated plaques and follow up studies are underway to connect peripheral and central changes in vivo.

Disclosures: A. Lee-Gosselin: None. **K.A. Russ:** None. **S. Brown:** None. **C. Jesudason:** None. **E. Mason:** None. **L.G. Apostolova:** None. **K.N.H. Nudelman:** None. **A.J. Saykin:** None. **S. Chu:** None. **T. Richardson:** None. **A. Palkowitz:** None. **J.L. Dage:** None.

Poster

198. Alzheimer's Disease: Biomarkers III

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Topic: C.02. Alzheimer's Disease and Other Dementias

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PREADAPT project: EU Joint Programs for Neurodegenerative Diseases Research (JPND) PERSOMED
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ISCIII grant: AC19/00097

Title: Increased TAM receptors in CSF: Protection against Alzheimer's disease?

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Abstract: The role of inflammation in Alzheimer’s disease (AD) is well established. Recently, we showed that subjects who contain elevated soluble TAM receptors, Tyro3 and Axl, in their cerebrospinal fluid (CSF) had larger cortical volume and were more stable in cognition at follow-up (Brosseron et. al., 2021). In the current study, we used the Olink Explore platform for biomarker measurements in human CSF samples of N=489 and N=784 from the DELCODE (Germany) and Fundació ACE (Spain) cohorts respectively. The biomarker levels were stratified corresponding to pathological amyloid-β (Aβ) and tau levels in patients. Here, we first validated our previous results that Tyro3 and Axl are significantly increased in tau-positive patients using the Olink platform. Additionally, we have uncovered novel biomarkers that are related to the TAM signaling pathway namely, MerTK, Protein S, and Gas6 that showed similar effects. Statistical analyses for the human proteomics were performed and visualized in R software. To functionally validate our clinical findings *in vitro*, we used three human monocytic leukemia (THP-1) cell lines: Wild-type (Control), Tyro3-overexpressing (Tyro3OE), and Axl-overexpressing (AxlOE). Since tau and Aβ are critical proteins in AD, we checked the influence of 3h tau exposure on Aβ phagocytosis. Statistical analyses for N=3-8 independent *in vitro* experiments were done in GraphPad PRISM 8. We observed a reduction of Aβ phagocytosis in the presence of tau in control THP-1 cells, but this effect was absent in Tyro3OE cells. To examine the NLRP3 inflammasome activity in these cells, we treated them with tau for 3h and Aβ for 24h. We found a significant reduction in IL-1β release in the Tyro3OE when compared with control THP-1 cells. This was supported by a reduced IL-1β mRNA expression in Tyro3OE cells. STAT1 phosphorylation in Tyro3OE cells was significantly increased in western blot

analysis, which when inhibited by JAK1/2 inhibitor Ruxolitinib, partially restored IL-1β release in Tyro3OE. Hence, STAT1 phosphorylation is key for Tyro3OE-mediated immunosuppression in models of AD. These *in vitro* findings suggest that patients with elevated TAM receptors may use the Tyro3-STAT1-IL-1β pathway to suppress inflammation and enhance Aβ phagocytosis thereby conferring subtle protection against AD. Future studies could harness this ability of Tyro3 to disarm inflammatory cells and ameliorate pathogenesis in AD.

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Poster

198. Alzheimer's Disease: Biomarkers III

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 198.06

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: The Galen and Hilary Weston Foundation

Title: Measuring cellular alteration of neural progenitors exposed to serum from individuals with mild cognitive impairment: a prognostic biomarker for Alzheimer’s disease

Authors: *E. SILAJDZIC[1], H. LEE[1], T. FLADBY[3], D. AARSLAND[2], S. THURET[1]; [1]Dept. of Basic & Clin. Neurosci., [2]Dept. of Old Age Psychiatry, Inst. of Psychiatry, Psychology, and Neurosci., Kings Col. London, London, United Kingdom; [3]Div. of Med. and Lab. Sci., Univ. of Oslo, Oslo, Norway

Abstract: Alterations in adult hippocampal neurogenesis (HN) occur early in Alzheimer’s disease (AD). Previously, we developed a cell-based assay to study the process of HN in vitro and utilised it to predict the progression of individuals with mild cognitive impairment (MCI) to AD. In the current study, we aimed to validate the prognostic power of our assay in a larger, independent cohort. To this end, we treated a human hippocampal progenitor cell line (HPC0A07/03C, ReNeuron) with baseline serum samples from the placebo arm of the Ginkgo Evaluation of Memory (GEM) Study for either 48 hours (proliferation assay) or 10 days (differentiation assay). Immunocytochemistry was performed to visualise cells positive for: proliferation (Ki67), apoptosis (cleaved caspase 3, CC3), neural stem cell (Nestin and Sox2), neuroblast (doublecortin, DCX), and neuronal differentiation (microtubule-associated protein 2, MAP2) markers. The percentage of cells positive for these markers was quantified in a semi-

automated manner using a high-content imaging platform (Opera Phenix, Perkin Elmer). Preliminary analysis was performed on neurogenic markers while being blinded to the demographic and clinical information of GEM study participants (n = 293). Regardless of assay type, % Ki67-positive and % CC3-positive cells varied widely across individuals. In contrast, there was no significant differences between individuals for % Nestin&Sox2-positive cells in the proliferation assay, while % MAP2-positive and % DCX-positive cells showed high variance between individuals in the differentiation assay. After unblinding, the full dataset will be fitted to predictive model(s) to assess whether the previously observed prognostic power of our cellular assay can be replicated. We also aim to compare 'elderly cognitively healthy' and 'individuals with MCI remaining cognitively stable' groups in hopes of detecting the earliest changes in the neurogenic process. Finally, the relationship between the altered neurogenic process and other AD-associated factors (e.g. hippocampal volume, APOE4 allele frequency, and cerebrospinal fluid Aβ42 and tau) will be ascertained.

Disclosures: E. Silajdzic: None. **H. Lee:** None. **T. Fladby:** None. **D. Aarsland:** None. **S. Thuret:** None.

Poster

198. Alzheimer's Disease: Biomarkers III

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Program #/Poster #: 198.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Conacyt AS1-42600

Title: Could the bacterial amyloid protein curli be a factor associated of Alzheimer's disease?

Authors: *D. F. DE LIMA-MAR[1], V. SANCHEZ-VALLE[1], L. CHAVEZ-DUEÑAS[2], E. F. NAVARRO-GARCIA[2], C. PEREZ-CRUZ[1];
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Abstract: Could the bacterial amyloid protein curli be a factor associated of Alzheimer's disease?D. F. de Lima-Mar[1], V. Sanchez-Valle[1], L. Chavez[2], E. F. Navarro-Garcia[2], C. Perez-Cruz[1]*.[1] Department of Pharmacology, CINVESTAV-IPN. Mexico City, Mexico[2] Department of Celular Biology, CINVESTAV-IPN. Mexico City, Mexico*email: cperezc@cinvestav.mx
The increase of senile population is accompanied by an enhanced incidence of neurodegenerative diseases. Research has been focused in underlying etiological mechanisms at the central level. However, recent data show that alterations in the periphery can be associated with onset of neurodegeneration, years before clinical signs of dementia. The microbiota-gut-brain axis has become a relevant topic, as dietary patterns, infections or antibiotics. They all cause alteration in the gut microbiota or dysbiosis and in the brain function. Dysbiosis is associated with amyloid beta peptide (AB) aggregation in the brain, one of the main characteristics of Alzheimer's Disease (AD).Transgenic (Tg) mouse models for AD (i.e. *APP/PS1* mice) develop a greater

accumulation of AB in the brain and periphery. Moreover, Tg mice developed gut dysbiosis like humans with AD. In AD patients and Tg mice, there is an increased abundance of bacteria from the *phylum* Enterobacteriaceae, such as *Escherichia coli* (*E. coli*). *E. coli* produces curli protein, a bacterial amyloid protein (BAP) rich in β-sheet molecule assembled into a highly stable cross-β structure. Curli is recognized as a pathogen-associated molecular pattern (PAMPs) inducing Toll-like receptor activation (TLR-2) and secretion of inflammatory cytokines. AD patients and Tg mice show a chronic low-grade inflammation associated with the gut dysbiosis. Until now it is not known whether gut dysbiosis and the resultant *E. coli* abundance leads to an overexpression of curli protein in Tg mice. The aim of this work was to describe the presence of *cgsA* gene and curli protein in fecal samples of 8 months-old male Tg and wild-type mice. Our data show *cgsA* and curli expression in both strains, but abundance was genotype dependent. Inflammation markers like TLR2/4 were related to curli presence. Thus, our data supports previous reports and contribute to the understanding of microbiota-gut-brain axis and its relation to AD.
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Disclosures: D.F. De Lima-Mar: None. **V. Sanchez-Valle:** None. **L. Chavez-Dueñas:** None. **E.F. Navarro-Garcia:** None. **C. Perez-Cruz:** None.

Poster

198. Alzheimer's Disease: Biomarkers III

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 198.08

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: Quantitation of neurodegenerative biomarkers in plasma using SMC high sensitivity immunoassays

Authors: *J. HWANG[1], L. CHEN[2], A. SAPORITA[2], M. BANERJEE[2], A. KO[3], E. ADKISSON[3], Q. XIAO[2];
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Abstract: Quantification of protein biomarkers in patients with Alzheimer’s Disease (AD) and Parkinson’s disease (PD) is important for monitoring neurodegeneration. Current understanding of AD and PD centers around monitoring neurodegenerative biomarkers in cerebrospinal fluid (CSF). However, due to the invasive nature of collecting CSF samples, new blood biomarkers are needed. Many neurodegenerative disease biomarkers, however, are not detectable in some blood samples due to low abundance and thus require higher sensitivity immunoassays. To this end, we have developed Aβ40, Aβ42, Tau, phosphorylated Tau (T181), TDP-43, SNAP-25, GFAP, NPTX2, UCHL1, alpha-synuclein and phosphorylated alpha-synuclein (S129) high sensitivity immunoassay kits that can accurately quantitate these biomarkers in human blood samples. Here we report our results from screening CSF and plasma biomarkers, most commonly associated with neurodegenerative diseases using Single Molecule Counting (SMC) high

sensitivity immunoassays. Using samples purchased from BioIVT, Precision and Discovery Life Science, our data confirms that Aβ42, Tau, phosphorylated Tau (T181), TDP-43 and SNAP-25 show significant difference in CSF samples from normal versus AD patients. However, in normal versus AD plasma samples, no significant difference was observed for Tau and SNAP-25. The most significant difference in normal versus AD plasma samples was observed for Aβ42 ($p < 0.03$), TDP-43 ($p < 0.04$), Aβ40 ($p < 0.05$) and phosphorylated Tau ($p < 0.05$). In summary, SMC high sensitivity immunoassay kits can provide a powerful non-invasive biomarker tool for monitoring the progression of neurodegenerative diseases such as Alzheimer's and Parkinson's disease.

Disclosures: **J. Hwang:** A. Employment/Salary (full or part-time):; MilliporeSigma. **L. Chen:** A. Employment/Salary (full or part-time):; MilliporeSigma. **A. Saporita:** A. Employment/Salary (full or part-time):; MilliporeSigma. **M. Banerjee:** A. Employment/Salary (full or part-time):; MilliporeSigma. **A. Ko:** A. Employment/Salary (full or part-time):; MilliporeSigma. **E. Adkisson:** A. Employment/Salary (full or part-time):; MilliporeSigma. **Q. Xiao:** A. Employment/Salary (full or part-time):; MilliporeSigma.

Poster

198. Alzheimer's Disease: Biomarkers III

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 198.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Nasal exhaled breath proteome in Alzheimer's Disease

Authors: ***G. LANE**[1], S. G. LEHMANN[1], A. SHERIFF[1], Q. YANG[2], B. BONAKDARPOUR[1], J. A. MASTRIANNI[4], J. JAMKA[1], J. N. SAVAS[1], K. HAUNER[3], T. J. NOTO[1], C. ZELANO[1]; [1]Dept. of Neurol., Northwestern Univ., Chicago, IL; [2]Interdepartmental Neurosci. Program, Northwestern Univ., EVANSTON, IL; [3]Med. Social Sci., Northwestern Univ., Chicago, IL; [4]Neurol., Univ. of Chicago, Chicago, IL

Abstract: Olfactory deficits are present early in the course of neurodegenerative disease, making the olfactory system an attractive target for identification of early biomarkers, including in Alzheimer's Disease. The peripheral ends of olfactory sensory neurons touch air in the nose, and their central ends touch the brain. Thus, when air moves through the nasal cavities during natural breathing, it comes into contact with olfactory sensory neurons. During nasal exhalation, turbulent air flowing over the olfactory neurons could volatilize proteins from the surface of the olfactory epithelium into exhaled breath, which could then be captured and analyzed. Collection of exhaled breath is noninvasive and simple to perform. Furthermore, the proteome of exhaled breath differs from other extensively studied sample types like CSF and blood; thus, characterization of proteins in exhaled breath may contribute distinct diagnostic and mechanistic information about Alzheimer's Disease, potentially relating to changes in the olfactory epithelium. In an exploratory pilot study, using a new nasal breath collection technique

developed in our lab, we collected 60 exhaled breath samples from healthy young controls, healthy age-matched controls, and Alzheimer's Disease patients, and analyzed samples using mass spectrometry. Preliminary results suggest that we were able to detect in breath proteins that are present in the olfactory epithelium. We found that healthy aging increases the amount of proteins present in nasal breath, and that Alzheimer's Disease is associated with changes, compared to age-matched controls, in several proteins associated with mitochondrial function and epithelial protection. These preliminary results suggest altered protein composition on exhaled breath in Alzheimer's Disease, indicating exhaled breath as a promising sample type for the identification of new biomarkers for Alzheimer's Disease.

Disclosures: **G. Lane:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder. **S.G. Lehmann:** None. **A. Sheriff:** None. **Q. Yang:** None. **B. Bonakdarpour:** None. **J.A. Mastrianni:** None. **J. Jamka:** None. **J.N. Savas:** None. **K. Hauner:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder. **T.J. Noto:** None. **C. Zelano:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder.

Poster

198. Alzheimer's Disease: Biomarkers III

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Program #/Poster #: 198.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Effects of neighboring phosphorylation events on the affinities of pThr181-tau antibodies

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder for which there are no disease-modifying treatments. Neuropathological hallmarks of AD brain include extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs) enriched with amyloid-beta peptide and hyperphosphorylated forms of the microtubule-binding protein tau, respectively. Clinical symptoms of AD, including a loss of memory and executive function, are thought to occur decades after the onset of pathology, and at this stage it is too late for effective therapy. Accordingly, a major priority in the AD field is to identify peripheral biomarkers that report on the initial phase of the disease and, therefore, can be assayed to enable earlier diagnosis. A tau variant phosphorylated on threonine 181 (pThr181-tau) has been widely investigated as a potential AD biomarker in cerebrospinal fluid (CSF) and blood. pThr181-tau is present in NFTs of AD brains, and CSF levels of pThr181-tau correlate with overall NFT burden. The detection of pThr181-tau in human biofluids relies heavily on the use of pThr181-

tau antibodies. However, the impact of phosphorylation at sites near Thr181 (a modification known to occur in the brains of AD patients) on the affinity of these antibodies for their cognate antigen has not been characterized. Here, we used a bio-layer interferometry (BLI) assay to assess the degree to which the affinity of pThr181-tau antibodies is altered by the phosphorylation of nearby serine and threonine residues. A panel of tau peptides phosphorylated on Thr181 as well as different combinations of Thr175, Ser184, and Ser185 was synthesized and immobilized on BLI biosensors. The binding of two commercial pThr181-tau antibodies, AT270 and D9F4G, to the immobilized peptides was monitored via BLI, and a dissociation constant for antibody binding to each peptide target was determined. Our results revealed that the affinity of each antibody for pThr181-tau was affected to a different extent by neighboring phosphorylation events. These results demonstrate for the first time that the binding of pThr181-tau antibodies to their target is influenced by the phosphorylation of adjacent residues, highlighting the importance of selecting antibodies with well-characterized recognition properties for accurate biomarker quantitation.

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Poster

198. Alzheimer's Disease: Biomarkers III

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Program #/Poster #: 198.11

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: AARF-17-530186
AG058748
AG072328

Title: The impact of monoamine synthesis capacity on depression symptoms depends on individual differences in Alzheimer's disease pathology

Authors: *T. MARKOVA[1], C. J. CIAMPA[2], J. H. PARENT[3], J. L. COWAN[4], T. M. HARRISON[5], W. J. JAGUST[6], A. S. BERRY[2];
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Abstract: The serotonin-producing raphe nuclei and the amygdala are highly connected and are both sites of age, Alzheimer’s disease (AD), and depression-related changes. We measured monoamine synthesis capacity in cognitively normal older adults (mean age = 77.10, n = 49) using [18-F]Fluoro-m-tyrosine ([18-F]FMT) positron emission tomography (PET) to examine relationships between the neurochemical health of the raphe, cross-sectional tau (as measured by [18-F]Flortaucipir PET), retrospective longitudinal cortical thickness changes, and retrospective longitudinal changes in depression symptoms assessed using the geriatric depression scale

(GDS). Higher dorsal raphe (DRN) [18-F]FMT net tracer influx (Ki) and higher amygdala [18-F]Flortaucipir standardized uptake value ratio (SUVR) were each associated with atrophy in the left banks of the superior temporal sulcus (BanksSTS) (Monte Carlo, $p < 0.05$, adjusting for age and sex). Additionally, higher amygdala tau was associated with decreasing amygdala volume over time ($t(31) = 4.284$, $p < 0.001$, $b = -0.610$, adjusting for age and sex). These results are consistent with higher DRN synthesis capacity and amygdala tau each being indicators of worse aging trajectories. Increasing GDS was also associated with widespread cortical atrophy, including in the left BanksSTS. The relationship between atrophy and increasing GDS was strongest in individuals with higher levels of DRN [18-F]FMT Ki ($t(29) = -9.9018$, $p = 0.0036$, adjusting for age, sex, and beta-amyloid status, measured with [11-C]Pittsburg Compound B PET)). However, the effect [18-F]FMT Ki had on these relationships was moderated by amygdala tau levels ($F(1,22) = 5.7185$, $p = 0.0258$, adjusting for age and sex). When both [18-F]FMT Ki and amygdala tau levels were high, increasing cortical atrophy predicted worsening GDS scores ($t(22) = 2.7079$, $p = 0.0128$, $b = -25.5777$). Together, these findings suggest the [18-F]FMT Ki measure of DRN monoamine synthesis capacity is a promising tool for studying AD pathological and psychiatric changes in aging.

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Poster

198. Alzheimer's Disease: Biomarkers III

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Program #/Poster #: 198.12

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH Grant R01 NS092865

Title: Mth1, a biomarker for monitoring normal human astrocytes response to amyloid-beta activation

Authors: *H. CHEN, J. XU;
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Abstract: Early detection and intervention might hold the promise for curing Alzheimer disease (AD). Neuroinflammation mediated by Amyloid-Beta peptide (1-42) (Aβ42) has been revealed as an early step of neurodegeneration and eventually leads to AD. Thus, monitoring biomarkers involved in Aβ42-mediated neuroinflammation may help early AD diagnosis. However, MTH1, a molecule involved in oxidative stress, is unclear whether it plays any role in neuroinflamation or is regulated by Aβ42. Here we characterize a radiotracer, [^{3}H]TH287, that can be used in a binding assay to measure MTH1 level in normal human astrocytes (NHA) (K_d = 2.58 nM, B_{max} = 1024.40 fmol/mg protein, Hill coefficient: 0.93). Furthermore, NHA and astrocytes-derived glioblastoma cell line U251MG were treated with Aβ42 or Aβ40 for 24 hours to mimic the

astrocyte cell environment in AD; expression of MTH1 was then measured through the aforementioned radioligand binding assay. Our data shows that MTH1 dramatically upregulated in Aβ42-treated NHA in both time-dependent and dose-dependent manners (Aβ42 treated vs. control at 24 hours: 13.07 fold increase, $P<0.05$), while its expression in Aβ42 treated U251MG exhibit much less increase (Aβ42 treated vs. control at 24 hours: 1.21 fold increase, $P<0.05$), suggesting NHA might be a better cell model for studying astrocytes functions in AD than U251MG. Additionally, Aβ40 treatment leads to minimal expression of MTH1 in NHA (Aβ40 treated vs. control at 24 hours: 1.89 fold increase, $P<0.05$). Taken together, these data show that MTH1 can be upregulated by Aβ42 in NHA for the first time, suggesting that MTH1 is a valuable biomarker for monitoring astrocytes response to Aβ42 activation in human brain; and its ligand, TH287, is a valuable radiotracer for measuring MTH1 involved in AD.

Disclosures: H. Chen: None. **J. Xu:** None.

Poster

198. Alzheimer's Disease: Biomarkers III

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Program #/Poster #: 198.13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR

Title: ^{18}F-track, a pet radioligand of trk b/c receptors: automated radiosynthesis, preclinical evaluation in tgf344 rat, and initial in vivo distribution in human

Authors: *J.-P. SOUCY[1], R. SCHIRRMACHER[2], S. BLINDER[1], P. ROSA-NETO[1], A. KOSTIKOV[1], A. THIEL[3];
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Abstract: The receptor family tropomyosine kinases (TrkA/B/C), the target of a number of neurotrophins, is involved in the regulation of neuronal differentiation, growth and survival. Decreased density of Trk in the brain have been reported for many neurodegenerative conditions, including Alzheimer' disease. Our group recently described a PET radiopharmaceutical for that system, [^{18}F]TRACK, and we also described its first use in human (ACS Chem. Neurosci. 2019, 10, 2697−2702). Here, we present a new, fully automated, GMP-compliant radiosynthesis approach, as well as results from preclinical studies in WT (3) and TgF344 (2) rats, and finally initial whole-body distribution studies in 6 normal human subjects (22-61 y.o., 3 ♀). Synthesis was implemented on a Scintomics GRP module (Fürstenfeldbruck, Germany). On 6 successive runs, average radiochemical efficiency was 5.0±1.4% without decay correction, with a total activity from 3500-5700 MBq, radiochemical purity of >99% and molar activity of 250±75 GBq/μmol. All batches passed standard QC, including pyrogenicity and sterility testing.

WT rats showed the highest SUVmax (1.56 ±0.28, at 10 minutes post-injection) in the thalamus, as expected from previous studies. In two TgF344 rats' thalamic SUVmax were 1.5 and 1.4, and there was a tendency to also have lower values than those of WT animals in the frontal cortex and striatum. In humans (injected with 129 to 147 MBq of [^{18}F]TRACK), SUVmax for the whole brain was was obtained between 115 and 210 seconds post injection. It should be noted however that whole brain SUVmax values varied significantly, from 2.00 to 4.08. Whole brain average SUV decreased to 0.73±0.167 at 80 minutes. The regions showing the highest average SUVmax were the cortex (4.8±1.34) and the thalamus (5.6±1.77) Uptake in white matter was significantly lower, with an average SUVmax of 2.7±1.06. With an automated, well controlled synthesis method, resulting in high molar activity values, and with favorable brain kinetics, [^{18}F]TRACK is a promising agent for the evaluation of the Trk-B/C system of the brain. Potential applications include diagnosis of neurodegenerative conditions, as well as research on, and follow-up of, potential therapies neurotrophic for those diseases.

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Poster

198. Alzheimer's Disease: Biomarkers III

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 198.14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Swedish Research Council 2017-06105
Center for Innovative Medicine (CIMED) Region Stockholm FoUI-954431
Marie Skłodowska-Curie Action: Innovative Training Network Grant Agreement 860197
Multi-omics Interdisciplinary Research Integration to Address Dementia diagnosis (MIRIADE)

Title: Relation of different cerebrospinal fluid biosignatures to clinical phenotypes in a memory clinic cohort

Authors: *V. ALANKO[1], S. MRAVINACOVÁ[2], G. HAGMAN[3], P. NILSSON[2], M. KIVIPELTO[1,3,4,5], A. MÅNBERG[2], A. SANDEBRING-MATTON[1,4];
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Abstract: Due to the heterogeneous nature of Alzheimer's disease (AD), stratification of patients into biological subtypes employing multiple biomarker analyses may prove to be crucial

for finding the most suitable prevention and treatment options. Despite advancements in subtype identification, we need a better understanding of how clinical characteristics distribute between subgroups across the dementia spectrum. To this end, 288 cerebrospinal fluid (CSF) samples from patients examined at the Karolinska University Hospital memory clinic in Solna, Sweden, were analyzed. Patients have been diagnosed with subjective cognitive impairment (SCI), mild cognitive impairment (MCI), AD, and other dementias. The cohort is deeply phenotyped with thorough neuropsychological tests, brain imaging, physical examinations, routine blood tests, and AD CSF biomarkers. Using an antibody-based suspension bead array, levels of 53 proteins were measured. An unsupervised clustering algorithm was used to identify subtypes based on the protein measurement. The assessed panel revealed differences in patterns of protein levels within diagnostic groups and, by applying clustering analyses, subgroups across the dementia spectrum could be identified. Our ongoing analyses explore clinical features, e.g., decreased cognition, AD biomarker levels, and co-morbidities of the clusters. The analyzed proteins may further reflect the (patho)physiological state of the brains of the patients. To conclude, AD, which falls under the umbrella term dementia, could in fact also be considered an umbrella itself with distinct subgroups. In the current study, we demonstrate that measuring only tens of proteins in the CSF is adequate to stratify patients into significant subgroups with distinct pathological markers.

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Poster

198. Alzheimer's Disease: Biomarkers III

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 198.15

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: U01AG068057
ZEN-20-644609

Title: Longitudinal MRI atrophy biomarkers and their relation to CSF Tau and Aβ in the ADNI3 cohort

Authors: *C. BOYLE[1], C. R. K. CHING[1], S. I. THOMOPOULOS[1], M. A. BERNSTEIN[3], B. J. BOROWSKI[3], C. R. JACK, JR.[4], M. W. WEINER[5], P. M. THOMPSON[2];
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Abstract: Objective and Rationale: Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by the accumulation ofcerebrospinal fluid (CSF) tau and Aβ over time (Jack et al., 2018) as well as other biomarkers including sum-of-boxes Clinical Dementia Rating (sobCDR). Analysis of longitudinal data has attempted to definethe relationship between these markers and

atrophic rates. We used tensor-based morphometry (TBM), a sensitive, high-throughput biomarker for tracking disease progression in large imaging studies, to derive these rates and resulting statistical maps revealed significant associations. **Methods:** We identified ADNI3 longitudinal MRI scans with a minimum 24-month interval, due to a stronger signal-to-noise ratio (SNR) for change. We included those subjects with populated values for amyloid status based on standardized cut-off values (Royse et al., 2021) and tau levels. The final dataset included 407 subject-visit scans from 117 healthy controls (CN) (age at screening: 72.6±5.6 y, 82F/35M), 29 individuals with MCI (75.2 ± 7.0 y, 12 F/17 M), and 16 individuals with AD (age: 78.7±8.5 y, 9 F/7 M).We applied TBM (Leow et al., 2009; Hua et al., 2011) to produce 3D Jacobian (tissue loss rate) maps representing relative expansion or contraction at each brain voxel by measuring relative volumedifferences between each participant's annual follow-up and baseline scan. At each voxel in the brain, linear regression models were fit to relate regional brain volume change rates to baseline amyloidstatus, tau, and sobCDR. We adjusted for age, sex and inter-scan interval from baseline to latest follow-up scan.**Results and Conclusion:** TBM-derived atrophic rates correlated with amyloid positive status and elevated tau levels at baseline,and higher sobCDR. Each variable of interest showed significance (amyloid positive status; p= 0.0003; tau levels; p= 0.002; sobCDR; p=.004), passing FDR correction at <.05 across the whole brain (Benjamini et al., 1995). TBM is a sensitive, high-throughput biomarker for tracking disease progression in large imaging studies.

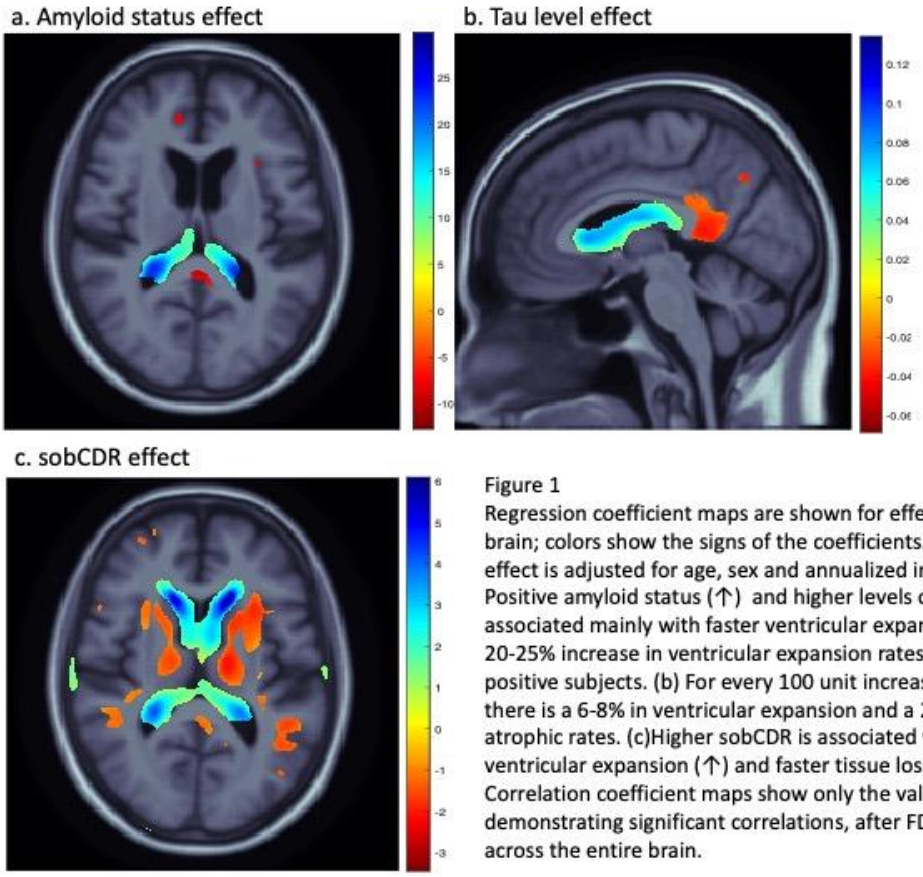

Figure 1
Regression coefficient maps are shown for effects across the brain; colors show the signs of the coefficients. The map of each effect is adjusted for age, sex and annualized inter-scan interval. Positive amyloid status (↑) and higher levels of tau (↑) are associated mainly with faster ventricular expansion. (a) There is a 20-25% increase in ventricular expansion rates for amyloid positive subjects. (b) For every 100 unit increase in tau levels, there is a 6-8% in ventricular expansion and a 2-6% increase in atrophic rates. (c)Higher sobCDR is associated with faster ventricular expansion (↑) and faster tissue loss rates (↓). Correlation coefficient maps show only the values in regions demonstrating significant correlations, after FDR correction across the entire brain.

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Poster

198. Alzheimer's Disease: Biomarkers III

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Biofluid biomarkers of the blood-brain barrier and brain vascular dysfunction in APOE4 carriers

Authors: *A. SAGARE[1], A. MONTAGNE[1], D. A. NATION[2], G. BARISANO[1], M. SWEENEY[1], J. STANLEY[1], M. HARRINGTON[3], H. CHUI[1], J. RINGMAN[1], H. YASSINE[1], L. SCHNEIDER[1], J. PA[1], T. BENZINGER[4], A. FAGAN[4], J. MORRIS[4], E. REIMAN[5], R. CASELLI[6], A. TOGA[1], B. ZLOKOVIC[1];
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Abstract: Recent studies suggest that brain vascular dysfunction and breakdown of the blood-brain barrier (BBB) may contribute to neuronal dysfunction and accelerate age-related cognitive decline and dementia due to Alzheimer's disease (AD). Advances in neuroimaging techniques and the development of ultrasensitive platforms for reliable and reproducible measurement of biofluid biomarkers of vascular injury and amyloid and tau biomarkers in cerebrospinal fluid (CSF) and blood now enable us to better understand the role of vascular dysfunction in the pathophysiology of AD. Here, we developed sensitive antibody-based assays to analyze novel biofluid markers of vascular injury in CSF obtained from human research participants ≥45 years of age recruited at the University of Southern California Alzheimer's Disease Research Center (ADRC), Huntington Medical Research Institutes, the Washington University Knight ADRC, Banner Alzheimer's Institute and Mayo Clinic Arizona. We then measured levels of CSF biomarkers, including soluble platelet-derived growth factor-β (sPDGFRβ), a marker associated with brain capillary pericyte damage, CSF matrix metalloproteinase-9 (MMP9), cyclophilin A

(CypA) and conventional biomarkers of BBB breakdown including CSF/plasma albumin ratio (Qalb) and CSF fibrinogen. We stratified participants by *APOE* genotype as *APOE4* carriers (Ɛ3/Ɛ4 and Ɛ4/Ɛ4) or *APOE4* non-carriers (Ɛ3/Ɛ3). Our biomarker analyses considering participants' cognitive and genetic status showed that high baseline levels of the sPDGFRβ in CSF predicted future cognitive decline in *APOE4* carriers but not in non-carriers, even after controlling for amyloid-β and tau status and correlated positively with CSF MMP9 and CypA levels. These findings suggest that activation of the BBB-degrading CypA-MMP9 pathway and injury to brain capillary pericytes contributes to *APOE4*-associated cognitive decline that may serve as a therapeutic target in *APOE4* carriers. Further development of surrogate biomarkers of BBB dysfunction in plasma that may predict future cognitive dysfunction is underway.

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Poster

198. Alzheimer's Disease: Biomarkers III

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 198.17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: IITP 2017-0-00432

Title: Using phase-locked power to detect biomarkers for Alzheimer's disease in a small trial attention and inhibition task

Authors: *S.-K. KIM, L. KIM;
Ctr. for Bionics, Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: Pharmacological treatment for early Alzheimer's disease (AD) and mild cognitive impairment (MCI) can slow disease progression. Therefore, the prognosis of AD at the MCI stage is very important. Neuroimaging systems, such as electroencephalography (EEG), functional magnetic resonance imaging, and functional near-infrared spectroscopy detect neural biomarkers of AD. EEG is widely used to measure cognitive ability owing to its high temporal resolution and low cost. However, often in EEG, several hundred trials are repeated to obtain clearer results by minimizing the response to noise or non-target events, making it problematic for patients with cognitive impairment, who have difficulty performing the same task for a long time, possibly affecting the results. The aim of this study was to identify biomarkers in a minimal trial during a cognitive task for elderly people, including patients with AD. We analyzed the phase-locked power that removed the influence on the non-phase-locked power that occurred in addition to the cognitive task to distinguish subjective cognitive decline (SCD), amnestic MCI

(aMCI), and AD when performing cognitive tasks. We enrolled 13 patients with subjective cognitive decline (SCD; age, 63.07 years; six men and seven women), 15 patients with aMCI (age, 64.73 years; seven men and eight women), and five patients with early AD (age, 67.75 years; one man and four women). The diagnosis was established according to the Seoul Neuropsychological Screening Battery, Mini-Mental State Examination, and clinical dementia rating scale. Among the cognitive function indicators for diagnosing AD, 10 trial visual attention tasks and 16 trial visual go/no-go tasks (go, 8; no-go, 8) were conducted to measure the attention and inhibition abilities. Each trial was presented for 2 s after 1-2 s of fixation on a computer monitor, and total time for each cognitive task was approximately 1 min. The phase-locked wavelet power after removing the non-phase-locked power revealed novel features. In the visual attention task, the theta increase and alpha decrease in the occipital region, which are biomarkers of visual attention, were more strongly expressed in the SCD and aMCI groups than in the AD group 0-400 ms after stimulus presentation. In addition, in the visual go/no-go task, the SCD and aMCI groups showed a stronger theta increase in the no-go trial than in the go trial 300-700 ms after stimulus presentation in the frontal and central regions, which is a biomarker of inhibition. In conclusion, using phase-locked power could help detect biomarkers of attention and inhibition in SCD, aMCI, and AD even in a small trial.

Disclosures: S. Kim: None. **L. Kim:** None.

Poster

198. Alzheimer's Disease: Biomarkers III

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 198.18

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: Validation of Highly Sensitive NF-L, Ab1-42, Ab1-40, GFAP Multiplex and Phospho-Tau 181 (p-Tau181) Immunoassays, and Quantitation in Alzheimer’s Disease Plasma

Authors: *A. CHENNA[1], B. LEE[2], J. W. WINSLOW[2], C. J. PETROPOULOS[2];
[1]Monogram BioSciences Inc.-LabCorp,, S San Francisco, CA; [2]Monogram BioSciences Inc.-LabCorp, S San Francisco, CA

Abstract: In the US, over 6 million people, most of them age 65 or older, are afflicted with dementia that may be caused by Alzheimer’s disease (AD). Emerging therapeutic approaches to AD have led to the development of assays for the early detection of AD pathology in blood and cerebral spinal fluid (CSF). Changes in the levels of neuronal proteins neurofilament light chain (NF-L), amyloid beta1-40 (Aβ1-40), amyloid beta1-42 (Aβ1-42), glial fibrillary acid protein (GFAP), and phosphorylated tau (p-Tau181) in the blood are associated with brain pathophysiology and may be used as prognostic/diagnostic biomarkers of AD. Several of these biomarkers are present at low concentrations in blood, thus we have applied a highly sensitive technology, single molecular array (Simoa) immunoassay from Quanterix, for quantitative measurements. We conducted validations for the Neurology 4-Plex E (NF-L, Aβ1-42, Aβ1-40,

GFAP) and the p-Tau181 assays based on the following validation parameters: Standard curve precision and accuracy, control precision and accuracy, endogenous levels/detectability, reproducibility, parallelism, sample stability, and interfering substances. We have used the validated assays to measure biomarker levels and correlative relationships in AD plasma samples (n=95), relative to healthy control plasma samples (n=64). AD sample groups represent patients exhibiting mild (n=30, MMSE >23-30), moderate (n=24, MMSE=16-22) and severe (n=40, MMSE<16) cognitive impairment. Analysis of NF-L, Aβ1-42, Aβ1-40, GFAP and p-Tau181 revealed significant increases in median levels in the combined AD group (mild, moderate, severe), relative to controls ($p<0.0001$; Mann-Whitney t-test). The median plasma Aβ1-42/Aβ1-40 ratio was significantly lower in the combined AD group relative to controls ($p= 0.043$). The levels of all biomarkers were elevated in the moderate and severe AD patient subgroups relative to controls ($p<0.0001$). Levels of NF-L, p-Tau181 and GFAP were significantly elevated in mild AD relative to controls ($p<0.0001$). NF-L, p-Tau181 and GFAP levels were positively correlated in the AD group (Spearman $r=0.475-0.718$, $p<0.0001$), whereas a significant inverse correlation was observed between Aβ1-42/40 ratio vs p-Tau181 ($r= -0.312$, $p=0.0049$), consistent with observations reported in the literature for AD CSF. This study supports the utility of ultra-sensitive multiplex immunoassays for the assessment of neurodegeneration biomarker levels in AD patient plasma, a more accessible sample matrix than CSF, and can potentially contribute to the diagnostic and prognostic evaluation of Alzheimer's patients.

Disclosures: **A. Chenna:** A. Employment/Salary (full or part-time):; Monogram_LabCorp. **B. Lee:** A. Employment/Salary (full or part-time):; Monogram_LabCorp. **J.W. Winslow:** A. Employment/Salary (full or part-time):; Monogram_LabCorp. **C.J. Petropoulos:** A. Employment/Salary (full or part-time):; Monogram_LabCorp.

Poster

198. Alzheimer's Disease: Biomarkers III

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 198.19

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant U01 AG024904
Boye Foundation

Title: Preclinical Detection and Monitoring of Alzheimer's Disease Using a Multi-Disease Diagnostic Platform Employing Autoantibodies as Blood-based Biomarkers

Authors: *C. DEMARSHALL[1], J. VIVIANO[1], S. EMRANI[2], U. THAYASIVAM[3], G. GODSEY[1], A. SARKAR[1], B. BELINKA[1], D. LIBON[4], R. NAGELE[1];
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Abstract: Evidence for the universal presence of serum autoantibodies and their potential diagnostic utility for detection and diagnosis of Alzheimer's disease (AD) and other neurodegenerative diseases has been extensively demonstrated by our laboratory. It is well known that AD-related pathological changes in the brain can begin up to a decade before patients experience telltale symptoms, yet preclinical detection remains an unmet goal. In the present study, we demonstrate the utility of a panel of 8 AD-related autoantibodies capable of detecting a broad range of pathology along the AD continuum, including preclinical AD (years before the onset of symptoms), prodromal AD (mild cognitive impairment, MCI), and mild-moderate AD. Sera from a total of 455 subjects, including 79 cognitively normal participants from ADNI (preclinical AD) who later progressed to MCI/AD within an average of 5 years, were screened using a custom Luminex xMAP® assay to measure relative titers of 8 AD-related autoantibodies. Subjects were randomly separated into Training and Testing Sets, each containing roughly equal numbers of samples from patients at preclinical, prodromal and mild-moderate AD[N1] as well as several different control groups and were evaluated for the presence of AD using *Random Forest* (*RF*) and Receiver Operating Characteristic curves to construct a diagnostic model. The panel of 8 AD-related autoantibody biomarkers predicted a patient's probability of an AD diagnosis with 79.3% accuracy and an area under the curve (AUC) of .85 (95% CI = 0.80-0.90) in the Testing Set subjects, using our markers alone. Inclusion of age and cognitive complaint parameters to the diagnostic model improved overall accuracy to 97.0% and the AUC to .99 (95% CI = 0.99-1). In addition to distinguishing subjects with AD pathology from cognitively normal controls, the panel readily distinguished these subjects from Parkinson's disease and breast cancer subjects, thus showing excellent disease specificity. These results demonstrate the utility of our custom Luminex xMAP® blood-based autoantibody biomarker panel as an accurate, non-invasive, and inexpensive diagnostic screener, not only for detection of prodromal AD and beyond, but also the earliest (preclinical) stages of pathology, several years before the onset of telltale clinical symptoms. Additionally, this appears to be a multi-disease diagnostic and disease-staging strategy, since it has already been shown to be useful for detecting a multitude of neurodegenerative diseases including both early-stage AD and PD, as well as Multiple Sclerosis, with other disease applications currently underway.

Disclosures: **C. DeMarshall:** A. Employment/Salary (full or part-time):; Durin Technologies Inc. **J. Viviano:** A. Employment/Salary (full or part-time):; Durin Technologies Inc.. **S. Emrani:** None. **U. Thayasivam:** F. Consulting Fees (e.g., advisory boards); Durin Technologies Inc. **G. Godsey:** A. Employment/Salary (full or part-time):; Durin Technologies Inc. **A. Sarkar:** A. Employment/Salary (full or part-time):; Durin Technologies Inc. **B. Belinka:** A. Employment/Salary (full or part-time):; Durin Technologies Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Durin Technologies Inc.. **D. Libon:** None. **R. Nagele:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Durin Technologies Inc.. F. Consulting Fees (e.g., advisory boards); Durin Technologies Inc..

Poster

199. Parkinson Disease: Novel Therapeutics

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 199.01

Topic: C.03. Parkinson's Disease

Support: NIH UH3-NS100553
NIH R01-NS119520
Michael J. Fox Foundation

Title: Greater improvement in motor function with directional versus circular deep brain stimulation - Results from the SUNDIAL study

Authors: *H. WALKER[1], M. WADE[1], D. KUHMAN[2], A. NAKHMANI[3], C. GONZALEZ[1], C. HURT[2];
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Abstract: Background: Directional brain stimulation provides greater control of radial current spread than conventional ring-shaped electrodes. Whether this added flexibility can improve motor function is unclear. Here we examine whether directional and circular stimulation differentially change motor performance in patients with Parkinson's disease.
Methods: We measured motor behaviors in 31 patients who underwent unilateral subthalamic nucleus brain stimulation surgery (SUNDIAL, NCT03353688) from each of 8 configurations (6 directional contacts and their corresponding rings) during device activation. Objective measures of dexterity, gait, and overall mobility were queried in a double-blind fashion in the practically defined "off" medication state versus preoperative baseline with stimulus amplitude at the center of the therapeutic window.
Results: The best versus the worst directional contact on a given row yields significant changes in performance across 5 motor tasks ($p<0.001$ each task). Specific stimulation directions can worsen function versus baseline, whereas the best direction yields greater improvement than ring stimulation ($p=0.005$, $p=0.001$, $p=0.007$, $p<0.001$, respectively, across tasks). Although directional DBS improves therapeutic window and side effect thresholds versus ring stimulation (0.40 ± 0.94 and 0.35 ± 0.51 mA, $p<0.001$, respectively), these correlate only modestly with motor improvements. Resting beta power did not predict motor improvements by directional DBS across any of the motor tasks.
Conclusions: Optimized directional subthalamic nucleus DBS yields better group-level motor performance than ring stimulation, in addition to known advantages related to tolerability. Prospective studies should evaluate whether these improvements persist over longer time intervals.

Disclosures: H. Walker: None. **M. Wade:** None. **D. Kuhman:** None. **A. Nakhmani:** None. **C. Gonzalez:** None. **C. Hurt:** None.

Poster

199. Parkinson Disease: Novel Therapeutics

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 199.02

Topic: C.03. Parkinson's Disease

Support: This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIT).(No. 2022R1A2C2006535)
The manufacturer provided a massage chair free of charge for this study

Title: Eeg study on the effect of mechanical massage in parkinson's disease

Authors: *W. HUR[1], M. PARK[2], K. CHOI[3], H. YOO[4];
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Abstract: While the number of Parkinson's disease (PD) patients is expected to rise by more than 12 million by 2040, treatment with dopaminergic drugs can slow but not stop the disease's progression. Due to the lack of treatment options, many patients seek complementary and alternative medicine treatment to manage their symptoms and improve their quality of life. Several recent studies have confirmed that various treatments for PD, such as massage, yoga, Tai Chi, cannabis, and art therapy, are effective in PD patients. Meanwhile, electroencephalography (EEG) studies of PD patients have shown that the incidence of EEG abnormalities is higher than in normal old individuals. The most common alteration in PD is generalized slowing of the EEG. The more severe the disorder, the better the occipital background slowing and the more severe akinesia, but there are studies suggesting that EEG abnormalities will appear in patients with Parkinson's disease without dementia, which will also affect the subcortical structure. In this study, the EEG measurements and interview results of 4 PD patients who received mechanical massage therapy will be discussed. There were 3 patients with Hoehn-Yahr Scale stage 1.5 and 1 with stage 2, and the average duration of PD was 6.5 years. Patients received a spinal massage treatment that included meditation, such as AI-guided deep breathing and brain relaxation. The massage was given flexibly at medium intensity for more than 40 minutes based on the patient's preferences. The EEG was measured before and after the treatment using a digital EEG with 19 channels of scalp electrodes by the international 10-20 system. As a result of ANOVA analysis (treatment x band x region, $F = 1.221$, $p = 0.27$), indicating that the effect of the treatment was not significant. Although alpha and beta waves were found to decrease after treatment, there is a limitation that a statistically significant effect cannot be confirmed due to the large individual deviation and the small number of cases. A post-massage interview, on the other hand, revealed that the massage had beneficial effects such as increasing emotional stability and insomnia relief. Furthermore, the patients were pleased that they could self-manipulate the massage program. The patients' subjective satisfaction was high and all hoped that the treatment would be continued. Therefore, further research into mechanical massage treatment as a home-care option for PD patients is required.

Disclosures: **W. Hur:** None. **M. Park:** None. **K. Choi:** None. **H. Yoo:** None.

Poster

199. Parkinson Disease: Novel Therapeutics

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 199.03

Topic: C.03. Parkinson's Disease

Title: A meta-analysis of exercise intervention and the effect on Parkinson's Disease Symptoms

Authors: *S. O. AHMAD[1], J. LONGHURST[1], D. STILES[2], L. DOWNARD[3], S. MARTIN[3], H. DINH[3];
[1]St. Louis Univ., [2]Doisy Col. of Hlth. Sci., [3]St. Louis Univ., Saint Louis, MO

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disease that is distinguished by weakness, tremors at rest, which progresses to uncontrollable shaking, paralysis, bradykinesia, and an inability to perform everyday activities. The non-motor symptoms that occur can be presented as pain, depression, cognitive dysfunction, sleep issues, and anxiety. Functionality is tremendously impaired by physical as well as non-motor symptoms. Recent treatment has begun to incorporate non-conventional interventions that are more functional and tailored to the patients with PD. The purpose of this meta-analysis is looking at different types of therapeutic exercise to determine which types are beneficial for individuals with PD. Most therapy is aerobic based, but there are many types of programs. This meta-analysis looks at aerobic, aquatic, balance, boxing, cycling, dance, Tai Chi, and walking as types of interventions to mediate PD symptoms. Research question: Do exercise interventions help with the management of Parkinson's Disease symptoms? Methods: Two reviewers screened the title and abstract records (n=668) found in the initial search. A total of 185 full-text articles were eligible for a more in depth review of inclusion criteria. A total of 25 articles were considered to be eligible. These articles were found by searching "Parkinson's Disease AND intervention AND exercise" in PubMed, SCOPUS, and CINAHL databases. Rayyan was then used to screen and categorize the articles that were found. Our review identified 25 studies which met inclusion criteria for meta-analysis. The interventions lasted from 4 to 26 weeks. Results indicated a positive overall effect of therapeutic exercise on patients with PD, where the overall d-index was .155. This was statistically significant with a CI95% of $0.0791 < \mu < 0.2308$, $p < .05$, as using this approach, a positive effect was indicated, as the CI lies to the right of, and doesn't include zero. Intervals that include zero are non-conclusive and non-significant. The homogeneity analysis was significant, $Q(24) = 268.3505$, $p < .05$, revealing that there was more variability in the d-indexes than would be expected due to sampling error alone, $Q(24) = 268.3505$, $p < .05$. The purpose of this study was to evaluate the effect of exercise interventions on people with Parkinson's Disease through a meta-analysis of current research utilizing UPDRS scores. The results of this meta-analysis demonstrated that exercise had an overall positive effect on Parkinson's Disease symptoms.

Disclosures: S.O. Ahmad: None. **J. Longhurst:** None. **D. Stiles:** None. **L. Downard:** None. **S. Martin:** None. **H. Dinh:** None.

Poster

199. Parkinson Disease: Novel Therapeutics

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 199.04

Topic: C.03. Parkinson's Disease

Support: Arizona Biomedical Research Commission (ABRC) Grant ADHS18-198846
NIH Grant NS122805-01
NIH Grant NS109608

Title: Repurposing of sub-anesthetic ketamine to treat L-DOPA-induced dyskinesia - Results from preclinical models and an open-label Phase I/II clinical trial

Authors: *T. FALK[1], M. J. BARTLETT[1], S. S. RICHARDS[1], A. LIND[1], C. LIU[2], C.-H. HSU[3], M. L. HEIEN[2], S. J. SHERMAN[1];
[1]Neurol., [2]Chem. and Biochem., [3]Epidemiology and Biostatistics, Univ. of Arizona, Tucson, AZ

Abstract: L-DOPA-induced dyskinesias (LID) are debilitating motor symptoms of dopamine-replacement therapy for Parkinson's disease (PD) emerging after years of L-DOPA treatment. Low-dose ketamine infusions are effective therapy for depression and pain, important comorbidities of PD. Our work shows that sub-anesthetic ketamine treatment has short-term antiparkinsonian activity and can reduce existing LID long-term after an initial 10-hr total treatment. Ketamine treatment in 6-hydroxydopamine hemi-lesioned PD rats both attenuated development of LID (n=9/group; $p < 0.05$) and reduced severity in established LID (n=10/group; $p < 0.05$) by 50%. Ketamine rapidly suppressed corticostriatal spectral signatures of LID and the long-term anti-dyskinetic effects of ketamine were dependent on brain-derived neurotrophic factor (BDNF). An open-label, dose-finding Phase I/II clinical trial was conducted to test safety and tolerability of low-dose ketamine infusion to treat LID, and to find an effective dose-range suitable for outpatient use. Two 5-hr low-dose ketamine infusions were given within a one-week period. Measured outcomes: reduction of dyskinesia, captured with UDysRS (Unified Dyskinesia Rating Scale), and effects on parkinsonian symptoms, captured with UPDRS (Unified Parkinson's Disease Rating Scale), Hamilton Depression and Pain (NRS) scales. Statistics: linear mixed effects models. Subject diaries of on/off symptoms were collected. Plasma samples were collected to analyze BDNF levels and for pharmacokinetic analysis of ketamine and metabolite. Analyses show safety and tolerability in a population of subjects with moderate to advanced PD, and indicate possible efficacy with a large effect size that warrants further study. We screened 13 subjects: 3 were screen failures, 10 enrolled in the study, 1 did not complete the infusion due to nausea, 9 had complete infusions. The target infusion rate was 0.30 mg/kg/hr. The maximum tolerated infusion rate ranged from 0.20-0.30 mg/kg/hr. The side effects that prompted reduction of the infusion rate were mostly discomfort due to dissociation or hypertension. No adverse events occurred post-infusion. UDysRS: 51% reduction from baseline during Infusion 2 (p=0.003), 49% at 3-week (p=0.006) and 41% at 3-month (p=0.011) post-ketamine. UPDRS: 27% reduction during Infusion 2 (p=0.057), 28% at 3-weeks (p=0.026) and 5% at 3-months (p=0.258). Further analyses are ongoing. Our results provide further support for the repurposing of sub-anesthetic ketamine for individuals with LID. A multi-center, double-

blind, placebo-controlled Phase II/III trial, with midazolam as an active-placebo, is planned to start in 2022.

Disclosures: **T. Falk:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); pending patent application for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, that has been licensed to PharmaTher Inc.. **M.J. Bartlett:** None. **S.S. Richards:** None. **A. Lind:** None. **C. Liu:** None. **C. Hsu:** None. **M.L. Heien:** None. **S.J. Sherman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); pending patent application for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, that has been licensed to PharmaTher Inc...

Poster

199. Parkinson Disease: Novel Therapeutics

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 199.05

Topic: C.03. Parkinson's Disease

Title: Within patient comparison of neurostimulator longevity; sequentially contrasting the Medtronic Activa PC to the Medtronic Percept PC utilizing in-device battery estimates.

Authors: *E. L. HARGREAVES[1], D. L. CAPUTO[2], D. DOLCE[1], R. J. DIPAOLA[2], S. F. DANISH[1];
[1]Neurosurg., [2]Neurol., Jersey Shore Univ. Med. Ctr. (JSUMC), Neptune, NJ

Abstract: Here, we examine the differences between actual Activa PC longevity and the estimated longevity of the Percept PC, utilizing identical contact configurations and programming parameters. The Activa PC 37601 released in May of 2009 was promoted as having a duration of 2-5 years. In our hands, the average longevity of 65 initially implanted Activa PCs was 3.90 years. In June of 2020 Medtronic received FDA approval for its Percept PC B35200, with claims that it would outlast its predecessor. We examined these claims by contrasting the actual longevity of 35 Activa PC implants, to their subsequent Percept PC implant estimates following device exchanges. Individuals were originally implanted with Activa PCs bilaterally targeting the subthalamic nucleus for Parkinson's. The final parameters (with current conversion if necessary) and contact configurations of the depleted Activa PC were carried over to the newly implanted replacement Percept PCs, starting from August of 2020 until May of 2022. At the initial post-exchange visit occurring within 30 days, the longevity estimate, number of device exchanges, contact configuration, and parameters were logged. Data was incomplete for 7/35 individuals, leaving the analyses to be performed upon 28 individuals. The average longevity of the Activa PCs was 3.78 years. The next visit after the device exchange in favor of Percept PCs exhibited an average estimated longevity of 4.56 years indicating an average increase of 7.4 months (p=0.012).Further analyses stratified the device longevity by the

number of exchanges. The initial Activa PCs lasted an average of 4.01 years (n=15), while Activa PCs that were already secondary devices lasted 3.66 years (n=9), while Activa PCs that were on their third exchange or greater lasted 3.19 years (n=4). Coincidently, Percept PCs that were the initial replacement had an estimated longevity of 4.08 years (n=15), while Percept PCs that were the third successive device had an estimated longevity of 5.04 years (n=9), and those that were even more tertiary replacements had an estimate of 5.4 years (n=4).In conclusion, the initial Activa PC duration in this retrospective study matched well with our previous initial device longevity findings and as reported by others subsequent Activa device duration decreases successively with further exchanges. In stark contrast, the Percept PCs' estimated longevity match those of their most immediate initial Activa predecessors, and then increased their estimated longevity, with successive exchanges. Thus, according to the Percept PC longevity estimates they should outlast their Activa PC predecessor given identical parameters and contact configurations.

Disclosures: E.L. Hargreaves: None. **D.L. Caputo:** None. **D. Dolce:** None. **R.J. DiPaola:** None. **S.F. Danish:** F. Consulting Fees (e.g., advisory boards); Medtronic.

Poster

199. Parkinson Disease: Novel Therapeutics

Location: SDCC Halls B-H

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Program #/Poster #: 199.06

Topic: C.03. Parkinson's Disease

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This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2019R1F1A1059310).

Title: Decision-making Ability of Parkinson's Patients who Performed Art Activities

Authors: *H.-R. YOO[1], **W. HUR**[2], M. PARK[3], S. PARK[3], J. LEEM[4];
[1]Dept. of Cardiol. and Neurol. of Korean Med., Daejeon Hosp. of Daejeon Univ., Daejeon, Korea, Republic of; [2]Daejeon Univ., Daejeon, Korea, Republic of; [3]Clin. Trial Ctr., Daejeon Korean Med. Hosp. of Daejeon Univ., Daejeon, Korea, Republic of; [4]Wonkwang Univ., Iksan, Korea, Republic of

Abstract: Parkinson's disease (PD) is caused by damage to the substantia nigra in the midbrain and is characterized by a dopamine deficiency, resulting in motor symptoms such as tremors and stiffness. In addition, non-motor symptoms such as constipation, sleep disorder, depression, and

lethargy precede. Indeed, non-motor symptoms dominate the clinical picture of advanced PD and contribute to severe disability, impaired quality of life, and shortened life expectancy. In recent years, it has become increasingly clear that PD patients do not only exhibit problems executing movements but also show marked impairments when deciding which movement to perform. This study examines the change in color making decisions before and after art activity in 9 PD patients who underwent art activity. Before and after 8 weeks of art activities using various color tools such as crayons and paints, a color selection task was conducted along with fNIRS. After extracting the RGB values of the 20-color felt pen, they were substituted into the RGB color wheel, and the saturation and brightness of each color were sorted in order. Arrange 5 high-saturation colors on the left, which is difficult to reach, 5 low-saturation colors on the right side that are easy to reach, and 5 colors on each side by mixing the high and low saturation levels in a conspicuous location. Task is close your eyes and rest for 30 seconds, then open your eyes, choose a color you like, and color one figure with four equal areas for 1 minute, then close your eyes and rest for 30 seconds. Repeat this 4 times. The color can be selected only once, and the screen is colored continuously for 1 minute. In the coloring task before and after art activity, statistically significant results of fNIRS could not be confirmed. However, after the art activity, the patients showed improvement in their motivation, such as not simply painting, but adding patterns to shapes, choosing various colors, and wanting to change colors while selecting several colors. There was no noticeable difference in the selection frequency according to saturation or color, but the patients generally preferred the pen on the right side, and the pen in the second position rather than the first one most often. The pens on both sides and the front end showed a low frequency of use once each. In addition, before the art activity, when selecting a color, they picked up the pens on either side or in front of the eyes to color in a diagonal position. Accordingly, it was confirmed that art activities change the achievement desire and visual information collection method of PD patients. Therefore, it is necessary to improve the limitations of this task and expand the related research.

Disclosures: **H. Yoo:** None. **W. Hur:** None. **M. Park:** None. **S. Park:** None. **J. Leem:** None.

Poster

199. Parkinson Disease: Novel Therapeutics

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Program #/Poster #: 199.07

Topic: C.03. Parkinson’s Disease

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Title: Feasibility and safety of regenerative peripheral nerve tissue delivery to the nucleus basalis of Meynert for cognitive decline in patients with Parkinson's disease

Authors: J. E. QUINTERO[1], L. KOEHL[2], A. LEDREUX[4], A. GILMORE[4], J. A. GURWELL[2], J. T. SLEVIN[2], A.-C. GRANHOLM[4], *G. A. GERHARDT[3], C. G. VAN HORNE[1]; [1]Neurosurg., [2]Neurol., [3]Neurosci., Univ. of Kentucky Med. Ctr., Lexington, KY; [4]Neurosurg., Univ. of Colorado Anschutz Campus, Denver, CO

Abstract: Previously, we have shown safety and feasibility evidence for autologous peripheral nerve tissue (PNT) deployment into the substantia nigra in conjunction with standard deep brain stimulation surgery (DBS). Our overarching hypothesis is that regenerative PNT delivery to the nucleus basalis of Meynert (NBM) could support cholinergic cell survival and result in stable cognitive outcomes. In the current study, the primary objectives were to examine the safety and feasibility of a unilateral PNT (autologous sural nerve) delivery to the NBM at the time of DBS surgery in participants with Parkinson's disease (PD). Secondary objectives were to assess the cognitive and motor outcomes. As part of the standard of care, all participants received bilateral globus pallidus interna (GPi) DBS. All participants (n=7) received PNT to the NBM area. There were no serious adverse events related to the study procedures. The most common study-related adverse event was paresthesia in the lateral aspect of the ankle and foot ipsilateral to the sural nerve transection. Outcome data were analyzed. Post-DBS plus PNT neurocognitive evaluations were on average 12.4 months post-surgery (SD = 3.05). Pre- to post-DBS Plus cognitive scores were compared via paired sample t-tests. No significant changes were noted in verbal memory, working memory, processing speed, or executive functions; phonemic fluency neared significance (FAS $t = 2.295$; $p = 0.061$). On- and off-state Unified Parkinson's Disease Rating Scale (UPDRS) scores were obtained 12 months post-surgery. Twelve-month UPDRS Part III OFF-state scores indicated no change from enrollment (mean difference = 1.9; 95% CI: -4.6 to +8.3); ON-state scores remained stable ($M = 22.0$; $SD = 2.7$). Two participants died of unrelated causes >12 months post-grafting and brain tissue was examined histologically. There was a significant growth of cholinergic fibers extending towards the PNT. DBS plus NBM participants showed stable cognition. There was no change in motor function, while pathology supported accurate deposition of NBM grafts. Overall, the procedure appears safe with potential for graft-induced preservation of cholinergic neurons that could have potential cognitive benefit in patients with cholinergic degeneration.

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Poster

199. Parkinson Disease: Novel Therapeutics

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 199.08

Topic: C.03. Parkinson's Disease

Support: Grammy Museum Foundation

Title: Association between clinical motor symptoms and peripheral inflammatory cytokines after group therapeutic singing in persons with Parkinson's disease.

Authors: *E. STEGEMOLLER, K. DIAZ-SANTANA, M. L. KOHUT;
Iowa State Univ., Ames, IA

Abstract: Research has revealed that group therapeutic singing (GTS) is an effective treatment strategy to improve motor symptoms as assessed by the MDS-UPDRS. Yet, the underlying mechanism that may explain the significant improvements with GTS remains unknown. The objective of this study was to examine changes in peripheral inflammation after one hour of group therapeutic singing in persons with Parkinson's disease (PD). Eleven participants with PD were tested on medication and had been singing in the group for a mean of 2.1 ± 1.7 years. Clinical motor symptoms and physiological data were collected prior to and after 1 hour of GTS. The motor section of the MDS-UPDRS was collected to assess clinical motor symptoms and later scored by two Movement Disorder Specialists masked to the study intervention and to pre/post collection. A blood sample was collected, and serum isolated. Key inflammation-related markers were analyzed (TNF-α, IFN-γ, IL-1β, IL-8, IL-2, IL-7, IL-5, IL-13, IL, 4, IL-10 IL-12p70, GM-CSF, and IL-6). Spearman correlations were completed to determine the relationship between the change in MDS-UPDRS and inflammatory cytokines. When examining the relationship between the change in inflammatory cytokines and the change in motor UPDRS, as clinical motor symptoms improved, the fold change in most inflammation-related cytokines increased. Specifically, significant associations between the change in motor UPDRS were revealed with the fold changes in IL8 (R = -0.755, p = 0.007) and IL1β (R = -0.619, p = 0.019). A trend was revealed with IL10 (R = -0.555, p = 0.077). No other associations were revealed. These results suggest that an increase in inflammation-related cytokines in serum after GTS may be related to improvements in motor symptoms. Further research is needed to identify the cell source of the changes in cytokines to better understand how GTS is influencing peripheral inflammation.

Disclosures: E. Stegemoller: None. **K. Diaz-Santana:** None. **M.L. Kohut:** None.

Poster

199. Parkinson Disease: Novel Therapeutics

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Program #/Poster #: 199.09

Topic: C.03. Parkinson's Disease

Support: GVSU CSCE

Title: Effect of Nordic walking on serum brain-derived neurotrophic factor in people with Parkinson's disease

Authors: *S. K. KHOO[1], C. COATES[1], Z. WALTERS[1], J. HALL[2], D. VAUGHAN[2], L. NELSON[2], M. BRECHBIEL[2], M. SHOEMAKER[3], C. HARRO[3];
[1]Cell and Mol. Biol., [2]Med. Lab. Sci. Program, [3]Physical Therapy, Grand Valley State Univ., Grand Rapids, MI

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder. To date, there is no cure for PD; current treatments only provide symptomatic relief that do not preserve mobility or slow disease progression. Thus, it is critical to research on neuroprotective interventions to benefit people with PD. Exercise is known to increase the levels of various neurotrophins, a family of proteins that support survival, development, and functions of neurons. Specifically, exercise increases brain-derived neurotrophic factor (BDNF) to stimulate growth of new circuits in neurons to promote neuronal and cognitive plasticity. BDNF protein expression is significantly reduced in PD brains and can be reflected in patients' blood serum in lower levels compared to healthy controls. Here, we investigated the effect of Nordic walking-a form of aerobic exercise with specialty poles to mimic full body movement of cross-country skiing-on serum BDNF levels in people with PD. We hypothesized that serum BDNF levels would increase and stay elevated with a 4.5-month Nordic walking exercise regimen. We recruited 12 individuals with PD with mild to moderate disease severity with the ability to safely and continuously walk a minimum distance of 500 feet independently without an assistive device. Our study design involved a 4-week baseline phase (no Nordic walking), followed by a 6-week Nordic walking training phase and a 3-month independent Nordic walking exercise phase. Blood samples were collected every 2 weeks for baseline and training phase (BL1, BL2, TS1, TS2, and TS3), and every month for the independent phase (TS4, TS5, and TS6). BDNF enzyme-linked immunosorbent assays (ELISA) was used to measure serum BDNF levels. We found BDNF levels increased significantly when comparing average baseline vs. TS6, TS1 vs. TS6, and TS3 vs. TS6 ($P<0.02$, $P<0.02$, and $p<0.0001$, respectively). This pilot study showed that long-term Nordic walking exercise may increase BDNF levels to improve brain health of people with PD.

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Poster

199. Parkinson Disease: Novel Therapeutics

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Program #/Poster #: 199.10

Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation (grant number 18159)

Title: Dynamic postural stability improves after multi-modal training in individuals with Parkinson's disease.

Authors: *A. PENKO[1], M. STREICHER[1], E. ZIMMERMAN[1], R. KAYA[1], J. L. ALBERTS[2]; [2]Cleveland Clin., [1]Cleveland Clin., Cleveland, OH

Abstract: Many individuals with Parkinson's disease (PD) exhibit postural instability and gait dysfunction in conjunction with cognitive impairment resulting from impaired basal ganglia function. These impairments worsen with advancing disease, and may increase fall risk. Multimodal training (MMT) or the simultaneous training of motor and cognitive task, has shown to improve fall frequency in individuals with PD. The impact of MMT on postural instability, specifically, dynamic balance, which is required for daily activities, remains unknown. This project aimed to evaluate the impact of MMT on dynamic postural stability and fall frequency in individuals with mild-moderate PD. Thirty seven (N=37; mean age 68.6±6.4) mild-moderate PD patients (mean MDS-UPDRS III 35.4±13.5) were randomized to a traditional MMT (n=18) group, with a physical therapist, or an augmented reality MMT group (n=19), delivered utilizing the Microsoft Hololens 2 augmented reality device. Both groups completed training 2x/week for 8 weeks. Outcome measures were captured at baseline, end of treatment (EOT) and EOT+8 weeks under single-task (assessing balance only utilizing a force platform and a limits of stability test) and dual-task conditions (assessing balance while simultaneously completing a cognitive task, serial 7's). The relationship between fall frequency and dynamic postural stability was evaluated at baseline, and a significant correlation was found ($r=-0.33$, $p=0.043$). A significant difference was found between single and dual-task conditions as the total area of postural stability during a limits of stability test was less under dual-task conditions ($p<0.001$). There was also a significant effect of time, with total area increasing (improved dynamic balance) for both groups over time ($p=0.0016$). No significant effects were found for fall frequency ($p>0.05$). In conclusion, the relationship between falls and balance was found to be significant, with greater falls being associated with poorer balance. For the intervention, both traditional and augmented reality MMT resulted in improved dynamic postural stability. This improvement may be related to improved function in informational processing in the basal ganglia, and likely has a positive impact on quality of life in individuals with PD.

Disclosures: A. Penko: None. **M. Streicher:** None. **E. Zimmerman:** None. **R. Kaya:** None. **J.L. Alberts:** None.

Poster

199. Parkinson Disease: Novel Therapeutics

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Program #/Poster #: 199.11

Topic: C.03. Parkinson's Disease

Support: Ministry of health grant 3000-14527

Title: A novel real time algorithm to predict freezing of gait in Parkinson's patients using wearable mobility sensors data

Authors: *N. GALOR[1], T. KRASOVSKY[2,4], S. HASSIN[3,5], B. HEIMLER[1], M. PLOTNIK[1,6,7]; [1]Ctr. of Advanced Technologies in Rehabil., [2]Pediatric Rehabil., [3]Movement Disorders Institute, Dept. of Neurol., Sheba Med. Ctr., Ramat Gan, Israel; [4]Dept. of Physical Therapy, Univ. of Haifa, Haifa, Israel; [5]Dept. of Neurol. and Neurosurgery, Fac. of Med., [6]Dept. of Physiol. and Pharmacology, Fac. of Med., [7]Sagol Sch. of Neurosci., Tel-Aviv Univ., Tel Aviv, Israel

Abstract: Freezing of gait (FOG) is a common motor symptom of advanced stages of Parkinson's disease (PD) causing a transient inability to walk despite the intention to keep walking. Previous studies have shown that FOG events can be detected with high precision by analyzing gait kinematics collected through wearable motion sensors. However, until now, very few algorithms aimed at real-time prediction of FOG, i.e., before its occurrence. Here we present a novel algorithm for real-time FOG prediction based on the online monitoring of the stepping coherence between the two legs, as extracted from signals obtained from 21 PD patients wearing motion sensors on their shanks during gait trials with FOG-triggering events (e.g., turns). This measure was chosen due to previous evidence demonstrating impaired bilateral coordination of gait (BCG) in relation to FOG, with some additional works documenting BCG occurring together with a transient functional decoupling between cortical and sub-cortical regions around freezing events, ultimately suggesting BCG might be a direct behavioral manifestation of FOG-related brain processes. Our algorithm calculated BCG via wavelet analysis (calculating the coherence of the wavelet transform of gait signals, specifically the anteroposterior angular velocity). Then the algorithm detected the time points where the legs' coordination was strongly violated and 'flagged' them as FOG-alert events. Here we present preliminary results based on simulated real-time streaming of the collected data. Out of 103 FOG events that were present in the collected data, the algorithm predicted 96 events (sensitivity of 93.2%), on average 1.5 seconds (SD= ± 0.75 seconds) prior to the event. The algorithm often flagged also turns (i.e., in 146 out of 252 turns, i.e., 58%). However, while turning-related false positives remain a challenge for this potential FOG prediction solution, it is known that BCG is typically altered during turning, with many evidence documenting an increase in FOG propensity around turns. Thus 'false alarms' in this case may not be a major limitation. Overall, these results suggest this algorithm has the potential to be incorporated within a wearable system for FOG prediction, i.e., potential FOG-triggering events are identified by the algorithm, and strategies such as external auditory cueing could be delivered to avert/limit FOG occurrences.

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Poster

199. Parkinson Disease: Novel Therapeutics

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Program #/Poster #: 199.12

Topic: C.03. Parkinson's Disease

Title: Revolutionary new class of therapeutics derived from the human gut microbiome displaying neuromodulatory properties could offer a promising treatment for Parkinson's disease

Authors: *S. CHETAL, A. ZHU, I. ROBERTSON, S. REID, N. VINCY-JOSE, S. AHMED, C. MOORE, L. MARKINSON, I. E. MULDER;
4D Pharma Res. Ltd., Aberdeen, United Kingdom

Abstract: The gut-brain-axis is emerging as an important modulator of neurodegenerative diseases, such as Parkinson's disease (PD). Accumulating evidence links the gut microbiome to PD symptomatology and pathophysiology. In PD, non-motor gastrointestinal symptoms and alterations of the enteric nervous system (ENS) often precede the onset of motor symptoms by decades. Gut microbiome-derived therapeutics that restore functional gut-brain-axis communication could potentially have disease-modifying properties, to slow or even prevent neurodegenerative processes. 4D pharma, a clinical-stage microbiome company, utilising their proprietary MicroRx® discovery platform, have identified two live biotherapeutic products (LBPs), *Parabacteroides distasonis* MRx0005 and *Megasphaera massiliensis* MRx0029, for the treatment of PD. MRx0005 and MRx0029 appear to have complimentary mechanisms of action. Pre-clinically, the LBPs affect different aspects of the gut-brain-axis, with MRx0005 displaying a strong anti-(neuro)inflammatory profile, while MRx0029 protected neurons from neurotoxin-induced cytotoxicity, modulated intestinal barrier function and promoted neurodifferentiation markers *in vitro*. Furthermore, the disease-modifying potential of MRx0005 and MRx0029 was demonstrated in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model. MPTP administration produced deficits in dopaminergic function. MRx0005 reduced deficits in striatal DA from 77% to 59% and striatal DOPAC from 44% to 17%. MRx0029 protected nigral TH^+ neurons by reducing MPTP-induced deficits in TH^+ cell numbers from 46% to 11%. The impact of MRx0029 and MRx0005 on the microbiome and metabolomics were also assessed. Increased bacterial diversity was found in animals treated with MRx0029, and changes in several compounds associated with histone acetylation, oxidative stress and protein aggregation were also observed. The FDA has approved an IND application for a first-in-human Phase I multi-centre, randomized, double-blind, placebo-controlled cross-over design with MRx0005 or MRx0029 in PD patients. The study will evaluate the safety and tolerability of the strains in separate patient cohorts and will also measure biomarkers relating to the proposed mechanisms of action of the LBPs. These findings potentially indicate that in the not-too-distant future, diseases of the central nervous system (CNS) may be treated by therapeutics derived from living gut bacteria. 4D pharma is continuing to build on research into the gut-brain axis and gut-targeted LBPs such as MRx0005 and MRx0029 offer a promising new way to treat neurodegenerative disorders such as PD.

Disclosures: **S. Chetal:** A. Employment/Salary (full or part-time):; 4D Pharma PLC. **A. Zhu:** A. Employment/Salary (full or part-time):; 4D Pharma PLC. **I. Robertson:** A. Employment/Salary (full or part-time):; 4D Pharma PLC. **S. Reid:** A. Employment/Salary (full or part-time):; 4D Pharma PLC. **N. Vincy-Jose:** A. Employment/Salary (full or part-time):; 4D Pharma PLC. **S. Ahmed:** A. Employment/Salary (full or part-time):; 4D Pharma PLC. **C. Moore:** A. Employment/Salary (full or part-time):; 4D Pharma PLC. **L. Markinson:** A. Employment/Salary (full or part-time):; 4D Pharma PLC. **I.E. Mulder:** A. Employment/Salary (full or part-time):; 4D Pharma PLC.

Poster

199. Parkinson Disease: Novel Therapeutics

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 199.13

Topic: C.03. Parkinson's Disease

Support: Mitacs IT20279 (Scholaship)
Chronic Pain Network CIHR 358108
Canadian Foundation for Innovation 10071

Title: Noninvasive neurostimulation studies in non-human primates and in patients with Parkinson's disease

Authors: *E. GOURIOU, T. DI PAOLO, C. SCHNEIDER;
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Abstract: Parkinson's disease (PD) is the second largest neurodegenerative disease worldwide in terms of occurrence. The known motor and non-motor symptoms are induced by the disruption of the loop between basal ganglia, thalamus and cerebral cortex. L-Dopa, the main symptomatic treatment for PD, induces over time disabling involuntary movements called L-Dopa-induced dyskinesias (LID). Glutamatergic overactivity is well documented in PD and LID. But treatments for LID are limited. E.g., amantadine can have more debilitating side effects than a long duration of action and not all PD patients are responders. Therefore, non-pharmacological approaches that normalize glutamatergic function in brain without side effects noninvasive stimulation are of interest, like the transcranial direct current electrical stimulation (tDCS) over the primary motor cortex (M1) and the peripheral repetitive magnetic stimulation (rPMS of muscles). The pre-clinical arm of our study (to reduce dyskinesias and improve motor symptoms after tDCS) is conducted in non-human primates (NHP) lesioned with MPTP and treated with L-Dopa as a model of PD with LID (n= 3 NHP). One tDCS session induced some LID decrease without deterioration of the beneficial effects of L-Dopa on motor symptoms. These effects are now tested with 10 tDCS sessions (5 sham sessions, 5 real tDCS sessions) and the underlying mechanisms will be deciphered following post-mortem analyses brain tissues. In parallel, patients (n=4 to date) are tested with tDCS (sham and real, 5 sessions each) over M1 contralateral to the most affected side of the body. It is also explored if the combination of tDCS with rPMS increases the gains more than tDCS alone. The first results are presented here for the scores of the Unified Parkinson Disease Rating scale part III (UPDRS III). The 5 sessions of tDCS alone induced a 11-point decrease (14,5% improvement) and the 5 sessions of tDCS+rPMS induced another 9-point decrease (12%). Sham did not yield any effect. Changes of daily life activities and of posturo-motor control, specifically for bradykinesia and rigidity, were maintained over a month post-stimulation and changes of M1 excitability denoted cortical plasticity. Non-motor symptoms were not deteriorated (depression, sleep quality).These preliminary data are promising and studies will contribute to knowledge in this specific research field. The approach could have a clinical impact, as for instance the possibility to reduce doses and side effects of medication if tDCS or tDCS+rPMS actually reduces dyskinesias and improves the posturo-motor function.

Disclosures: **E. Gouriou:** None. **T. Di Paolo:** None. **C. Schneider:** None.

Poster

199. Parkinson Disease: Novel Therapeutics

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Program #/Poster #: 199.14

Topic: C.03. Parkinson's Disease

Support: Farmer Family Foundation

Title: Deep brain stimulation leads to spatiotemporally variable upper extremity muscle responses which are affected by muscle activation and stimulation amplitude

Authors: *C. TOTH[1], B. CAMPBELL[1,4], L. FAVI BOCCA[2,3], K. BAKER[1], K. MOTT[2], D. CUNNINGHAM[5,6], A. MACHADO[1,2,3], K. BAKER[1];
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Abstract: Deep brain stimulation (DBS) is a standard-of-care (SoC) treatment for Parkinson's disease (PD) and essential tremor (ET). The mechanisms by which DBS modulates the motor circuit of the central nervous system is only partially understood; even less is known about how stimulation influences corticomotor output. To understand the effects of DBS on muscle activity, we recorded electromyographic (EMG) activity as subjects tracked a visually-presented, alternating (5 seconds each, contract/relax) square-wave pattern via isometric gripping a dynamometer, with additional resting state trials recorded. EMG was recorded bilaterally from the biceps, triceps, flexor carpi radialis, and extensor digitorum communis. DBS was delivered at amplitudes below, at, or above each participant's SoC therapeutic level. Motor evoked potentials (MEPs) were calculated for each combination of behavioral state and stimulation amplitude, and each was classified as either responsive or non-responsive to DBS. Data were recorded from twelve PD patients with subthalamic nucleus (STN) DBS implants and seven ET patients with ventral intermediate nucleus (VIM) implants. In both populations, and consistent with anatomical pathways, DBS elicited a myogenic response most commonly, but not exclusively, contralateral to the implanted hemisphere, with distal muscles modulated more than proximal muscles. Responses were more prevalent in the contracted state compared to the resting state, providing evidence that muscle activation facilitated propagation of DBS into the muscle. DBS at SoC amplitude led to more widespread modulation of upper extremity muscles than higher or lower amplitudes. MEPs exhibited both short latency (<50 ms) and longer latency peaks; short latency peaks were more consistent across subjects and stimulation amplitudes than longer latency peaks. Short latency peaks may be caused by activation of internal capsule fibers by DBS, although our findings could implicate more complex pathways as well. Longer latency peaks may represent a combination of more complex motor circuit activation and signal rebound from muscles via reflex arcs. Further work is needed to characterize the physiological

mechanisms of EMG modulation via DBS and its relationship to therapeutic benefit and side-effects.

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Poster

199. Parkinson Disease: Novel Therapeutics

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Topic: C.03. Parkinson's Disease

Support: Farmer Family Foundation
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Title: Subthalamic activity evoked by electrical stimulation reveals link between circuit resonance, beta-band oscillations and levodopa in Parkinson's disease

Authors: *S. MESBAH[1], B. CAMPBELL[2,3], L. BOCCA[3], S. NAGEL[3], R. RAMMO[3], A. MACHADO[3,1], K. BAKER[2,3], D. ESCOBAR[1,3];
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Abstract: Characterizing changes in circuit-wide neural dynamics related to the therapeutic effect of levodopa medication in Parkinson's disease (PD) is key to better understanding how PD alters brain circuits and developing neuromodulation approaches that optimally restore circuit function. Previous studies have shown that single electrical stimulation pulses delivered in the subthalamic nucleus (STN) evoked 10-30 Hz ("beta-band") oscillations in scalp EEG recordings over the primary motor cortex, and the damping (resonance measure) of these beta-band responses increased after levodopa administration. Clarifying whether STN stimulation also evokes beta oscillations in the STN and how levodopa alters the damping of these STN oscillations is relevant to better understanding the effect of levodopa on the basal ganglia. We tested the hypotheses that 1) levodopa administration is associated with a reduction in the amplitude of STN beta-band activity evoked by stimulation of the STN, and 2) this reduction in amplitude can be explained by an increase in damping in the dynamics of the stimulation-evoked responses (ERs). Local field potentials (LFPs) during 2.93 Hz stimulation were recorded from the STN of five PD patients 2-7 days after DBS implantation surgery during the on- and off-medication states to characterize the ER dynamics. ERs exhibited their highest power in the beta-band in all the subjects, and levodopa administration was associated with a significant reduction in the ER amplitudes in the beta-band ($p<0.01$). Our analysis with data-driven ER mathematical models indicates that levodopa-related reductions in the ER amplitude in the beta-band can be explained by an increase in damping in the ER dynamics. Together our results suggest that a

decrease in damping in circuitry connected to the STN plays a critical role in the generation of beta-band oscillations observed in the basal ganglia of PD patients, and an increase in damping is associated with the therapeutic benefit of levodopa. While the mechanisms underlying resonance in the basal ganglia are unclear, we argue that the susceptibility of STN neurons to fire in the beta-band in parkinsonism, associated with changes in synaptic plasticity in the striato-pallidal, pallido-subthalamic, and cortico-subthalamic connections, is also linked to the changes in ER damping. Our results highlight the relevance of developing neuromodulation systems that reshape plasticity and damping in the basal ganglia-thalamocortical circuit to characterize the causal role of resonance in the manifestation of PD, and potentially restore motor function with precision based on each patient circuit dynamics.

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Poster

199. Parkinson Disease: Novel Therapeutics

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Support: National Institute of Neurological Disorders and Stroke of the National Institutes of Health under award number NS092730
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Title: Decoding of neural oscillatory changes based on machine-learning resultant from directional STN-DBS: A new path for intelligent adaptive DBS

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Abstract: Background: Subthalamic nucleus deep brain stimulation (STN-DBS) is an established, standard of care alternative approach for ameliorating the motor symptoms of Parkinson's disease (PD). However, overall clinical efficacy depends largely on stimulation targeting modulating sensorimotor pathways (e.g., dorsolateral STN) while minimizing spread to non-motor regions of STN and adjacent pathways. Novel, directional leads are designed to facilitate targeted stimulation delivery, but the additional adaptability potentially comes at the cost of further programming effort. Our work endeavors to apply magnetoencephalographic (MEG) techniques to identify PD-specific biomarkers to facilitate both acute DBS programming and, ultimately, to sub-serve demand-dependent adaptive DBS. **Methods:** We recorded MEG following STN-DBS and conducted source estimation to identify the neuro-oscillatory activity

resulting from the stimulation of various sites in the area of the STN using directional and omnidirectional protocols. We further characterized the power changes particularly in the beta-band during STN-DBS. The cortical source localization and power changes (PD biomarkers) were used as features to train machine learning algorithms to discriminate between optimal and non-optimal stimulation effects in PD patients. **Results:** The volume of activated tissue (VTA) was calculated in order to discretize the optimal configurations related to the motor STN sub-region activation. Stimulation with optimal DBS configurations related to motor STN sub-area revealed source activations close to the motor cortex, whereas non-optimal stimulations related to STN associative sub-area localized neural sources in other frontal brain regions. In addition, the power had the strongest intensity in the beta-band following optimal STN-DBS, but the non-optimal directions had less oscillatory beta-power. Machine learning algorithms classified optimal versus non-optimal settings with high accuracy. **Conclusion:** This study reveals MEG capability in detecting and differentiating cortical activities resultant from stimulating various sub-regions relative to the STN, further supporting the functionally-segregated model of this nucleus. **Significance:** These results based on MEG-source localization, neural oscillatory, and machine learning for decoding brain signals from neural time-series could represent an objective biomarker of clinical response. This may further expand the clinical utility of aDBS protocols based in artificial intelligence.

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Poster

199. Parkinson Disease: Novel Therapeutics

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 199.17

Topic: C.03. Parkinson’s Disease

Support: PDF Grant PF-RCE-1948

Title: Probing the differential vulnerability of neurons in Parkinson’s disease by single nucleus analysis

Authors: *A. YADAV[1], Z. CHATILA[1], X. E. FLOWERS[1], T. YUN[1], A. F. TEICH[1], R. M. COSTA[2], P. L. DE JAGER[3], E. AREA-GOMEZ[5], G. J. MARTINS[4], R. ALCALAY[6], J.-P. VONSATTEL[4], S. E. PRZEDBORSKI[4], E. BRADSHAW[4], V. MENON[1];
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Abstract: A striking neuropathological feature of Parkinson's disease (PD) is the loss of only specific subpopulations of neurons, and, within these, only subsets of neurons are affected. The molecular basis for this remarkable differential susceptibility remains to be established. To begin shedding light on this important aspect of PD neurodegeneration, herein we applied a single nucleus sequencing approaches to four selected brain regions that are known to be differentially affected in PD using postmortem samples from 19 sporadic PD and 14 controls. We integrated the single nucleus RNA-seq datasets from different region of the brain to identify the major cell types in the brain such as Neurons, Oligodendrocytes, Microglia, and Astrocytes. We further interrogated neurons in order to identify the subpopulations that were region-specific as well as overlapping across the four brain regions. We compared our results to a recent study reported for dopaminergic neurons (DA) by Kamath et al., Nat Neuroscience 2022. We found expression of multiple DA markers from Kamath et al. 2022, such as SLC6A3, TH, SLC18A2, CALB1, NR4A2, and SOX6 in our DA neuron clusters. Interestingly, we also observed expression of AGTR1 which corresponds to highly susceptible population of neurons in the substantia nigra in PD. We are further interrogating region specific clusters and how it translates to our understanding of PD. Our ongoing studies aim at integrating our neuronal with non-neuronal genomic data to try to generate

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Poster

199. Parkinson Disease: Novel Therapeutics

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Topic: C.03. Parkinson's Disease

Support: PDF Grant PDF PF-RCE-1948

Title: Using microglial signatures from single-nucleus profiling to identify candidates for disease-modifying therapy for Parkinson Disease

Authors: *Z. K. CHATILA[1], A. YADAV[1], X. E. FLOWERS[2], T. D. YUN[1], A. F. TEICH[3], R. M. COSTA[4], P. L. DE JAGER[5], E. AREA GOMEZ[8], G. J. MARTINS[4], R. N. ALCALAY[9], J. P. VONSATTEL[6], S. E. PRZEDBORSKI[7], V. MENON[1], E. M. BRADSHAW[1];
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Abstract: All brain areas affected in Parkinson's disease (PD) show an abundance of microglia with an activated morphology together with increased expression of pro-inflammatory cytokines, raising the view that neuroinflammation, especially through microglia, may contribute to the neurodegenerative process in this common and incurable neurodegenerative disorder. To begin elucidating the mechanisms by which microglia may contribute to the demise of neighboring neurons in PD, we sought to capture the heterogeneity of the microglia by applying a 10x single nucleus sequencing approach to four selected brain regions that are known to be differentially affected in PD, using postmortem samples from sporadic PD and controls. We have generated an unbiased transcriptomic dataset to investigate microglial heterogeneity in 19 sporadic PD cases across the substantia nigra (SN), ventral tegmental area, substantia innominate, and hypothalamus, as well as in the SN of 14 controls. We identified 12 microglial subclusters ranging in cell number from 7,734 to 287, which are differentially represented across the four brain regions in PD and in the SN of controls. Our results demonstrate microglial heterogeneity across regions in PD, and highlight potential microglial subpopulations that may participate in PD or protect against disease. Specifically, our analysis revealed a microglial subpopulation that appears to be unique to the substantia nigra (SN) in PD, which interestingly differentially expresses PD-associated genetic markers when compared to other brain regions, including the PD associated locus that contains TMEM163 identified through GWAS and meta-analysis. We also found a microglial subpopulation that is more strongly represented in the SN of controls versus SN tissue from donors with PD, which differentially expresses heat-shock proteins that may be protective against the alpha synuclein proteinopathy implicated in PD processes. With these data, we can generate a PD-specific microglial signature, which can be used to develop biomarkers and devise safe and effective therapies aimed at targeting specific immune cell responses, rather than broadly suppressing immune function.

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Poster

199. Parkinson Disease: Novel Therapeutics

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National Science Foundation [Graduate Research Fellowship DGE-1845298 (W.J.G.V.)]
Michael J. Fox Foundation [Therapeutic Pipeline Program 9998.01 (D.K.C.)]

Title: Human tissue-engineered nigrostriatal pathway encased in hyaluronic acid for axon tract reconstruction in Parkinson's disease

Authors: *W. J. GORDIÁN-VÉLEZ[1,2,3], K. D. BROWNE[1,2], H. CHEN[1,2], J. E. DUDA[2,4], D. CULLEN[1,2,3];
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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease. Its motor symptoms are caused by the death of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc), the degeneration of their axons along the nigrostriatal pathway, and the loss of striatal dopamine. A potential treatment is the replacement of neurons in the SNpc and nigrostriatal axons to repair the native anatomy and connectivity and to restore proper DA signaling. Our method involves the tissue-engineered nigrostriatal pathways (TE-NSPs), which consist of a tubular hydrogel with a collagen/laminin core that encases an aggregate of DA neurons and its axon projections. These are fabricated *in vitro* to resemble the pathway and implanted to reconstruct it. Here our objective was to characterize TE-NSPs made with a biocompatible and tunable hyaluronic acid hydrogel and human induced pluripotent stem cell (iPSC)-derived DA neurons. These human TE-NSPs were implanted in 6-hydroxydopamine-lesioned athymic rats and tested for motor function, striatal dopamine with fast-scan cyclic voltammetry (FSCV), and survival and innervation with histology. Rat-scale human TE-NSPs (345/160 µm outer/inner diameter, 5-6 mm long, 3,300 cells) fully grew axons by 21 days, exhibited a discrete population of DA neurons projecting axon tracts, and expressed tyrosine hydroxylase (TH), β-tubulin III, and GIRK2. With *in vitro* FSCV, we measured 73-122 nM of electrically-evoked dopamine in the aggregate and axons. We could also make scaled-up TE-NSPs (973/500 µm, 1 cm, 100,000 cells), which released higher dopamine levels of ~588 nM and had axons integrating with and releasing dopamine within a striatal aggregate. Rat-scale human TE-NSPs exhibited robust survival when implanted for 3 months, with 82±14% preservation of the aggregate, expression of TH and human NCAM, and maintenance of long-distance axons. TE-NSP animals had significantly higher TH+ innervation in the dorsal striatum compared to acellular hydrogel implants and no repair groups combined (7.9±1.2 vs 3.5±1.1%, $p = 0.0237$). Higher dopamine was also detected with TE-NSPs with *ex vivo* FSCV in the dorsal

striatum (136±9 vs 62±21 nM, $p = 0.0396$). As proof-of-concept we implanted scaled-up TE-NSPs (556/300 µm, 5-6 mm, 55,000 cells) in rats for 6 months and observed preserved bundled TH+ axon tracts, robust growth into the dorsal striatum, and dopamine functionality at ~300 nM. We ultimately advanced the clinical relevance of the TE-NSPs and, with further optimization of their scalability and therapeutic benefits, they may offer a regenerative medicine solution to reconstruct the neuroanatomy and ameliorate symptoms in PD patients.

Disclosures: **W.J. Gordián-Vélez:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Provisional Patent App. 63/190,581 titled "Engineered neural networks in tailored hydrogel sheaths and methods for manufacturing the same". **K.D. Browne:** None. **H. Chen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Provisional Patent App. 16/093,036 titled "Implantable living electrodes and methods for the use thereof". **J.E. Duda:** None. **D. Cullen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); D.K.C. is a scientific co-founder of Innervace Inc., a University of Pennsylvania spin-out company focused on translation of advanced regenerative therapies to treat central nervous system disorders..

Poster

199. Parkinson Disease: Novel Therapeutics

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Topic: C.03. Parkinson's Disease

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Title: Evaluating the use of alpha synuclein deletion as a universal strategy for preventing pathology in cell-replacement therapy in Parkinson's Disease

Authors: *F. NILSSON[1], J. KAJTEZ[1], S. CORSI[2], Y. CHEN[3], A. BRUZELIUS[1], A. FIORENZANO[1], T. KUNATH[4], A. BJORKLUND[1], M. PARMAR[1];
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Abstract: Parkinson's disease (PD) affects approximately 1% of people over the age of 60. Due to mechanisms that are still insufficiently understood, the degeneration of dopaminergic neurons in the substantia nigra pars compacta leads to resting tremor, bradykinesia, and gait and balance deficits. The main pathological hallmark of PD is the presence of Lewy bodies and Lewy neurites, insoluble intraneuronal aggregates consisting of misfolded protein. The major component of both Lewy bodies and Lewy neurites is alpha synuclein (a-syn). Post-mortem analysis of transplanted patients in receipt of fetal tissue revealed accumulation of a-syn pathology in a small subset of transplanted cells over time, suggesting a host-to-graft disease

propagation. The number of affected cells were low and did not affect graft function. The advent of iPSCs has opened up the possibility to graft patient-specific cells, and they may be more prone to develop disease-associated pathology after grafting. If this is the case, gene-correction presents a solution for patients with known monogenetic mutations, but this approach is not applicable for the majority of PD patients, since 90% of all cases are sporadic. Instead, for sporadic patient lines alternative strategies need to be evaluated. The purpose of this study is to evaluate if disease-associated pathology can be avoided by knocking out SNCA, the gene encoding a-syn. Since a-syn is normally expressed in the brain and has a physiological role in synaptic vesicle transmission it is imperative to study the effect of SNCA deletion and if the cells still can remain fully functional and healthy. Using multiple knock-out clones we have shown that SNCA–/– cells differentiate into floor plate progenitors and mature into midbrain dopaminergic (mDA) neurons on par with their parental human pluripotent stem cells. Moreover, electrophysiological analysis using patch clamp electrophysiology shows that SNCA deletion does not prevent the cells from firing mature action potentials. Upon grafting into the rat brain, the cells innervate the striatum and receive afferent input from appropriate endogenous regions, including prefrontal cortex, striatum and thalamus. Moreover, upon transplantation in 6-OHDA lesioned rats This data suggests that removal of a-syn protein does not affect the differentiation or functionality of the mDA neurons and could thus be a promising strategy for autologous grafting that would give patients the clinical benefit of autologous cell therapy with reduced risk of developing Lewy body pathology in the transplant.

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Poster

199. Parkinson Disease: Novel Therapeutics

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 199.21

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant R56 AG059284
NIH Grant P51 OD011133

Title: Physical and Cognitive-Based Regenerative Rehabilitation in Parkinsonian Marmosets

Authors: E. W. DAADI[1], E. S. DAADI[1], T. OH[1], J. KIM[1], G. ROY-CHOUDHURY[1], K. STEECE-COLLIER[2], ***M. DAADI**[3];
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Abstract: Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder caused by the death of dopamine (DA) neurons in the substantia nigra pars compacta (SNpc). The loss

of DA neurons in the SNpc results in a range of debilitating motor symptoms such as bradykinesia, tremor, rigidity and postural instability. Induced pluripotent stem cells (iPSCs) are specialized cells that can be differentiated from adult somatic cells genetically 'reprogrammed' to an embryonic stem cell state and serve as a valuable source for cell-based replacement therapies. Evidence indicates that stem cell derived dopaminergic neurons are a promising avenue in the search for a viable long-term treatment of PD. Here, we report the isolation of self-renewable neural stem cells (NSCs) from human iPSCs derived from individuals with PD. The NSCs demonstrated the ability to proliferate in response to mitogenic growth factors. The isolated NSCs were further differentiated into neurons of dopaminergic lineage. The dopaminergic neurons co-expressed the tyrosine hydroxylase, β-tubulin and midbrain transcription factors (e.g.: FoxA2, Nurr1, Pitx3, Lmx1a, ALDH2, GIRK2). Differentiated neurons were transplanted into the putamen of MPTP-lesioned marmoset model of PD. The grafted animals were subjected to either a physical and cognitive training (PCT) or to sedentary conditions and monitored for a period of 6 months. This rehabilitation paradigm engages cognitive and fine visuomotor coordination. Using actigraphy, we observed that the grafted parkinsonian marmosets significantly improved their daily activity to normal baseline level, while the sedentary control group improved by 28.57%. The PD rating scale (PDRS) and object retrieval task with barrier detour demonstrated more significant sensorimotor and cognitive improvements in the PCT group compared to the sedentary group. These data suggest that PCT enhances sensorimotor and cognitive performances presumably by enhancing functional integration of the grafted dopaminergic neurons within the host striatum.

Disclosures: **E.W. Daadi:** None. **E.S. Daadi:** None. **T. Oh:** None. **J. Kim:** None. **G. Roy-Choudhury:** None. **K. Steece-Collier:** None. **M. Daadi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ownership interest, NeoNeuron LLC.

Poster

200. Spinocerebellar Ataxias

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 200.01

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Uehara Memorial Foundation
Research committee for Ataxic Disease
MEXT | Japan Society for the Promotion of Science (JSPS) JP21K15698
The research committee for Ataxic Disease

Title: Novel PRKCG mutations in Japanese autosomal dominant spinocerebellar ataxia

Authors: *Y. TADA[1], K. KUME[1], S. NOGUCHI[2], T. SEKIYA[3], K. NISHINAKA[3], H. ISHIGUCHI[4], J. KOH[4], S. EMORI[1,4], Y. NAKAYAMA[4], T. KURASHIGE[5], Y. IZUMI[6], H. ITO[4], N. SAKAI[2], H. KAWAKAMI[1];

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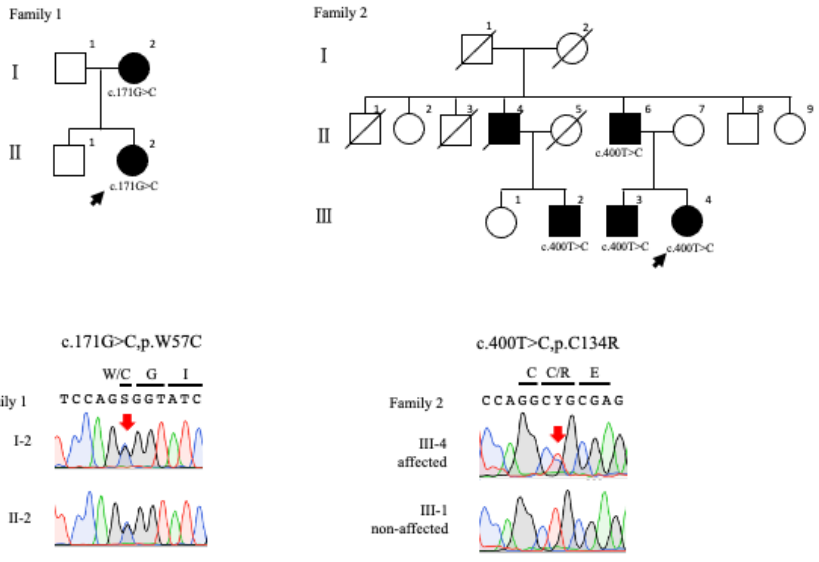
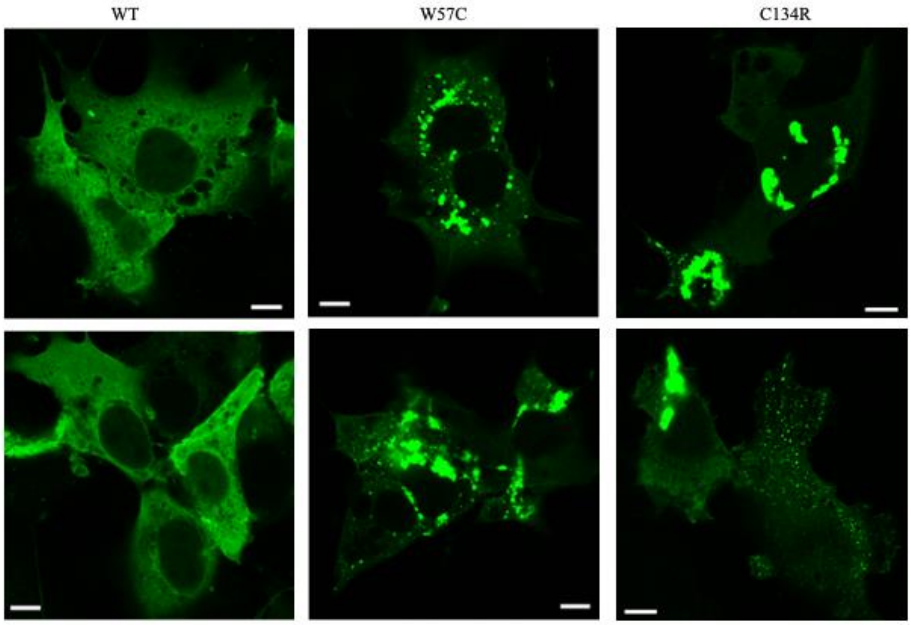
Abstract: Spinocerebellar ataxia type 14 (SCA14) is an autosomal dominant SCA caused by variatns in the PRKCG gene endoding protein kinase C gamma (PKCγ). Although the toxic gain of function mechanism is main cause of SCA14, the molecular pathophysiology remains unclear. Objective of this study is to analyze how novel variant influence PKCγ and to clarify the molecular pathogenesis of SCA14. Clinical symptoms and neurological findings of two Japanese families were evaluated by neurologists. Exome sequencing was performed by the BGI platform. GFP-tagged PRKCG harboring identified variants were transfected into the Hela cells. The aggregation of PKCγ was analyzed by confocal laser microscopy. Solubility of PKCγ was evaluated by proportions of 1% Triton-X insoluble fraction. Patients in family 1 presented only cerebellar atrophy without ataxia, and patients in family 2 had cerebellar ataxia, dystonia, and more severe cerebellar atrophy than patients in family 1.Exome sequencing identified two novel missense variants in PRKCG : c.171G>C,p.W57C (family 1) and c.400T>C,p.C134R (family 2). Both two mutant PKCγ was aggregated in cytoplasm. Although the solubility of C137R PKCγ was decreased compared to that of wild type, W57C PKCγ retained the solubility. We identified two novel variants in PRKCG. The difference in the severity of two families may be due to the difference in solubility changes between the two variants. Decreased solubility of PRKCG protein may play an important role in the pathogenesis of SCA14.





Disclosures: **Y. Tada:** None. **K. Kume:** None. **S. Noguchi:** None. **T. Sekiya:** None. **K. Nishinaka:** None. **H. Ishiguchi:** None. **J. Koh:** None. **S. Emori:** None. **Y. Nakayama:** None. **T. Kurashige:** None. **Y. Izumi:** None. **H. Ito:** None. **N. Sakai:** None. **H. Kawakami:** None.

Poster

200. Spinocerebellar Ataxias

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 200.02

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Mitochondrial DNA haplogroups and age at onset of Spinocerebellar ataxia type 2: a study in Indian SCA2 early and late onset patients.

Authors: *A. K. SONAKAR[1], M. SRIVASTAVA[2], M. FARUQ[3], A. K. SRIVASTAVA[1]; [1]Neurol., All India Inst. of Med. Sci., New Delhi, India; [2]Neurol., ALL INDIA INSTITUTE OF MEDICAL SCIENCES, NEW DELHI, India; [3]Genomics and Mol. Med., CSIR-IGIB, New Delhi, India

Abstract: Title: Mitochondrial DNA haplogroups and age at onset of Spinocerebellar ataxia type 2: a study in Indian SCA2 early and late onset patients. **Background:** Spinocerebellar Ataxia type 2 is an autosomal dominant polyglutamine ataxia caused by an unstable expansion of a CAG tract in the ATXN2 gene. Mitochondrial dysfunction has been linked to the AO modifiers of several neurodegenerative disorders, including SCA2. Expanded CAG repeats not entirely explains AO in SCA2 patients and show up the existence of disease modifiers of age onset. Mitochondrial DNA haplogroups have been linked to clinical manifestations in several polyglutamine disorders which are suggesting that they may act as disease modifiers in SCA2.**Methods:** To investigate the mtDNA haplogroups contribution of AO variation in SCA2 patients, we sequenced D-LOOP and hypervariable regions of Mitochondrial genome of 123 early and late onsets (70 and 53 respectively) patients from mixed Indian population. SCA2 patients were divided into two groups based on the AO & CAG numbers. Five years of onset gap was taken between the Early and late-onset group in SCA2 patients on the same CAG repeat size. mtDNA haplogroups were obtained after sequencing the mtDNA D-loop and hypervariable regions. The mtDNA haplogroups were obtained from 123 patients including 70 early-onset and 53 late-onset SCA2 patients which were classified into 13 phylogenetically related clusters. **Results:** The major haplogroups found were H, L, U, M, A, N, J I, T, R, D, W and D and the frequency of haplogroup in both early and late onset groups.AO was significantly different at the same expanded CAG repeats in SCA2 early and late onset patients, which are showing the existence of other non-CAG factors role in the AO modifiers. In the frequency distribution, we observed the most frequent mtDNA haplogroups as H and M in both early and late-onset SCA2 patient's groups. The frequency of H and M mtDNA haplogroups in the early onset SCA2 patients group was 53.19% and 46.81%. Whereas in the late-onset SCA2 patients the frequency distribution of H and M mtDNA haplogroups was 41.67% and 58.33% respectively. Further, we considered both the mtDNA haplogroups H and M to rule out as AO modifier factor in SCA2 patients. However, there was no significant association found between mtDNA haplogroup clusters and SCA2 early and late-onset patient's groups. **Conclusions:**These findings suggest that age onset modifiers of SCA2 early and late onset patients the mtDNA haplogroups analysis should perform in worldwide SCA2 patient's samples to exploration of mtDNA haplogroup role in disease age modifiers variability.**Keywords:** mtDNA,SCA2,genetic modifier,polyglutamine disorder, AO

Disclosures: A.K. Sonakar: None. **M. Srivastava:** None. **M. Faruq:** None. **A.K. Srivastava:** None.

Poster

200. Spinocerebellar Ataxias

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 200.03

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: JSPS KAKENHI Grant Number JP19K07976

Title: Identification of synucleinopathy associated microglia in a novel mouse model of multiple system atrophy (MSA)-cerebellar type

Authors: *H. YAMAGUCHI[1,2], Y. NISHIMURA[1], D. MATSUSE[1], K. MASAKI[1], T. TANAKA[1], T. SAIGA[1], M. HARADA[1], K. F. TANAKA[3], R. YAMASAKI[1], N. ISOBE[1], J.-I. KIRA[1,4];
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Abstract: Aim: Early intrathecal glial activation is associated with aggressive progression of multiple system atrophy (MSA), though precise roles of glial activation remain elusive even in mouse models. We aimed to clarify the glial activation and identify glial subpopulations contributing to MSA pathology. **Methods:** We developed a novel mouse model of MSA-cerebellar type (MSA-C) by over-expression of human mutant α-syn in oligodendrocyte from 8 weeks of age by removing doxycycline (DOX) from the diet using Tet-off system. **Results:** Transgenic (Tg) mice showed age dependent increase of phosphorylated-α-syn (p-α-syn) accumulation in the brainstem and spinal cord and age dependent exacerbation of motor symptom, mainly cerebellar ataxia. The lesions showed pronounced demyelination with infiltration of Iba1, arginase1 (Arg1), and toll-like receptor 2 (Tlr2)-positive microglia and glial fibrillary acidic protein-positive astrogliosis. Re-inhibition of human mutant α-syn expression by DOX diet at 23 weeks (23WDOX+) resulted in full recovery but re-inhibition at 27 weeks (27WDOX+) showed partial recovery of the symptom. P-α-syn accumulation markedly decreased in both 23 and 27WDOX+ while demyelination improved in 23WDOX+ but persisted in 27WDOX+. Arg 1-positive microglia disappeared in the lesions of 23WDOX+ mice but partially decreased in those of 27WDOX+. Single cell RNA-sequence (scRNA-seq) of CD11b-positive cells isolated from the brainstem and spinal cord at 22 and 26 weeks uncovered for the first time distinct Sdc4, Tgm2, Arg1 and Tlr2-positive microglial subpopulation with pro-inflammatory cytokines (synucleinopathy associated microglia) in Tg mice. Prophylactically colony stimulating factor 1 receptor (CSF1R) inhibitor-treated Tg mice showed more rapidly deterioration in rotarod time than vehicle-treated Tg mice with the increase of both p-α-syn accumulation and demyelinated areas in the brainstem and spinal cord. scRNA-seq revealed that prophylactic CSF1R inhibitor treatment induced relative increase of synucleinopathy associated microglia with higher expression of Ccl12 and Msr1, which may contribute to exacerbation of MSA pathology. **Conclusion:** The synucleinopathy associated microglia could be a new target of MSA therapy.

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Poster

200. Spinocerebellar Ataxias

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 200.04

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Fellowship SNI CONACyT to L-BP
Fellowship SNI CONACyT to C-MV

Title: Effects of sensory manipulation by weight on the torso on gait and balance in patients with spinocerebellar ataxia type

Authors: *L. BELTRAN-PARRAZAL[1], S. ROSAS-NAVARRO[1], M.-L. LOPEZ-MERAZ[1], C. A. PEREZ-ESTUDILLO[1], C. MORGADO-VALLE[2];
[1]Inst. de Investigaciones Cerebrales, [2]Insituto de Investigaciones Cerebrales, Univ. Veracruzana, Xalapa, Mexico

Abstract: Spinocerebellar ataxia type 7 (SCA7) is an autosomal dominant neurodegenerative disease. Its clinical presentation is a progressive cerebellar ataxia associated with cone and retinal dystrophy. SCA7 etiology is an abnormal CAG repeat expansion in the ataxin-7 gene (ATXN7); this mutation causes the degeneration of the brain stem cells, retina and cerebellum. Currently, SCA7 does not have pharmacological treatment or physical rehabilitation to control or stop neurodegeneration. Physical therapists use weights, either on the limbs or on the axial skeleton as an intervention to assist patients to control extraneous movements. The use of vests with weights distributed on the torso of patients with other neurodegenerative diseases e.g., multiple lateral atrophy, who show symptoms of ataxia, produces an improvement in gait speed and postural control in the first week of use. In this study, we measured the baseline SARA score, axial and appendicular movements, gait, and postural balance in patients with SCA7. Then, we applied continuous weighted sensory manipulation with a weight-compensating device in the form of a vest. A net weight of 800 g was distributed in specific areas. We measured the impact on axial and appendicular movements, gait, and postural balance, with the SARA test and inertial sensors for 6 months. We found statistically significant differences against baseline on days 1, 10, 15, 30, 60 and 90 in SARA; days 10, 15 and 30 in axial movements; days 15, 30 and 90 in appendicular movements and day 15 in postural balance. We did not find significant differences in the march parameters. Our data show that continuous sensory manipulation with weight improves the SARA score, axial, and appendicular movements. We discuss how this sensory manipulation could improve the quality of life of SCA7 patients.

Disclosures: L. Beltran-Parrazal: None. **S. Rosas-Navarro:** None. **M. Lopez-Meraz:** None. **C.A. Perez-Estudillo:** None. **C. Morgado-Valle:** None.

Poster

200. Spinocerebellar Ataxias

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 200.05

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH grant R21NS081182
NIH grant R01NS097903
NIH grant R37NS033123
NIH grant U01NS103883
NIH grant R35NS127253

Title: Targeting Staufen 1 with antisense oligonucleotides (ASOs) for treating ALS and SCA2

Authors: D. R. SCOLES, S. PAUL, W. DANSITHONG, *K. P. FIGUEROA, M. GANDELMAN, F. ROYZEN, A. PRICE, C. J. ANDERSON, S. M. PULST;
Univ. of Utah, SALT LAKE CITY, UT

Abstract: Staufen1 (STAU1) is a multifunctional RNA binding protein that controls mRNA degradation and subcellular localization. STAU1 interacts with the ATXN2 protein, that is polyglutamine expanded in spinocerebellar ataxia type 2 (SCA2). We previously showed that STAU1 is elevated and aggregated in cells from SCA2 patients, cells from amyotrophic lateral sclerosis (ALS) patients, and in SCA2 and ALS mouse models. We also found that reduction of STAU1 abundance *in vivo* by genetic interaction improved motor behavior in an SCA2 mouse model, normalized the levels of several SCA2-related proteins, and reduced aggregation of polyglutamine-expanded ATXN2. Here we developed antisense oligonucleotides (ASOs) lowering STAU1 expression toward developing a therapeutic that may be effective for treating SCA2 and ALS. We performed a screen of 118 20mer phosphorothioate 2'-*O*-methoxyethyl (MOE) ASO gapmers targeting across the *STAU1* mRNA coding region for lowering STAU1 expression in HEK-293 cells. ASO lowering STAU1 by greater than 45 % were rescreened in SCA2 patient fibroblasts, and 10 of these were tested for lowering STAU1 abundance *in vivo* in a new BAC-STAU1 mouse model. This identified efficacious ASOs targeting human *STAU1 in vivo* that normalized autophagy marker proteins. We chose ASO-45 that targets human and mouse *Stau1* for further *in vivo* testing. When delivered by intracerebroventricular (ICV) injection, ASO-45 normalized autophagy markers and abnormal mRNA abundances in cerebella of ATXN2-Q127 SCA2 mice. In *Thy1*-TDP-43 transgenic mice, a model for ALS, ASO-45 also reduced STAU1, increased CHAT and, NEUN abundance, and reduced cleaved caspase-3 levels in spinal cord extracts. Targeting *STAU1* may be an effective strategy for treating ALS and SCA2 as well as other disorders characterized by STAU1 overabundance or impairment of autophagic flux.

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Poster

200. Spinocerebellar Ataxias

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 200.06

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH NS083706
NIH NS088321
NIH MH119803
NIH AG066447
Lo Graduate Fellowship
NINDS T32 NS041228

Title: Elucidating mechanisms underlying cerebellar neuronal vulnerability in SCA1

Authors: *K. LUTTIK[1], V. OLMOS[2], A. OWENS[1], J. YUN[3], A. KHAN[3], T. DRIESSEN[2], J. LIM[1,2];
[1]Neurosci., [2]Genet., Yale Univ., New Haven, CT; [3]Yale Col., New Haven, CT

Abstract: Identifying Region-Specific Disease Signatures in Spinocerebellar Ataxia type 1 (SCA1)
Selective regional vulnerability is observed across many neurodegenerative diseases, including spinocerebellar ataxia type 1 (SCA1), however the mechanisms underlying this selective neuronal loss is not well understood. SCA1 is a monogenic neurodegenerative disorder, characterized by progressive ataxia, gait impairment, cognitive deficits, and respiratory dysfunction, which is replicated in SCA1 mouse models. Interestingly, in SCA1, the disease-causing gene ataxin-1 is fairly ubiquitously expressed across brain regions and cell types, but is characterized by degeneration of specific cellular populations, including cerebellar Purkinje cell (PC) neurons. The functional impact of polyglutamine (polyQ)-expanded ataxin-1 (the cause of SCA1) in extra-cerebellar brain regions, and mechanisms underlying physiological deficits including cognitive decline in patients, are not well understood. To identify mechanisms in which specific brain regions are selectively vulnerable in SCA1, here we investigated the functional impact of polyQ-ataxin-1 expression in diverse extra-cerebellar brain regions. We characterized pathology in extra-cerebellar brain regions in SCA1, and utilized bulk RNA sequencing to compare the transcriptomes of the motor cortex and the cerebellum in order to identify region-specific differences in disease-signatures. We identified a region-specific dysregulation of kinase expression. These analyses also include a novel characterization of pathology in the SCA1 mouse cortex, which has identified the cortex as differently-affected, rather than completely unaffected or protected, and provides novel insights into SCA1 disease pathogenesis.
Acknowledgments We thank all members of the Lim laboratory for useful feedback, critiques, and comments. We also thank Xinran Liu and the Yale EM core for their help with the EM imaging. This work was supported by National Institutes of Health grants NS083706 (J.L.), NS088321 (J.L.), MH119803 (J.L.), AG066447 (J.L.), the Lo Graduate Fellowship (K.L.), and T32 NS041228 (K.L.).

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Poster

200. Spinocerebellar Ataxias

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 200.07

Title: WITHDRAWN

Poster

200. Spinocerebellar Ataxias

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 200.08

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Beneficial effects by administration of human mesenchymal stem cells in a mouse model of spinocerebellar ataxia type 2

Authors: *C. SHARMA[1], U. JUNG[3], S. KIM, PHD[2];
[1]Kyungpook Natl. Univ., [2]Sch. of Life Sci. & Biotech., Kyungpook Natl. Univ., Daegu, Korea, Republic of; [3]Pukyong Natl. Univ., Busan, Korea, Republic of

Abstract: We have investigated the therapeutic potential of human mesenchymal stem cells (hMSCs), transplanted intrathecally (IT), in a transgenic mice model bearing a polyQ mutation in the ataxin-2 gene. Our results showed that IT transplantation of hMSCs at 26 weeks old could induce a significant improvement of abnormal motor function, measured by the ataxic scoring system, shown in spinocerebellar ataxia type 2 (SCA2) mice until 24 weeks after hMSCs administration. In addition, we observed that hMSCs administration protected the loss of purkinje cells through production of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF), and inhibition of cerebellar inflammatory responses by production of anti-inflammatory molecules such as tumor necrosis factor stimulated gene-6 (TSG-6) and Follistatin-like 1 (FSTL1), respectively. Altogether, the administration of hMSCs improved motor behavior and ataxia-pathology alleviation by stimulating endogenous regeneration and suppressing inflammatory responses. Notably, the results of this study strongly support further exploration of the feasibility to design new clinical approaches for SCA2 patients.

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Poster

200. Spinocerebellar Ataxias

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 200.09

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: MITO2i Graduate Student Scholarship

Title: C-terminus of HSP70 Interacting Protein (CHIP) in mitochondrial homeostasis

Authors: *R. EARNSHAW[1,2], S. HUI[1,2], M. KAPADIA[1], Y. T. ZHANG[1,2], G. HEYMANN[1,2], K. FUJISAWA[1], L. V. KALIA[1,2,3], S. K. KALIA[1,2,4];
[1]Univ. Hlth. Network, Univ. Hlth. Network, Toronto, ON, Canada; [2]Lab. Med. and Pathobiology, [3]Dept. of Medicine, Div. of Neurol., [4]Dept. of Surgery, Div. of Neurosurg., Univ. of Toronto, Toronto, ON, Canada

Abstract: C-terminus of HSP70 Interacting Protein (CHIP) is a co-chaperone protein and E3 ubiquitin ligase which is involved in targeting many neurodegenerative disease-associated proteins for degradation via the ubiquitin-proteasome and autophagy-lysosome pathways. Mutations within the gene encoding CHIP are the cause of two recently identified cerebellar ataxias, spinocerebellar ataxia autosomal dominant type 48 (SCA48) and spinocerebellar ataxia autosomal recessive type 16 (SCAR16). Both SCA48 and SCAR16 are incurable debilitating neurodegenerative diseases involving significant loss of cerebellar Purkinje neurons, ataxia, cognitive impairment, and a wide range of variable additional symptoms. The mechanism(s) underlying CHIP-associated diseases are currently unknown.
Mitochondrial dysfunction is increasingly being implicated in neurodegenerative diseases and regulating mitochondrial quality control pathways has been suggested as a possible strategy for reducing or preventing neurodegeneration. In our previous work, we have identified chaperone networks that regulate mitochondrial control. Here, we identify CHIP as a regulator of mitochondrial dynamics, indicating that mitochondrial dysfunction may play a role in CHIP-associated neurodegeneration. Utilizing cell lines expressing the mtKeima reporter, a mitochondrially localized pH-dependent fluorescence protein used to monitor mitochondrial autophagy (mitophagy), a process by which mitochondria are selectively degraded via the autophagy-lysosome pathway, we have demonstrated that CHIP is able to regulate mitophagy *in vitro*. Additionally, utilizing *Caenorhabditis elegans* expressing the mtRosella mitophagy reporter protein pan-neuronally, another mitochondrially localized pH-dependent fluorescent reporter, we have demonstrated that loss of CHIP results in dysfunction within neuronal mitophagy *in vivo*. Our findings suggest that dysfunction of mitophagy may play a role in SCA48 and SCAR16 and modulating mitophagy may present a potential therapeutic avenue for reducing or preventing CHIP-mediated neurodegeneration

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Poster

200. Spinocerebellar Ataxias

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Program #/Poster #: 200.10

Topic: C.04. Movement Disorders other than Parkinson's Disease

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McGill SURA award (MR)
Healthy Brains for Healthy Lives fellowship (AAC)

Title: Deficits in BDNF-TrkB signaling contribute to cerebellar deficits in spinocerebellar ataxia type 6

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Abstract: Spinocerebellar ataxia type 6 (SCA6) is an inherited ataxia that presents with mid-life onset of motor coordination impairment and subsequent cerebellar degeneration. There is no cure and treatment options are limited. We used a knock in mouse model of SCA6 ($SCA6^{84Q/84Q}$) to characterize SCA6 pathophysiology and identify potential therapeutics. At 7 months these mice display significant deficits in motor coordination as well as alterations in Purkinje cell firing. We identified a reduction in the level of brain-derived neurotrophic factor (BDNF) and its receptor TrkB in the cerebellum of $SCA6^{84Q/84Q}$ mice. Furthermore, we found evidence for BDNF mis-trafficking due to an accumulation of BDNF in the early endosomes of Purkinje cells, which could further reduce the availability of free BDNF to bind TrkB receptors. The reduction in the level of cerebellar BDNF in $SCA6^{84Q/84Q}$ mice could be rescued by one month of voluntary exercise, an intervention which also rescued deficits in motor behaviour and Purkinje cell firing frequency. We next aimed to remedy the effects of reduced BDNF in $SCA6^{84Q/84Q}$ mice pharmacologically, using a BDNF mimetic. 7,8-dihydroxyflavone (7,8-DHF) is an orally-bioavailable small molecule agonist of the TrkB receptor. Chronic oral 7,8-DHF administration rescued deficits in both motor coordination and Purkinje cell firing frequency. We also observed an increase in Akt activation after 7,8-DHF treatment, indicating that 7,8-DHF activates signaling pathways downstream of the TrkB receptor. Treatment with 7,8-DHF was most effective when it was started early in disease progression, and could continue to rescue deficits for several months. By identifying BDNF-TrkB signaling as a locus of pathology in SCA6, we have identified novel therapeutic targets for SCA6.

Disclosures: **A.A. Cook:** None. **S. Jayabal:** None. **J. Sheng:** None. **M. Rice:** None. **A.J. Watt:** None.

Poster

200. Spinocerebellar Ataxias

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 200.11

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Cortical correlates of gait in pre-manifest and early spinocerebellar ataxia

Authors: *M. MANCINI[1], V. V. SHAH[1], F. HORAK[1], D. SAFARPOUR[1], C. M. GOMEZ[2]; [1]Oregon Hlth. and Sci. Univ., Portland, OR; [2]Univ. of Chicago, Chicago, IL

Abstract: Spinocerebellar ataxia (SCA) causes characteristic impairments of gait and balance that greatly impair quality of life. It has been hypothesized that compensation for impaired cerebellar control of gait and balance in SCA occurs via increased prefrontal cortex (PFC) control. Wireless, functional near-infrared spectroscopy (fNIRS) provides direct, physiological measures of PFC activity while performing actual movements. Only a few studies have examined prefrontal activity in SCAs while walking and none in pre-manifest SCA (SARA<3). Here, we hypothesized that prefrontal cortex activity will be increased during walking in pre-manifest and early SCA compared to healthy controls. Seven participants with genetically-determined SCA (age: 48±11) and 7 healthy controls (age: 46.8±13) participated in the study. The Scale for the Rating and Assessment of Ataxia (SARA) was administered by a movement disorders specialist before the gait assessment. An 8-channel, mobile fNIRS, with two reference channels, was used to record changes in oxygenated hemoglobin (HbO_2) and deoxygenated hemoglobin within the PFC. Participants walked for 2-minutes at a comfortable pace back and forth over a 10-meter distance, with a 180-degree turn at each end while wearing wireless, inertial sensors to derive gait and turning characteristics. Of the 7 individuals with SCA, three were classified as pre-manifest (SARA<3) and 4 as early SCA (SARA<10). PFC activity (HbO_2) while walking was larger than controls of similar age in every patient with SCA. In addition, pilot findings showed that increased PFC activity was also present even in the pre-manifest stage of SCA. Our pilot data suggest that PFC activity is increased in pre-manifest SCA, even when clinical scores are normal. Increased PFC activity is consistent with requirement for less automatic, cortical control of gait to compensate for impaired automatic, cerebellar control. Thus, we predict that gait characteristics will be related to increased PFC activity. fNIRS during functional activities could be employed to select SCA subjects to enroll and/or as an outcome measure in clinical trials for early treatments of these gait disorders. Studies in larger populations and test-retest reliability are needed to confirm these promising pilot findings.

Disclosures: **M. Mancini:** None. **V.V. Shah:** None. **F. Horak:** A. Employment/Salary (full or part-time):; Dr. Horak has significant financial interest in APDM Wearable Technologies, a company that may have a commercial interest in the results of this research and technology. This individual conflict has b. **D. Safarpour:** None. **C.M. Gomez:** None.

Poster

200. Spinocerebellar Ataxias

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 200.12

Topic: C.04. Movement Disorders other than Parkinson's Disease

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FCT PhD Scholarship 2020.00528.BD
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Title: The spinocerebellar ataxia type 37 ATTTC repeat insertion impacts zebrafish CNS development

Authors: *A. F. CASTRO[1,2,3], J. R. LOUREIRO[1,2], J. M. FERREIRA[2,3,4], A. M. VALENTIM[2,4], J. BESSA[2,5], I. SILVEIRA[1,2];
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Abstract: Spinocerebellar ataxias (SCAs) are autosomal-dominant neurodegenerative diseases characterized by progressive gait, limb and speech incoordination due to cerebellar atrophy. We found a pathogenic $(ATTTC)_n$ insertion into a non-pathogenic $(ATTTT)_n$ in a 5'UTR intron of *DAB1*, a *reelin adaptor* gene involved in neuronal cell migration, causing SCA37. We reported that $(ATTTC)_n$ overexpression triggers formation of abnormal nuclear RNA aggregates and $(AUUUC)_n$ microinjection in zebrafish embryos leads to significant lethality as early as 24 hours postfertilization (hpf). Moreover, $(ATTTC)_n$ insertions in six cerebellar expressed genes cause familial cortical myoclonic epilepsies. As the mechanism underlying $(ATTTC)_n$ diseases is unknown, we aim to generate a zebrafish developmental model to investigate $(AUUUC)_n$ pathogenesis and targeted treatment strategies. At least 100 embryos/condition/replicate (7 replicates) were microinjected with the $(AUUUC)_{58}$ or non-pathogenic $(AUUUU)_7$ RNAs (100ng/µL); H_2O was microinjected as negative control. To assess zebrafish development, we quantified viability and hatching rates from 24 - 96 hpf. The hatching rate in $(AUUUC)_{58}$ embryos was significantly lower (67.9% for H_2O, 63.4% for $(AUUUU)_7$, 29.3% for $(AUUUC)_{58}$; Log Rank test, $p<0.0001$) and the survival rate in $(AUUUC)_{58}$ embryos was significantly decreased compared with controls (74.7% for H_2O, 73.3% for $(AUUUU)_7$, 46.1% for $(AUUUC)_{58}$; Log Rank test, $p<0.0001$), at 96 hpf. To characterize early morphological abnormalities, we scored zebrafish head and/or tail malformations from 0 (no defects) to 3, according to their severity, at 24 hpf. The $(AUUUC)_{58}$ significantly increased severe malformations (score 0: 98.8% for H_2O, 89.7% for $(AUUUU)_7$, 55.2% for $(AUUUC)_{58}$; score 1: 0.3% for H_2O, 7.9% for $(AUUUU)_7$, 20.8% for $(AUUUC)_{58}$; score 2: 0.7% for H_2O, 2.2% for $(AUUUU)_7$, 22.9% for $(AUUUC)_{58}$; score 3: 0.2% for H_2O, 0.2% for $(AUUUU)_7$, 1.1% for $(AUUUC)_{58}$; Kruskal-Wallis test post-hoc pairwise comparisons, $p<0.0001$). To investigate neurotoxicity, we quantified the spontaneous tail coiling in embryos without defects, at 24 hpf. We detected a significant increase of involuntary tail movements in the $(AUUUC)_{58}$ embryos, compared with controls (3.1±0.9/min for H_2O, 2.7±0.8/min for $(AUUUU)_7$, 5.0±1.6/min for $(AUUUC)_{58}$; Kruskal-Wallis test post-hoc pairwise comparisons, $p<0.0001$). We are now assessing long term motor, anxiety and memory alterations in these aged animals. Our results suggest that the $(AUUUC)_{58}$ affects hatching and motor development in zebrafish. This model will be useful to further establish a proof-of-concept for $(AUUUC)_n$-targeting therapeutic strategies.

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Poster

200. Spinocerebellar Ataxias

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 200.13

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Effects of human mesenchymal stem cells in Ara-C-induced animal model of cerebellar ataxia

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Abstract: This study investigated the therapeutic effects of transplanting human mesenchymal stem cells (hMSCs) into wild-type (WT) mice intraperitoneally administered with cytosine arabinoside (Ara-C) to develop cerebellar ataxia (CA) during the first 3 postnatal days. hMSCs were injected intrathecally into 10-week-old mice one or three times at 4-week intervals. The hMSC administered mice showed improved motor and balance coordination compared to non-treated mice as measured using the Rota-rod, open field, and ataxic scoring assessments. Additionally, an increase in protein levels of neuronal nuclear protein (NeuN) and doublecortin (DCX), responsible for neurogenesis was observed in hMSC-treated mice compared with non-treated mice. Furthermore, hMSCs implantation significantly elevated neurotrophic factors including brain-derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF), and suppressed TNF-α and IL-1β mediated pro-inflammatory responses. Collectively, our results demonstrated that hMSCs exhibit therapeutic potential for Ara-C - induced CA by protecting neurons by stimulating neurotrophic factors and inhibiting cerebellar inflammatory responses which improved motor behavior and ataxia-pathology alleviation. In summary, this study suggests that hMSCs are effective for treating ataxia-related symptoms.

Disclosures: **P. Narae:** None. **S. Kim, PhD:** None.

Poster

200. Spinocerebellar Ataxias

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 200.14

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant 5R01NS085054-09

Title: A shared mechanism of olivary disease in SCA1 and Hypertrophic Olivary Degeneration

Authors: *L. M. MORRISON[1,2], H. P. HANDLER[4,5], H. HUANG[2], M. FU[2], S. S. PAPPAS[3], H. T. ORR[7,6], V. G. SHAKKOTTAI[2];
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Abstract: The inferior olive (IO) is a nucleus in the medullary brainstem that sends powerful excitatory projections into the cerebellum. These projections, known as climbing fibers, wrap around the vast dendritic arbors of Purkinje cells, influencing cerebellar output and, ultimately, motor control. A striking feature of IO neurons is that they are uniquely susceptible to degenerative hypertrophy. This distinct form of neurodegeneration, known as hypertrophic olivary degeneration (HOD), has previously only been observed after a loss of inhibitory synaptic input to the IO due to lesions in the brainstem. For the first time, we have identified degenerative hypertrophy of the IO in another context: spinocerebellar ataxia type 1 (SCA1), an inherited neurodegenerative disorder known to cause IO degeneration. Based on our recent findings, we hypothesized that this SCA1-related degenerative hypertrophy is not due to inhibitory deafferentation, but is instead the result of a net increase in intrinsic excitability. We investigated this hypothesis by examining IO disease in the genetically-precise SCA1 knock-in mouse model (SCA1-KI) using patch-clamp electrophysiology in brainstem slices from SCA1-KI mice. We found that SCA1-KI IO neurons were hypertrophic, exhibiting a significant increase in both dendrite length and complexity. In addition, a significant loss of calbindin-positive neurons in the IO, characteristic of IO degeneration, accompanied this hypertrophy. Interestingly, patch-clamp electrophysiology experiments showed that SCA1-KI IO neurons are also hyperexcitable. Further studies demonstrate that this SCA1-KI phenotype is not the result of inhibitory denervation (as in HOD, as it is historically understood), but a loss of certain potassium channels whose role is to decrease intrinsic IO neuron excitability. These results, together with our current knowledge of HOD, reveals neuronal excitability as a potential link between disparate causes of IO degenerative hypertrophy. This suggests that HOD is not solely the result of denervation, but by any cause, intrinsic or extrinsic, that increases excitability in IO neurons. This decoupling of HOD from denervation would be highly significant, as it would allow for the design of therapeutic agents that reduce intrinsic IO excitability as a shared therapeutic strategy in a wide variety of degenerative disorders of the IO.

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Poster

201. Neuroprotection I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 201.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Expression of the Wnt/ß-catenin pathway in cortex and cerebellum in rats with epileptic seizures induced by the Kindling model subjected to caloric restriction

Authors: *V. G. MENA MICHEL;
Instituno Nacional de Neurología y Neurocirugía "Manuel Velasco Suárez", Ciudad de México, Mexico

Abstract: Expression of the Wnt/β-catenin pathway in cortex and cerebellum in rats with epileptic seizures induced by the Kindling model subjected to caloric restriction
Authors: V. MENA[1], M. RUBIO OSORNIO[2], L. HERNÁNDEZ[1], D. FLORES[1], E. URIBE[1], N. GALLARDO[1], C. RUBIO[1*]; [1]Instituto Nacional de Neurología y Neurocirugía MVS, Departamento de Neurofisiología, [2]Instituto Nacional de Neurología y Neurocirugía MVS Departamento de Neuroquímica**Diclosures:V. Mena:** None. **M. Rubio:** None. **L. Hernández:** None. **D. Flores:** None. **E. Uribe:** None. **N. Gallardo:** None. **M.C. Rubio:** None.**Abstract:**The epileptic activity produces changes in glucose metabolism. Therefore, it is inferred that because Wnt/β-catenin is a protein with function in glucose metabolism, it promotes its effects on the metabolism of ketone bodies, providing a stabilizing effect on the membrane, derived from caloric restriction in epileptic neurons of the cerebral cortex and cerebellum. Consequently, we sought to characterize the expression of Wnt/β-catenin pathway in neurons of the cerebral cortex and cerebellum of rats subjected to caloric restriction and epileptic activity induced by an experimental model of Kindling epilepsy. We used male wistar rats (n=24), which were divided into four groups: a) control group (n=6); b) group with epileptic seizures (n=6); c) group with caloric restriction and induction of the kindling epilepsy model (n=6); d) caloric restriction group without kindling (n=6). The rats in the groups induced by the kindling model underwent stereotaxic surgery to implant electrodes in the basolateral amygdaloidal nucleus and in the motor cortex and thus be able to carry out the stimulation. Once the experimentation phase was over, the rats were sacrificed to perform immunohistochemistry and Western Blot techniques for the analysis of the Wnt/β-catenin pathway. The effect of caloric restriction has been electrographically shown to increase the firing threshold of neurons and decrease neuronal excitability. The obtained results from this research protocol will provide data in relation to the cellular and molecular mechanisms that underlie the effect induced by kindling, which is characterized by being a model that shares mechanisms similar to the epileptic seizures that most patients with this pathology suffer.

Disclosures: V.G. Mena Michel: None.

Poster

201. Neuroprotection I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 201.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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CONACYT CF-214971
CONACYT PhD fellowship 1083209

Title: Neuroprotective effects of growth hormone (GH) in an optic nerve crush injury as an experimental model for glaucoma

Authors: *D. EPARDO, J. E. BALDERAS-MARQUEZ, M. CARRANZA, M. LUNA, J. AVILA MENDOZA, C. ARÁMBURO, C. G. MARTÍNEZ-MORENO;
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Abstract: Growth hormone (GH) exerts pleiotropic actions during development and maturation of the central nervous system. GH administration has been shown to induce neurotrophic effects such as neuroprotection, multi-activation of intracellular signaling pathways, synaptogenesis, axogenesis, and cell survival in several excitotoxic damage models in the chicken neural retina, both *in vivo* and *in vitro*. In this study, we analyzed the neuroprotective effects of GH treatment in the retina and the optic nerve, after optic nerve crush (ONC) injury, used as an acute model of glaucoma, in the rat. Five groups were established: 1) intact; 2) sham (eye surgery without optic nerve compression); 3) ONC;4) ONC+GH; and 5) GH only. The lesion was induced on the left eye of male wistar rats (6 wk-old) by compressing the optic nerve with self-closing forceps for 10 sec. GH was delivered subcutaneously (0.5mg/kg) every 12 h for 14 d or 24 h. Three days before sacrifice, Cholera Toxin Subunit B (CTB) was intravitreally injected as an anterograde axonal transport tracer. At the end of the treatment, retinas and optic nerves were collected for the evaluation of neurotrophic, synaptogenic and gliosis markers by RT-qPCR or for histological analysis. As expected, Brn3a immunohistochemistry of the retina showed that 14 d after ONC, more than 85% of Brn3a+ retinal ganglion cells were lost. However, the ONC+GH group lost only around 70% of the cells, suggesting a survival role induced by GH treatment. CTB axonal labeling showed some regeneration in the optic nerve since a few labeled axons posterior to the lesion were observed in the ONC+GH group, in comparison to the ONC group where all the axons were lost in that area. Furthermore, GH treatment partially restored the mRNA expression levels of NT-3, CNTF, NGF, Gap43, SNAP35, Bcl-2 and IL-6 24 h after the damage. Finally, it was found that 14 d after the lesion, GH downregulated the mRNA levels of GFAP, IL-6 and NGF while upregulated Gap43, as compared to the ONC group. These results suggest that GH treatment promotes neurotrophic effects that include neuroprotection and anti-inflammatory actions after an optic nerve injury.

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Poster

201. Neuroprotection I

Location: SDCC Halls B-H

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Program #/Poster #: 201.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01NS103940

Title: Single-nucleus RNA-seq of normal-appearing multiple sclerosis brains identified perturbation of sphingolipid metabolic genes

Authors: *Y. KIHARA[1], D. JONNALAGADDA[2], C. PALMER[3], R. DUTTA[4], B. D. TRAPP[5], J. CHUN[2];
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Abstract: Multiple sclerosis (MS) is an immune-mediated demyelinating disease in the central nervous system (CNS), affecting people in the prime of life. The most common condition with relapses and remissions (RRMS; relapsing-remitting MS) can also develop into secondary progressive MS (SPMS). Here, we applied single-nucleus RNA sequencing (snRNA-seq) to the prefrontal cortices of "normal-appearing, MS-affected" brains that showed no demyelination. This approach yielded 33,197 high-quality nuclei that were clustered into major CNS cell types including neurons, oligodendrocyte, astrocytes, microglia. Gene expression profiles of RRMS brains exhibited neuronal vulnerability, stressed oligodendrocytes, a reactive astrocyte phenotype and microglial activation, whereas those of SPMS brains showed oligodendroglial vulnerability and impairment of their progenitors' maturation. Furthermore, SPMS brains, as compared to RRMS brains, showed perturbation in the sphingolipid pathway, particularly the downregulation of sphingosine kinases (*SPHK1/2*), whose deficiency in mouse astrocytes diminished the fingolimod-P efficacy in an animal model of MS. These results provided insights into the cell type-specific gene expression divergence between RRMS vs. SPMS, as well as into clinical ramifications of approved disease-modifying drugs targeting sphingosine 1-phosphate (S1P) receptors including fingolimod, siponimod and ozanimod.

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Poster

201. Neuroprotection I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 201.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONACYT Grant 285184
CONACYT Postdoctoral Fellowship I1200/224/2021
and DGAPA-PAPIIT, UNAM (216422)

Title: The neuroprotective effect of the endocannabinoid metabolites of cytochrome P450 during the staurosporine-induced neuronal death

Authors: *C. M. NAVARRO-MABARAK, G. DOMINGUEZ-MACOUZET, J. E. R. MORÁN;
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Abstract: Cytochrome P450 (CYP) epoxygenases can metabolize the anandamide (AEA) and its products, the epoxyeicosatrienoic acid ethanolamides (EET-EAs), are biologically active and capable to activate the cannabinoid signaling pathway. The EET-EAs can be hydrolyzed by the soluble epoxide hydrolase enzyme (sEH) to form the dihydroxyeicosatrienoic acid ethanolamides (DHET-EAs), metabolites with low biological activity. It is known that the modulation of the endocannabinoid system has a therapeutic potential in the CNS diseases, since its activation has anti-inflammatory and antioxidant effects. We are, therefore, interested in studying the neuroprotective effects of EET-EAs in the neuronal death, as well as the mechanisms involved in this neuroprotection. To this purpose, we used primary cultures of cerebellar granule neurons and induced cell death with staurosporine (0.5 μM) treatment. To enrich the content of EET-EA in the cultures, we pre-treated cells during 2h with TPPU (100 μM), a specific inhibitor of soluble epoxide hydrolase (sEH) and with anandamide (20 μM). To determine the involvement of the cannabinoid signaling in the EET-EA-mediated neuroprotection, we used AM251 (1 μM), a CB1 cannabinoid receptor antagonist. Cell viability was assessed by measuring MTT reduction, as well as calcein/propidium iodide staining. Under these conditions, we found that TPPU and anandamide pre-treatment individually and in co-treatment showed a marked neuroprotective effect against staurosporine-induced neuronal death. Although we found that both anandamide and its CYP-derived metabolites resulted neuroprotective, we did not find a synergistic effect. Interestingly, we found that the observed neuroprotective effect of both conditions was reversed by the CB1 antagonist AM251. These results suggest that endocannabinoid metabolites of cytochrome P450 could play a significant role in the neuroprotection. Additionally, the observed neuroprotection could be mediated through an autocrine or paracrine mechanism.

Disclosures: C.M. Navarro-Mabarak: A. Employment/Salary (full or part-time):; CONACYT Postdoctoral Fellowship (I1200/224/2021) CVU (288339). **G. Dominguez-Macouzet:** None. **J.E.R. Morán:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Grants CONACYT (285184) and DGAPA-PAPIIT, UNAM (216422).

Poster

201. Neuroprotection I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 201.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant: RO1EY029823
Bright Focus
UNTHSC seed grant
NBAAD

Title: Novel Antioxidants protect Retina Ganglion Cells and Trabecular Meshwork Cells from stress induced cell death with potential to treat glaucomatous optic neuropathy.

Authors: *C. E. AMANKWA[1], J. FERGUSON[2], G. JOHNSON[3], L. LI[3], W. ZHANG[4], D. STANKOWSKA[3], S. ACHARYA[4];
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Abstract: Purpose: Scientific evidence suggests that oxidative stress-induced neurodegeneration is a hallmark feature in primary open angle glaucoma (POAG). Along with profound damage to optic nerve, retina, and trabecular meshwork (TM), POAG is reported to affect parts of the brain, notably, the hippocampus. Currently, treatment regimen does not fully address neurodegeneration and/or improvement of retina/hippocampus functionality. Our objective was to evaluate the cytoprotective effect of our in-house novel compounds SA-21 and SA-24 as hybrid superoxide dismutase and glutathione mimetic, using in vitro and in vivo cell death models.
Methods: The synthesis and characterization of SA-21 and SA-24 was confirmed by proton magnetic resonance and mass spectrometry. Normal human TM (NTM-5) and mouse hippocampal HT-22 cells were oxidatively stressed with tert-butyl hydroperoxide (TBHP-350μM) in the presence of SA-21, SA-24 (10μM, 100μM, and 1000μM) for 24h. In a separate study, rat R28 cells were subjected to Oxygen Glucose Deprivation (OGD) for 6 hours or treated with TIII (cocktail of TNF-α, IL-1β and IFN-Ɣ) for 1h to mimic ischemia/reperfusion (I/R) injury followed by addition of SA-21 or SA-24 compounds (1, 10, 100μM) incubated overnight. Cell survival was assessed by MTT and LDH assays, ROS was assessed using DCFDA assay. In addition, C57BL6 male mice (12-weeks old, n=4-5) were anesthetized, underwent optic nerve crush (ONC) surgery, and at day 0 and 3 intravitreally injected with 1% SA-21 (2μl) or vehicle. On day 7, pattern electroretinogram (PERG) was performed, animals were euthanized, and number of surviving RBPMS-positive RGCs were counted. Using the microbead mouse model of ocular hyperextension (OHT), IOP was monitored after dosing twice a day with SA-21 and SA-24 eyedrops.
Results: SA-21 and SA-24 were not cytotoxic to cells at all concentrations. SA-21 and SA-24 were cytoprotective to HT-22 and NTM-5 cells (100μM, 1000μM respectively). SA-21 and SA-24 at10 μM treatment significantly decreased ROS production (48% ,25%) in R28 cells using OGD model. ONC produced a 48% loss of RGCs, which was decreased in SA-21 treated mice

(by ~10%) with a trend in increase in PERG amplitude. Preliminary studies show SA-24 eye drops reduce about 4 mmHg IOP in OHT model compared to untreated eyes.
Conclusions: Both SA-21 and SA-24 rescues TM and HT-22 cells from oxidative stress induced cell death and preserved viability of R28 cells. Intravitreally injected SA-21 at the selected dose in mice demonstrated trend in protecting RGC from ONC induced death and topically dosed SA-24 decreased IOP in OHT mouse eyes. Further dose response, pharmacokinetic studies are in progress.

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Poster

201. Neuroprotection I

Location: SDCC Halls B-H

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Program #/Poster #: 201.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Belfer, ABI Blefer gift, MIT cost object #2664034
ABI - Gamma Research Fund, MIT cost object #3830110

Title: Gamma entrainment using sensory stimuli alleviate cognitive deficits and demyelination induced by chemotherapy agents

Authors: *T. KIM, B. T. JAMES, M. C. KAHN, C. BLANCO-DUQUE, F. ABDURROB, M. ISLAM, N. S. LAVOIE, L.-H. TSAI;
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Abstract: Cancer patients undergoing chemotherapy treatments suffer from a neurological condition called chemotherapy induced cognitive impairment (CICI), or chemo brain, which may last for the rest of their life. Despite the increasing number of chemo brain patients, neither the mechanism nor treatment is well explored. Recent findings indicate that chemo brain shares multiple features with neurodegenerative diseases such as chronic neuroinflammation, DNA damage and synaptic loss. We tested if Gamma Entrainment Using Sensory stimuli (GENUS), which has been shown to be effective against Alzheimer's disease pathophysiology, can be utilized as a tool to treat chemo brain. Here we show GENUS alleviates cisplatin-induced symptoms such as neuroinflammation and neurodegenration. Also, we found that GENUS promotes oligodendrocyte survival during chemotherapy and preventes demyelination, which is tightly associated with cognitive impariment in cisplatin-induced chemo brain. These alterations indeed led to significant improvement of cognitive functions in the mouse model. Furthermore, we show the effect of GENUS is not limited to cisplatin-induced chemo brain, but also applies to methotrexate (MTX)-induced symptomology, demonstrating that GENUS can be a versatile treatment approach for a wide range of chemo brain patients.

Disclosures: **T. Kim:** None. **B.T. James:** None. **M.C. Kahn:** None. **C. Blanco-Duque:** None. **F. Abdurrob:** None. **M. Islam:** None. **N.S. Lavoie:** None. **L. Tsai:** None.

Poster

201. Neuroprotection I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 201.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Thompson Family Foundation Initiative at Columbia University
DOD Grant W81XWH-22-1-0127

Title: Cell culture based platform for high throughput screening of biomolecules with neuroprotective effect against bortezomib-induced peripheral neuropathy (BIPN)

Authors: *I. UTKINA-SOSUNOVA[1], H. LI[3], E. TATISHEV[4], C. KARAN[3], S. PRZEDBORSKI[2];
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Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is a severe adverse sequela of many antineoplastic drugs. Annually, more than 400,000 new cases of CIPN are reported, adding healthcare costs of $2.5 billion/year. Bortezomib (BTZ), a commonly used proteasome inhibitor to treat multiple myeloma and other blood cell-related tumors, causes CIPN in ~50% of patients. Up to now, attempts toward developing neuroprotective therapies against BTZ-induced CIPN (BIPN) yielded little to no success. In this study we developed a reliable neuronal culture based platform for identification of small molecules with neuroprotective activity against BIPN. The *in vitro* model of BIPN made of adult mouse dorsal root ganglia (DRG) sensory neurons amendable for high-throughput screening of biomolecules with neuroprotective effects. We generated PirtCre:tdTomato mice with robust expression of tdTomato under the Pirt promoter in >96% DRG neurons throughout the whole extent of the spinal cord. Cultured neurons enabled us to identify cell bodies and neurites in alive cells. Sensory neurons were plated in 384-well plates and imaged with InCell Analyser. Images were processed with MetaMorph software for axonal degeneration (neurite length) and cytotoxicity (cell body count). BTZ (3nM) applied for 48h provided a consistent and reproducible decrease in 50 ± 7% of neurite length and 10 ± 2% loss of cell bodies. Using this approach, we tested 1,120 biomolecules from Tocris Library in high throughput screening and found 12 molecules with significant protective effect against BIPN. To exclude interference with anti-neoplastic effect of BTZ, hits were tested in a counter-screening assay using three multiple myeloma cell lines. Nine of the 12 molecules did not alter the anti-cancer activity of BTZ and were selected for further testing in human DRG sensory neurons. We anticipate that the completion of this study will identify a set of valuable small molecules for the treatment of BIPN.

Disclosures: **I. Utkina-Sosunova:** None. **H. Li:** None. **E. Tatishev:** None. **C. Karan:** Other; Consultant for Darwin Healthcare, Consultant for Genetika Plus. **S. Przedborski:** Other; Reviewing Editor for eLife, Scientific Board Member of Luciole Parmaceuticals, Inc.

Poster

201. Neuroprotection I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 201.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 1R43NS122666

Title: Exclusive antagonists of extrasynaptic NMDA receptors for treatment of Huntington's disease

Authors: R. GULIA[1], A. GROMOVA[1], T. QUACH[1], B. CHA[1], A. SAVTCHENKO[2], A. R. LA SPADA[1], ***E. MOLOKANOVA**[2];
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Abstract: Huntington's disease (HD) is a progressive inherited neurodegenerative disorder, and there is no disease-modifying treatment that can slow or reverse the progression of HD. NMDA receptors (NMDARs) are a validated drug target for HD. Focusing the drug development efforts on antagonists of NMDARs located outside synapses offers a promising new path to avoid detrimental consequences of inhibition of synaptic NMDARs by generic NMDAR antagonists. With this task in mind, we engineered a novel, rationally-designed NMDAR antagonist (**AuM**) with exclusive selectivity toward extrasynaptic NMDARs by exploiting spatial differences in NMDAR extracellular localization. To evaluate neuroprotective properties of AuM in HD, we turned to a well-established system in our lab: primary cortical neurons from BAC-HD mice. We treated primary cortical neurons from BAC-HD mice or wild-type (WT) littermate control mice with 25 nM AuM for 24 hours, and demonstrated significant neuroprotection of primary cortical neurons treated with AuM. We also performed studies to evaluate neuroprotection from excitotoxicity by treating primary cortical neurons from WT and BAC-HD transgenic mice with quinolinic acid (QA). We found that AuM significantly blunted QA-induced neuron cell death in both WT and BAC-HD neurons. We examined the potential *in vivo* utility of AuM in a pilot study of 11-month-old BAC-HD mice. We delivered 5 µl of AuM or PBS via ICV injection, and after 4 weeks, we did not find any signs of neurological abnormalities or overt systemic side effects. We then assessed motor function by performing rotarod analysis, and noted that AuM-treated BAC-HD mice displayed latency to fall times ~30% longer than latency to fall times for PBS-treated BAC-HD mice, an obvious improvement. After euthanizing BAC-HD mice, we immunostained sections of cortex and striatum with anti-Htt antibody S830. We observed visibly decreased Htt aggregates in the striatum of AuM-treated BAC-HD mice and discovered ~50% reductions in mHtt cytoplasmic aggregates. Next, we administered 5 µl of AuM or PEGylated

gold nanoparticles to 6-week-old N171-HD 82Q mice and subjected these mice to a battery of behavioral tests. We determined that AuM progressively and significantly improved motor skills of treated mice. Subsequent neuropathological studies are underway to quantify the AuM effects in N171-HD 82Q mice. These exciting findings suggest that targeting extrasynaptic NMDA receptors in HD is a valid therapeutic strategy and that AuM is a strong candidate for treating and slowing the course of HD.

Disclosures: **R. Gulia:** None. **A. Gromova:** None. **T. Quach:** None. **A. Savtchenko:** A. Employment/Salary (full or part-time):; NeurANO Bioscience. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NeurANO Bioscience. **A.R. La Spada:** None. **E. Molokanova:** A. Employment/Salary (full or part-time):; NeurANO Bioscience. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NeurANO Bioscience.

Poster

201. Neuroprotection I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 201.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Ministerio de Educación Nacional, Ministerio de Industria, Comercio y Turismo, ICETEX and World Bank (792-2017 2a Convocatoria Ecosistema Científico—Colombia Científica para la financiación de proyectos de I+D+i).
O.F.S. received a postdoctoral fellowship from Departamento Administrativo de Ciencia, Tecnología e Innovación-COLCIENCIAS (#811-2018) and Pontificia Universidad Javeriana (ID 8867).

Title: Passiflora edulis f. edulis sims extract induce neuroprotection and neuronal plasticity and synergize anticancer chemotherapeutics in vitro

Authors: O. F. SÁNCHEZ, J. M. VELASCO-ESPAÑA, A. V. RODRIGUEZ, L. Y. ROJAS-FONSECA, J. J. SUTACHAN, G. M. COSTA, ***S. L. ALBARRACIN**;
Pontificia Univ. Javeriana, Bogota. D.C., Colombia

Abstract: Cancer-induced cognitive impairment and chemotherapeutic brain damage due to blood-brain barrier deterioration are recognized effects that cancer survivors face. Although many molecules and drugs are used to ameliorate these detrimental effects, there is interest in new alternatives with a natural origin to be summed to this list. Given the enormous plethora of biological activities present in plant extracts from the specie *Passiflora edulis*, such as anti-inflammatory, neuroprotective, antidepressant- and anti-anxiolytic-like effects associated with

diverse components like flavonoids and phenols present in the plant, we aim to characterize *in vitro* the neuroprotective, neuroplastic, and antiproliferative properties of crude aqueous and hydroethanolic extracts of *P. edulis* f. *edulis sims,* a less investigated variety of *P. edulis*. Aqueous and hydroethanolic crude extracts of *P. edulis* f. *edulis sims* showed at specific concentrations in the range from 0.01 and 1 μg mL^{-1} diverse properties such as neuroprotective effect against doxorubicin (Dox) and an increase in the dendritic outgrowth and complexity in rat cortical neurons. *P. edulis* aqueous extract showed to inhibit the activation of AKT in rat cortical neurons and to reduce the increased levels of p-AKT and p-ERK(1/2) derived from the Dox-treatment. Furthermore, the aqueous crude extract shows to synergize the cytotoxic effect of Dox or CTX cytotoxicity on human glioblastoma and neuroblastoma in an administration-dependent form. Being particularly increased the cytotoxic effect of the chemotherapeutic drug in co-administrated and post-chemotherapy drug schemes. In summary, our results suggest that *P. edulis* f. *edulis sims* extracts are a natural alternative able to reduce neuronal damage induced by chemotherapy drugs and can be alternatively used as an antiproliferative co-adjuvant of Dox and CTX for brain cancer treatment. Future assessment *in vivo* would indeed increase our comprehension of these properties

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Poster

201. Neuroprotection I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 201.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: James and Esther King Florida Biomedical Research Program 9JK10
NCCIH 1R21AT009734

Title: The use of M1 muscarinic receptor stimulation to attenuate chemotherapy-related cognitive deficits and attenuate tumor growth

Authors: R. BOTELHO[1], B. E. JOHNS[2], *R. PHILPOT[2];
[1]Psychiatry and Behavioral Neurosciences, Univ. of South Florida, Temple Terrace, FL; [2]Univ. of South Florida, Tampa, FL

Abstract: Most individuals who report cognitive deficits during or following cancer chemotherapy are women. Many chemotherapeutic agents suppress ovarian function, decreasing circulating estrogens. Because estradiol regulates high affinity choline uptake (HACU) and HACU is the rate-limiting step for acetylcholine (ACh) synthesis, chemotherapeutic agents can indirectly impair cholinergic mediated cognitive processes. We have demonstrated that cyclophosphamide (CYP) and doxorubicin (DOX), a commonly used chemotherapeutic combination, produces deficits in spatial memory and impairment of HACU in the striatum and

hippocampus of female mice. Although activation of nicotinic ACh receptors has been linked cancer cell proliferation and reduced antiproliferative effects of several chemotherapeutic agents, activation of muscarinic ACh receptors (mAChRs) has been shown to inhibit tumor cell proliferation and enhance the effects of chemotherapeutic agents. Thus, activation of muscarinic receptors may be a viable therapeutic approach to bypass deficits in ACh synthesis and treat CRCDs.The present study used non tumor-bearing (M-) and breast tumor-bearing MMTV-PyVT (M+) female mice to determine if the daily administration of the M1 muscarinic receptor agonist xanomeline (0.0, 0.3, 1.0 or 1.8 mg/kg; s.c.) or the M1 positive allosteric modulator VU-357017 (0.0, 1.0, 3.0 or 10 mg/kg; s.c) during chemotherapy could: 1) attenuate elevations of circulating cytokines induced by the presence of tumors and/or 4 weekly injections of CYP (66.7mg/kg i.v.; ≈200mg/m2/wk) and DOX (6.7mg/kg i.v.; ≈20mg/m2/wk); 2) prevent the manifestation of spatial memory deficits resulting from repeated exposure to CYP+DOX; and 3) suppress tumor growth and enhance the antineoplastic and antiproliferative effects of CYP+DOX treatment in female M+ mice. Results indicate CYP+DOX administration impaired spatial memory and resulted in persistent increases in circulating concentrations of macrophage-inflammatory protein 2 [MIP-2 (CXCL2)], which damages white matter and impairs cognitive function. Daily treatment with 1.0mg/kg and 1.8mg/kg xanomeline, or 1.0mg/kg VU-0357017, during the 4 weeks of CYP+DOX chemotherapy prevented the emergence of impaired spatial memory. Importantly, neither xanomeline nor VU-357017 interfered with the antitumorigenic and antiproliferative effects of CYP+DOX treatment and both agents attenuated tumor growth when administered alone suggesting that these agents may be effective adjuvants for the prevention of CRCDs during CYP+DOX chemotherapy.

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Poster

201. Neuroprotection I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 201.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DFG Grant 398214842
DFG Grant 499371712

Title: The human cytokine receptor CRLF3 is a neuroprotective EV-3 (erythropoietin) receptor

Authors: *D. Y. KNORR[1], I. RODRIGUEZ POLO[2,3], H. S. PIES[1], N. SCHWEDHELM-DOMEYER[1], S. PAULS[1], R. BEHR[3], R. HEINRICH[1];
[1]Cell. Neurobio., [2]Dept. of Developmental Biol., Univ. of Goettingen, Göttingen, Germany; [3]Res. Platform Degenerative Dis., German Primate Ctr., Göttingen, Germany

Abstract: The vertebrate-specific cytokine erythropoietin (Epo) is a major regulator of erythropoiesis and a potent cytoprotectant in various tissues including the nervous system. While

erythropoiesis is stimulated via the classical receptor EpoR, neuroprotection involves both EpoR and additional alternative Epo receptors. Certain ligands, including the natural Epo splice variant EV-3, cannot stimulate classical EpoR (and erythropoiesis) but mediate neuroprotection via unknown receptors. The cytokine receptor-like factor 3 (CRLF3) is a cytokine receptor that shares sequence similarity with EpoR and is present in all major taxa ranging from cnidarians to humans. Our previous studies demonstrated that insect CRLF3 initiates anti-apoptotic neuroprotective mechanisms upon stimulation with human Epo and EV-3. Human CRLF3 is expressed in various tissues and has been associated with proliferation, differentiation, cell survival and some diseases, though its concrete function remained elusive. We studied whether CRLF3 might represent an alternative Epo-receptor that stimulates neuroprotection in the human nervous system. We generated *CRLF3* knock out (KO) human induced pluripotent cell (iPSC) lines along with isogenic control lines (Ig-Ctrl), differentiated them into neurons, induced apoptosis by addition of rotenone and quantified cell survival via FACS measurements. CRLF3 was stimulated by EV-3 to exclude coactivation of EpoR. All experiments (5 independent experiments each) compared untreated, stressed and EV-3 treated cells in cultures of wild type, Ig-Ctrl and *CRLF3*-KO cells. To characterize the means by which EV-3 might protect human iPSC-derived neurons from apoptosis, we performed qPCR to quantify the expression of pro- and anti-apoptotic genes (5 independent experiments for each iPSC line) and calculated statistical relevance by Fishers pairwise permutation test. Validity of results was ensured by conducting all experiments with two individual hiPSC lines. We demonstrate that EV-3 protects WT and Ig-Ctrl iPSC-derived neurons from rotenone-induced apoptosis. In contrast, *CRLF3*-KO neurons are not protected, indicating that CRLF3 serves as neuroprotective receptor for EV-3 in human neurons. Moreover, EV-3/CRLF3 signalling regulates expression of pro- and anti-apoptotic genes to favor cell survival.
Altogether, we identify human CRLF3 as a neuroprotective receptor that can be stimulated by EV-3 independently of EpoR coactivation. Hence, CRLF3 can be selectively targeted by Epo-like ligands to counteract neurodegenerative diseases without co-promoting inappropriate erythropoiesis and tumor growth.

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Poster

201. Neuroprotection I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 201.12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Dr. Miriam and Sheldon G. Adelson Medical Research Foundation
NIH Intellectual and Developmental Disabilities Research Centers Imaging Core (HD018655)

Title: CaMKII promotes retinal ganglion cell survival but suppresses axon regeneration under pro-regenerative treatment

Authors: *C. SHI[1], L. XIE[2], C. STARR[3], B. CHEN[3], L. I. BENOWITZ[4];
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Abstract: Injury to the optic nerve often results in an irreversible loss of vision due to the inability of retinal ganglion cell (RGC) axons to regenerate. Critically, the degeneration of RGCs following axonal injury limits the regenerative capacity of the optic nerve even under pro-regenerative treatments. Prior research has demonstrated a neuroprotective role for Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), where overexpression of a constitutively active mutant of CaMKII in the mouse retina strongly improves the survival of RGCs following several types of insult (Guo et al., 2021, *Cell*). Here, we further explore the role of CaMKII in axonal regeneration following optic nerve injury. To achieve this, we performed optic nerve crush surgeries following AAV2-mediated gene transfer of the CaMKII mutant into mouse retinas, in combination with intravitreal injection of pro-regenerative treatments previously characterized by our lab. We report that CaMKII improves RGC survival following optic nerve injury when combined with zymosan or a combinatorial treatment of oncomodulin (Ocm), stromal cell-derived factor 1 (SDF1), and the non-hydrolyzable cAMP analog CPT-cAMP. However, CaMKII overexpression greatly diminishes the regenerative effect of zymosan or Ocm/SDF1/CPT-cAMP treatments after optic nerve injury. These results extend the current understanding of the neuroprotective effects of CaMKII while revealing a novel suppressive effect on regeneration. Further research will address the effect of CaMKII on other pro-regenerative treatments (e.g. *pten* deletion) as well as the mechanisms by which CaMKII-mediated regeneration suppression takes place, providing the basis for further studies on methods to simultaneously support high levels of RGC survival and axon regeneration.

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Poster

201. Neuroprotection I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 201.13

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Effect of resveratrol on the redox system during Cerebellar aging in Wistar rats

Authors: *D. JUÁREZ SERRANO[1], I. CESAR ARTEAGA[1], S. TREVINO MORA[1], J. MORALES MEDINA[2], A. D. DIAZ FONSECA[1];

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Abstract: Aging is a process of progressive deterioration and increases the prevalence of chronic-degenerative diseases. Several theories have been proposed to describe it, including the oxidative stress theory of ageing. The cerebellum is one of the regions most affected during aging as it is responsible for various motor and cognitive functions. At present, many studies describe resveratrol as an antiaging component. The aims of this work are to study the effect of resveratrol on behavior and the redox system during cerebellar aging in Wistar rats. Male Wistar rats (n=216, 3-month-old) were used, randomly divided into 3 groups: control, vehicle (ethanol 7.5%) and resveratrol (10mg/kg of weight) with oral administration. The administration periods were for 2, 4, 6, 8, 10, 12, 14, 16 and 18 months. The novel object recognition test and the balance beam test were performed and the levels of lipoperoxidation (malondialdehyde and 4-hydroxinonenal), nitrites, oxidized and reduced glutathione. In addition, the enzymatic activity of superoxide dismutase (SOD), catalase (CAT) and the glutathione system [glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione S-transferase (GST)] were quantified. Prolonged administration with resveratrol confirmed its antioxidant and antiaging effect since that the animals-maintained locomotor activity and short- and long-term memory even at advanced ages. Moreover, resveratrol reduced the levels of nitrites and lipoperoxidation products. It maintained the activity and efficiency of the enzymes of CAT, SOD, GPx, GR, GST, even at advanced ages and attenuates the neuronal disorganization of the cerebellum. These effects become evident when compared to the control groups. Chronic administration of resveratrol reduces behavioral changes and oxidative damage produced in the cerebellum of Wistar rats during the aging process. Therefore, this molecule is suggested as an important pharmacotherapeutic option for prevention and/or regeneration to delay cerebellar deterioration during aging and thus increase the quality of life of human beings.

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Poster

201. Neuroprotection I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 201.14

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF-2022R1A2C1012351
NRF-2019R1F1A1063005

Title: Attenuation of hydrogen peroxide-induced oxidative cell death by corticosterone

Authors: C. LEE, *J.-H. JANG;
Sch. of Medicine, Keimyung Univ., Daegu, Korea, Republic of

Abstract: Stress breaks body balance, which can cause diverse physiological disorders and worsen preexisting diseases. However, recent studies have reported that controllable stress and overcoming from stress reinforce resilience to resist against more intense stress afterwards. Therefore, in this study we have investigated the protective effect of corticosterone (CORT), a representative stress hormone against hydrogen peroxide (H_2O_2)-induced neuronal cell death and its underlying molecular mechanism. Particularly, we have focused on the role of REST (repressor element 1-silencing transcription factor) and/or neuron-restrictive silencer factor (NRSF). REST is a transcription factor induced in the aging brain and regulates a network of genes mediating stress resistance. H_2O_2-decreased cell viability was effectively restored by the pretreatment of CORT (30 nM for 72 hr) in SH-SY5Y human neuroblastoma cells. Under this experimental condition CORT induced REST expression and its translocation to the nucleus. H_2O_2-increased expression of apoptotic markers such as PUMA and Bim was decreased by CORT pretreatment. Furthermore, pretreatment of SH-SY5Y cells with CORT attenuated H_2O_2-mediated oxidative damages by upregulation of antioxidant enzymes via activation of NF-E2-related factor 2 (Nrf2). These finding suggest that CORT with eustressed condition increases REST expression and enhances intracellular self-defense against distress (H_2O_2)-mediated oxidative cell death suggesting a role of REST as one of the key molecules for resilience and neuronal cell survival.

Disclosures: C. Lee: None. **J. Jang:** None.

Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

Location: SDCC Halls B-H

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Program #/Poster #: 202.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

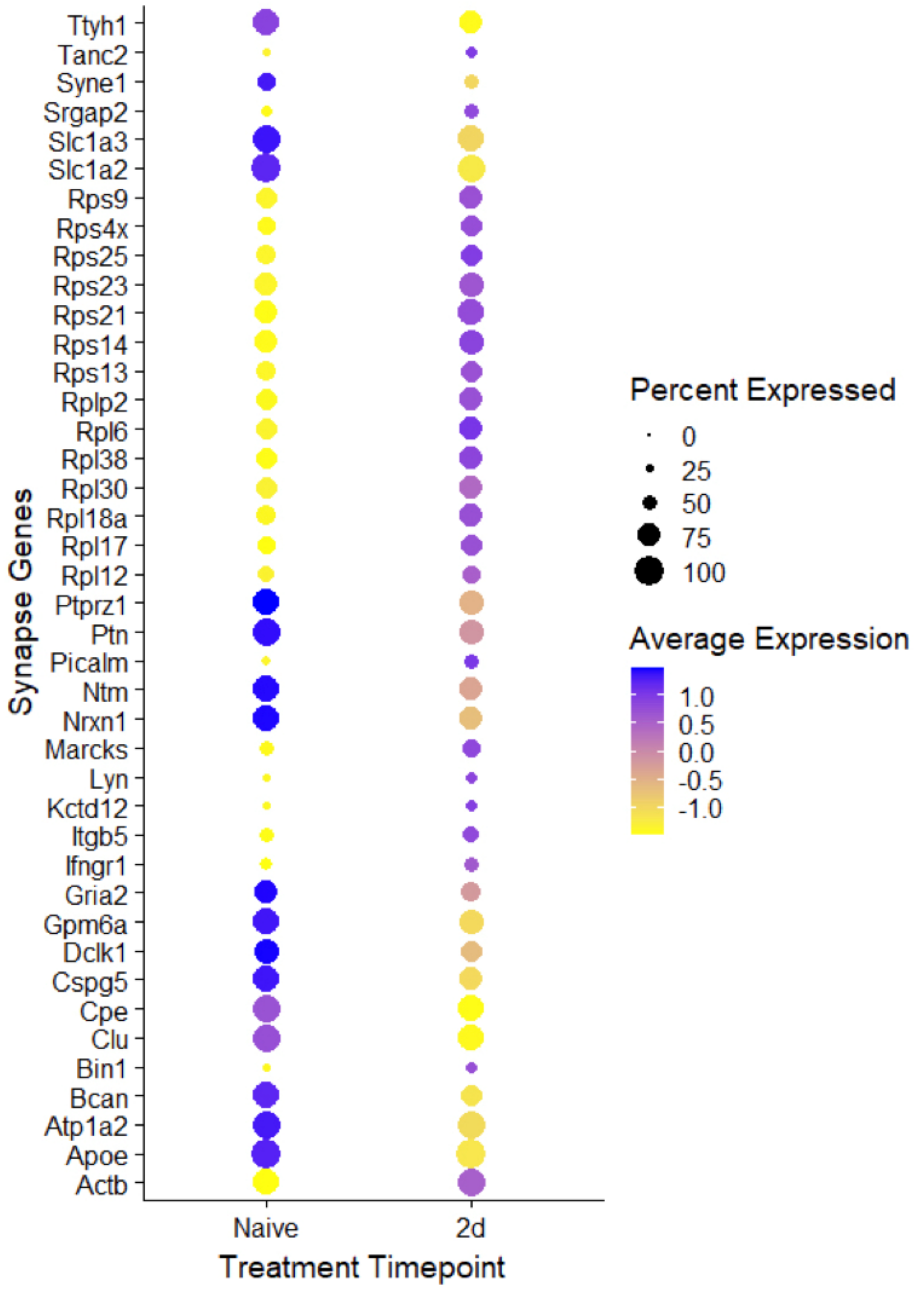
Support: NIH RO1AG038961
NIH RO1EB029338
NSF GRFP

Title: Single-cell RNA sequencing of hippocampal astrocytes following focused ultrasound-mediated blood-brain barrier opening reveals a potential role in modulating synaptic function

Authors: *R. NOEL, A. R. KLINE-SCHODER, A. J. BATTS, E. KONOFAGOU;
Biomed. Engin., Columbia Univ., New York, NY

Abstract: Focused Ultrasound (FUS) paired with systemically-introduced microbubbles is capable of opening the Blood-Brain Barrier (BBBO) in a reversible and targeted manner. This transient BBBO offers a technique for enhancing drug delivery to the brain, and activating supportive neural immune cells such as astrocytes and microglia. FUS has been shown to decrease Alzheimer's Disease (AD) pathology in addition to improving memory and behavioral deficits in animal models of AD. The mechanism by which these and other cognitive

improvements may result from FUS-BBBO is still under investigation, although recent studies implicate the activation of resident glial cells. Here we use single-cell RNA sequencing to characterize the response of hippocampal astrocytes two days after FUS-BBB opening in wild-type (WT) mice.16 WT mice were used for the present study. Eight mice were reserved as naïve, untreated controls, and the remaining mice received FUS-BBB treatment bilaterally targeting the hippocampus. The FUS-treated mice were sacrificed by cardiac perfusion two days following FUS-BBBO with sterile PBS. The hippocampi were dissected out, homogenized and processed with mechanical and enzymatic tissue digestion. These cells were then stained with a PE-conjugated ACSA-II antibody and a Live/Dead stain. Live, ACSA-II positive astrocytes were flow sorted and submitted for single-cell RNA sequencing. Sequencing analysis was performed using Seurat and TopGO gene ontology toolkits for R,Following quality filtering, 175 differentially expressed genes (DEG) ($P < 0.05$) were found in FUS-treated astrocytes compared to naïve. Gene ontology analysis revealed significant annotation to synaptic mechanisms in FUS-treated mice ($p = 1.64 * 10^{-11}$, Fisher Test). In particular, genes annotated to synaptic interactions, compared to naïve mice are shown in Figure 1.Remodeling of the neuronal synapses following activation by FUS-BBBO may endow astrocytes with an important regulatory role in maintaining the health of the neurovascular unit, and by extension neuronal circuits and the CNS at large.



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Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 202.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant F31

Title: Comparative proteomics of LPS-induced inflammation in different brain cell types captured by TurboID proximity labeling

Authors: *S. CUNNINGHAM, H. ZENG, P. KUMAR, P. BAGCHI, N. T. SEYFRIED, S. RANGARAJU;
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Abstract: Chronic neuroinflammation is central to the etiology of neurodegenerative disease, but there is currently a critical gap in our understanding of how inflammatory challenges impact distinct cellular proteomes. Proximity biotin ligase TurboID, coupled with Lipopolysaccharide (LPS) challenge and mass spectrometry, can purify distinct cellular proteomes from adult mice undergoing inflammatory challenges. To test the hypothesis first *in vitro*, we generated BV2 and N2A cell lines stably expressing TurboID containing a nuclear export sequence. We treated cells with 1μg/mL LPS and 200μM biotin for 48 hours and generated whole cell lysates (inputs) and streptavidin affinity-purification (AP) fractions (n=4/group) for label free quantitative MS (LFQ-MS). *In vitro*, TurboID biotinylated 60-65% of the entire proteome identified by LFQ-MS, with 1,754 proteins significantly enriched in the BV2 AP proteome and 2,011 proteins in the N2A AP proteome. Principal component analysis of the AP proteomes revealed that effect of LPS was robust on BV2 AP proteomes but minimal on N2A AP proteomes. Differentially expressed proteins by LPS treatment (LPS DEPs) were identified in both BV2 AP (>500 proteins) and N2A AP (>100 proteins) samples. K-means clustering of BV2 AP proteomes revealed 5 clusters of proteins, with cluster 1 (C1) representing LPS-driven proteomic changes shared between AP and input proteomes, and cluster 3 (C3) representing down-regulated changes in response to LPS. Gene set enrichment analysis showed C1 proteins correlating with peroxisome, phagosome, cytokine secretion and amoebiasis, and C3 proteins associated with terms involving cellular homeostasis. Protein interaction network analysis of N2A LPS DEPs identified clusters of proteins involved with proteasomal machinery identified by local network clustering. Next to investigate the effects of systemic LPS on neuronal proteomes, we have successfully directed TurboID to CamKIIa and PV interneurons in adult mice undergoing LPS challenge (I.P. inj. x4 days) and pharmacological ablation of microglia with PLX3397 chow (290 ppm, 5 wks.). Our quantitative imaging and immunoblotting results validate the efficacy of microglial ablation in WT mice (n=10, ~50% by Iba1 count), and confirm robust biotinylation of proteins in CamKIIa-Cre/TurboID$^{fl/-}$ mice. Our *in vitro* studies confirm that TurboID biotinylation of proteins can capture unique biological responses of neurons and microglia to inflammatory challenge. Our ongoing *in vivo* studies will identify differential effects of neuroinflammation on two classes of neurons, and determine the contribution of microglia to these changes.

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Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 202.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: KDDF Grant HN22C0307
NRF Grant 2021R1I1A1A01047750
NRF Grant 2020R1F1A1074104

Title: Downregulation of Mammalian Target of Rapamycin (mTOR) Signaling Pathway by Injecting rAAV-shmTOR to Alleviate Pain Hypersensitivity in a Rat Spared Nerve Injury Model

Authors: *M. PARK[1,2], H.-N. WOO[3], C. KOH[1], H. CHANG[1], J. KIM[3], K. PARK[4], J. CHANG[1,2], H. LEE[3], H. JUNG[1];
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Abstract: The activation of mammalian target of rapamycin (mTOR), a serine/threonine protein kinase, has been known as a one of the contributing factors in nociceptive sensitization after peripheral injury. Notably, its activation followed by the phosphorylation of downstream effectors causes the hyperexcitability of primary sensory neurons located in dorsal root ganglion (DRG). We investigated whether the inhibition of mTOR activation by directly injecting rAAV-shmTOR at the sciatic nerve of spared nerve injury (SNI) neuropathic pain model may decrease neuronal hyperexcitability in the DRG, along with downregulation of downstream effectors. Adult male Sprague-Dawley rats (200g-220g) were used. Rats were assigned into 4 groups: shmTOR (n=29), shCON (n=23), Sham (n=9), Normal (n=8). On post-operative 16th day (POD), shmTOR and shCON group were injected with rAAV-shmTOR and rAAV-shCON, respectively. Behavioral responsiveness was measured using von Frey test before modeling SNI, on POD14, PID3, 7, 14, 21, and 28 (PID: post-injection day). Rats were sacrificed on PID 21, and both spinal cord and DRG tissues were harvested for western blot analyses.The differences in mechanical withdrawal threshold were remarkable between shmTOR and shCON group. shmTOR group showed gradually increased threshold compared to shCON group (PID 21, ****$p<0.0001$). In consistent with the behavioral data, western blot analysis showed that mTOR complex 1 (mTOR p-2448; shmTOR vs. shCON, *$p<0.05$) and complex 2 (mTOR p-2481; shmTOR vs. sham, *$p<0.05$) were both downregulated in DRG compared to shCON and sham group on PID 21. For its downstream effectors, p-4EBP1 and p-PKCα were also highly downregulated compared to shCON group (p-4EBP1; shmTOR vs. shCON, ****$p<0.0001$, p-PKCα; shmTOR vs. shCON, ***$p<0.001$).This research has a significance in that we observed AAV treatment effects on a long-term basis and consequently showed downregulation of both mTORC1 and mTORC2 as well as behavioral hypersensitivity. This study may provide a new insight into understanding the underlying pathological mechanism of mTOR in neuropathic pain and bring us one step closer to establish clinical strategy for gene therapy in neuropathic pain.

Disclosures: M. Park: None. **H. Woo:** None. **C. Koh:** None. **H. Chang:** None. **J. Kim:** None. **K. Park:** None. **J. Chang:** None. **H. Lee:** None. **H. Jung:** None.

Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 202.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Interleukin-6 permeates and alters choroid plexus organoids derived from neuropsychiatric lupus mice

Authors: *J. A. REYNOLDS[1], L. TORZ[2], N. PETERSEN[2], C. PUTTERMAN[1];
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Abstract: Cognitive and emotional symptoms affect up to 50% of patients with systemic lupus erythematosus (SLE); these neurological sequelae (NPSLE) occur via an unknown mechanism. Lymphocyte infiltration of the choroid plexus (CP)—epithelia producing cerebrospinal fluid (CSF)—and CSF elevations of inflammatory molecules—primarily interleukin-6 (IL-6)—in NPSLE patients led us to study increased CP permeability as a possible mechanism for access of systemic inflammatory mediators into the brain. Using mouse-explant CP organoids, we aimed to not only uncover the pathogenesis of NPSLE but also to assess possible leakage of systemic neurotoxins via the CP as a trigger for other neuroinflammatory conditions. Our lab is the first to apply a CP organoid model (Petersen et al., 2020) to lupus mice and permeability studies. Before our experiments, we confirmed expected morphology, gene expression, and physiology of CP organoids derived from 16-week-old, female lupus mice (Figure 1A-D). Mice of this age and sex exhibit significant disease. We used whole-mount immunostaining to quantify organoid permeability to IL-6. Secondary-only and IL-6 knockout organoid controls ensured we stained only exogenous IL-6. Two trials (n = 3 - 5) showed IL-6 in the vacuoles (Figure 1E). IL-6 can alter epithelial functions and is produced by the CP infiltrate, so we hypothesized that IL-6 increases CP permeability. Using two cohorts with technical replicates, we incubated non-lupus organoids with a fluorescent tracer then treated them with the positive control egtazic acid (EGTA; disrupt tight junctions; n = 20), or IL-6 (n = 40), or the negative control PBS (n = 40). Serial confocal images were taken, and permeability to tracer was calculated (Figure 1F). By 15 minutes, IL-6 differed from PBS ($p = 0.014$) and resembled EGTA ($p = 0.964$). Our results reinforce using this novel organoid model to study the CP in NPSLE and other inflammatory conditions. Additionally, IL-6 likely crosses the CP in murine lupus and appears to promote CP leakiness. Our findings begin to uncover the role of CP permeability in NPSLE pathogenesis.

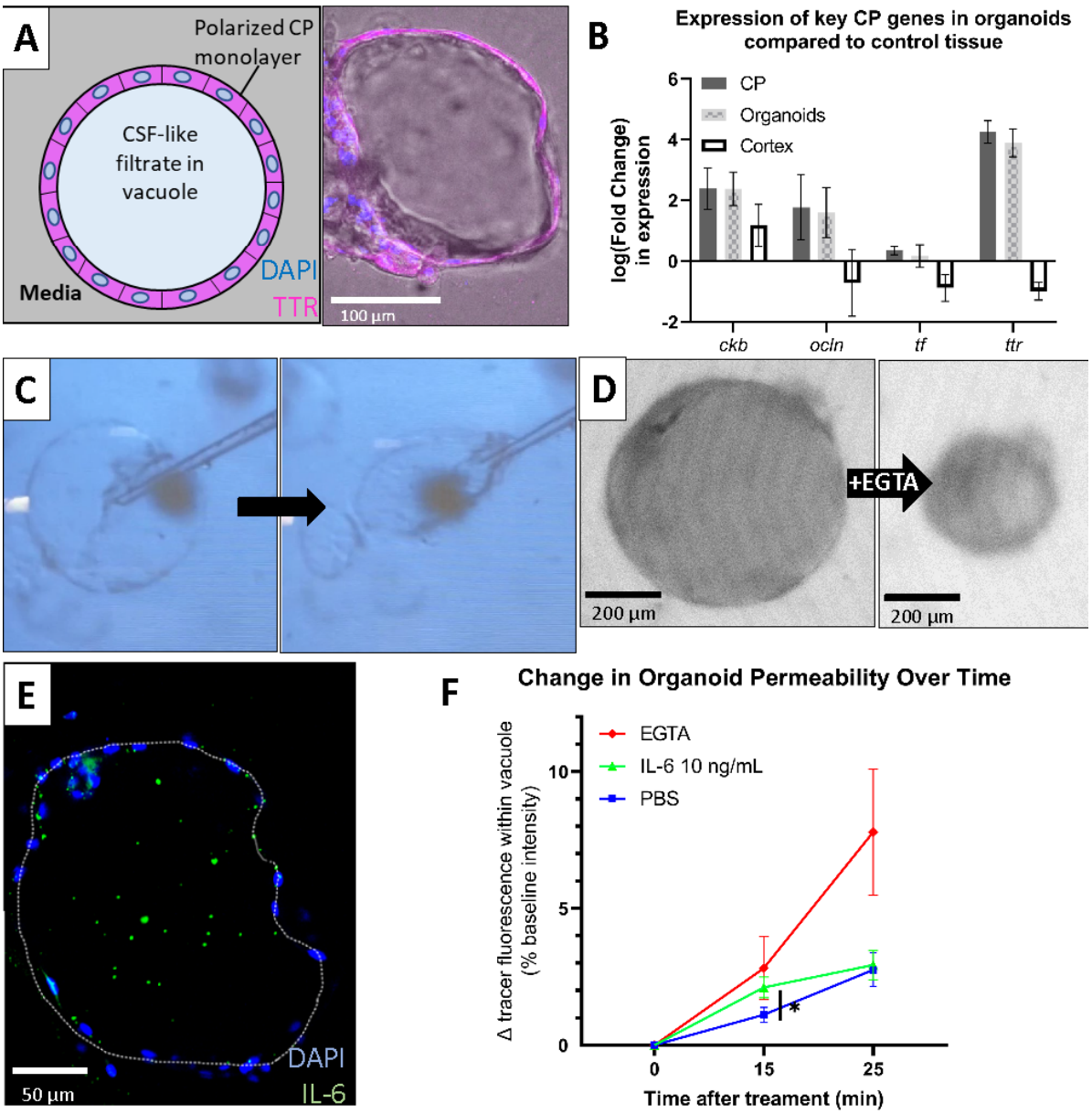

Figure 1. Validation of choroid plexus organoid physiology and testing inflammatory response. Spherical organoids derived from explanted choroid plexus (CP) tissue from adult mice. A) Diagram and immunofluorescent (IF) image of representative organoid expressing the classic CP marker transthyretin (TTR). B) q-PCR quantification of CP gene (*ckb* = creatine kinase b, *ocln* = occludin, *tf* = transferrin, *ttr* = *transthyretin)* expression in organoids compared to controls (positive = CP, negative = Cortex). C) Micro-aspiration of central fluid collapses organoid. D) Calcium chelation using egtazic acid (EGTA) collapses organoid in 40 minutes. E) Confocal IF microscopy shows exogenous interleukin-6 (IL-6) enters the central vacuole of organoid derived from lupus mice. F) Impact of treatments (n = 20-40 per group) on organoid permeability to a fluorescent paracellular tracer. * = $p < 0.05$

Disclosures: **J.A. Reynolds:** None. **L. Torz:** None. **N. Petersen:** None. **C. Putterman:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Equillium. F. Consulting Fees (e.g., advisory boards); Equillium, Kidneycure, Progentec.

Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 202.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant NS122242
NIH grant NS105981

Title: A neuroinflammatory assessment of TDP-43 proteinopathy in amyotrophic lateral sclerosis

Authors: ***B. EVANGELISTA**[1], R. B. MEEKER[2], N. STANLEY[3], X. LI[4], T. J. COHEN[5]; [1]Univ. of North Carolina Chapel Hill, Chapel Hill, NC; [2]Neurol., [3]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [4]Neurol., Duke Univ., Durham, NC; [5]Neurol., Univ. of North Carolina - Chapel Hill, Chapel Hill, NC

Abstract: Sporadic Amyotrophic Lateral Sclerosis (sALS) is the most common and fatal motor-neuron disease world-wide, affecting 3 in 100,000 individuals 50 years or older. Approximately 97% of sALS cases are pathologically characterized by the presence of hyper-phosphorylated, insoluble aggregates of the master RNA regulator protein, Transactive Response DNA Binding Protein (TDP-43). However, the exact molecular mechanism by which aggregated TDP-43 imparts neurotoxicity has not yet been determined. Recent evidence implicates innate and adaptive immune cells as a source of maladaptive neuroinflammation when stimulated by circulating protein aggregates. This neuroinflammatory response is thought to compromise neuron integrity and drive neurodegeneration. In this work, we assessed the ability of cultured macrophages to internalize and react to TDP-43 aggregates generated in-house using a novel immuno-purification technique. We observed macrophage reactivity and T-cell activation following TDP-43 aggregate treatment *in vitro*. Finally, we generated a multiplex immunohistochemical platform to perform predictive modeling of disease-modifying immune networks surrounding TDP-43 pathology in human sALS patient spinal cord. Presently, our work serves to investigate the mechanistic link between TDP-43 aggregation and sALS pathogenesis through a neuro-immunological lens. At large, this work serves to unveil previously unexplored immuno-diagnostic and -therapeutic avenues for thousands of patients world-wide.

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Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

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Program #/Poster #: 202.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NARSAD Grant #26730
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Fondation Vaincre Alzheimer FR-18008
Fondation France Alzheimer #1624
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Title: Cytokine-induced DNA breaks, a new player in chronic inflammation-induced behavioral impairment?

Authors: B. SCHMITT[1], M. BELLOY[1], C. PAUT[1], F. MARTY[1], R. ECALARD[2], R. BOURSEREAU[1], N. BLANCHARD[1], ***E. SUBERBIELLE**[1];
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Abstract: Neuroinflammation accompanies many neuropsychiatric and neurodegenerative diseases. Proinflammatory cytokines, such as interleukin-1 beta (IL-1b), are detected at elevated levels in the blood and the brain in these chronic diseases, yet the contribution of this cytokine to the pathological outcome remains largely unstudied. In acute inflammation, increased IL-1b levels disrupt behavior and cognition notably by impairing glutamate balance in the brain. However, the mechanisms by which the cytokine may specifically and durably cause behavioral abnormalities in chronic inflammation are unknown. Since epigenetic processes are critical player in neuronal plasticity and function, we hypothesized that durable effects of IL-1b in the brain may rely on epigenetic mechanisms. DNA double-strand break (DSB) response has emerged as major processes in the control of epigenetic mechanisms underlying cognition. The balance between DSB production and repair is tightly regulated in neurons in response to neuronal activity and DSB may regulate activity-dependent gene expression. Here, we explored whether chronic exposure to IL-1b caused behavioral deficits by altering the DNA DSB response in neurons. Using chronic exposure of adult mice to IL-1b, thanks to subcutaneously implanted osmotic minipumps, we investigated the impact of chronic IL-1b on innate and cognitive behaviors, on the distribution of brain cells populations, on DNA DSB response markers, and on IL-1b related signaling in neuronal cells. We showed that chronic exposure to IL-1b impaired consolidation of spatial memory, without any overt changes in glial populations nor neurogenesis. Despite a peripheral infusion of the cytokine, by disrupting IL-1b signaling through its receptor in neurons, we showed that chronic IL-1b-induced cognitive impairment were due to a direct effect of the cytokine on the neuron. Finally we found that chronic exposure to IL-1b increases DSB levels in neurons and that DSB response signaling in neurons was critical to IL-1b-induced behavioral deficits. Our results shed light on novel pathological

mechanisms in inflammation control of gene expression in neurons that could apply to a wide array of neurological diseases.

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Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

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Program #/Poster #: 202.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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NIH Grant MH072567
NIH Grant HL126559
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NIH Grant DA050528

Title: Antiviral immune responses, alterations of mitochondria dynamics, and neurological deficits are associated with the levels of occludin expression

Authors: *S. TORICES, N. FATTAKHOV, K. FRYDLOVA, T. TEGLAS, O. NARANJO, M. TOBOREK;
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Abstract: Occludin (ocln) is a tetraspan redox-sensitive protein associated with tight junctions of the blood brain barrier (BBB). It plays a key role in maintaining the integrity of the BBB and it has been described as a multifunctional protein. By high-throughput RNA sequencing, we identified changes in gene expression-related to ocln modifications in human brain pericytes, one of the main regulatory cells of the BBB integrity. After ocln silencing, we found an alteration in several genes of the antiviral retinoic-acid-inducible gene-1 (RIG-1) signal pathway and the immune system response pathway when compared with non-treated cells. Several studies have demonstrated the capacity of ocln to control HIV-1 infection of human pericytes. Here, we show an ocln antiviral role in HIV infection *in vivo* and *In vitro*. Mechanistically, we provide evidence that cellular ocln level can modulate HIV-1 infection by controlling the expression levels of several INF-stimulated genes such as ISG15, MX2, or IFIT1 through JAK/STAT signaling by influencing interferon regulatory factors (IRF) expression levels and STAT-1 activation. In addition, ocln can regulate mitochondrial dynamics and autophagy, potentially by its influence on the RIG-1 signaling pathway, which functions as a regulator of the cytoplasmic sensors of mitochondrial antiviral signaling protein (MAVS). Modulation of ocln expression levels can affect mitochondrial respiration and mitochondrial fission and fusion balance. Regulation of HIV

infection by ocln level was confirmed in a mouse model. Unexpectedly, we observed for the first time that ocln deficient mice present neurological deficits when compared to heterozygous or wild type mice. Overall, these results are important to a better understanding of the molecular mechanisms for viral infection in the brain and describe previously unrecognized role of the protein ocln as a key factor in the control of innate immune response.

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Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 202.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Neuroinflammation caused by peripheral Mycobacterium tuberculosis infection

Authors: *A. LATHAM*[1], C. GEER[2], I. ANDERSON[2], D. F. ACKART[3], A. D. HINES[1], B. PODELL[3], J. ELF[1], R. J. BASARABA[3], J. A. MORENO[1];
[1]Envrn. Hlth. and Radiological Sci., [2]Envrn. Hlth. and Radiological Sciences; Microbiology, Immunology, and Pathology, [3]Microbiology, Immunology, and Pathology, Colorado State Univ., Fort Collins, CO

Abstract: Tuberculosis (TB), a disease caused by the bacterium Mycobacterium tuberculosis (Mtb), is the second leading cause of death by an infectious disease and the World Health Organization (WHO) estimates that almost a third of the world's population has latent TB. Epidemiological studies demonstrate that pulmonary tuberculosis predisposes individuals to catastrophic neurodegenerative diseases, including Parkinson's Disease (PD). Additionally, co-infection with human immunodeficiency virus (HIV) and TB exacerbates cognitive deficits compared to HIV infection alone. Through studies examining the neurological effects of low-dose Mtb exposure by aerosol, we identify pathological markers of inflammation in the central nervous system (CNS) of female, Dunkin Hartley guinea pigs. This includes migration and proliferation of microglia followed by activation of astrocytes in multiple brain regions. Furthermore, other activated immune cells, such as mast cells, are seen in the brain tissue of animals with peripheral Mtb infection. Interestingly, these pathologies are present despite the absence of detectible bacteria in the brains of infected animals. These results are not only found in guinea pigs, a well-established model of pathological TB progression, but are also evident in Mtb infected CD-1 mice and non-human primates. Through this data, we aim to fully characterize the inflammation and subsequent neurotoxicity that accompanies systemic infection with Mtb. These studies will allow us to obtain a better understanding of the disease progression in the hopes of alleviating permanent cognitive impairments in human patients.

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Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

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Program #/Poster #: 202.09

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH grant R21NS081182
NIH grant R01NS097903
NIH grant R37NS033123
NIH grant U01NS103883
NIH grant R35NS127253

Title: Normalization of TDP-43 pathology in vivo by targeting Staufen 1

Authors: *D. R. SCOLES, S. PAUL, W. DANSITHONG, M. GANDELMAN, K. FIGUEROA, S. M. PULST;
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Abstract: TDP-43 aggregation is a pathological hallmark of amyotrophic lateral sclerosis (ALS), and is common in Alzheimer's disease (AD) and frontotemporal dementia (FTD), devastating diseases affecting a significant segment of the population. We have previously shown elevated mTOR and other autophagy-related proteins in fibroblast cells derived from patients with ALS/FTD and other neurodegenerative diseases, associated with overabundance of the RNA binding protein Staufen1 (STAU1). The objectives of this study are to determine if STAU1 functions mechanistically in regulating mTOR expression and if targeting STAU1 could restore TDP-43-related phenotypes *in vivo*. Previous studies had demonstrated that STAU1 interactions with 5'-UTRs of mRNAs enhance translation. We performed blot overlay assays demonstrating that STAU1 directly interacted with the 5'-UTR of mTOR. Following this, we established a reporter assay with the mTOR 5'-UTR upstream of luciferase, and consistent with increased translation, STAU1 overexpression increased the luciferase signal while the mRNA abundance remained constant. Next, to determine whether reducing STAU1 abundance could modify TDP-43 -related autophagy phenotypes *in vivo*, we crossed TDP-43 transgenic (tg) mice with *Stau1* knockout mice. By western blotting, we observed normalized protein abundances for each of mTOR, p-mTOR, p-S6K, p62 and LC3-II in the spinal cords of 22-wk old *TDP-43* tg mice haploinsufficient for Stau1. We also produced an antisense oligonucleotide targeting *Stau1*. TDP-43 transgenic mice treated with *Stau1* ASO had restored protein abundances for TDP-43, the motor neuron marker ChAT, the pan-neuronal marker NeuN and the astrocyte reactivity marker GFAP. Histological analysis revealed normalized levels of TDP-43 and CHAT in spinal cord motor neurons of TDP-43 transgenic mice treated with *Stau1* ASO. We conclude that Stau1

overabundance leads to abnormal autophagy and related toxic pathways associated with TDP-43 proteinopathy that can be restored by targeting *Stau1* expression. These data support STAU1 as a therapeutic target for TDP-43-related ALS as well as other neurodegenerative diseases in which STAU1 is overabundant.

Disclosures: D.R. Scoles: None. **S. Paul:** None. **W. Dansithong:** None. **M. Gandelman:** None. **K. Figueroa:** None. **S.M. Pulst:** None.

Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

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Program #/Poster #: 202.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01AG044404(JCL)
R01HL141255

Title: Biogenesis and function of extracellular vesicles derived from astrocytes treated with docosahexaenoic acid and arachidonic acid

Authors: *X. GENG, J.-W. SHIN, J. C. LEE;
Univ. of Illinois at Chicago, Chicago, IL

Abstract: Docosahexaenoic acid (DHA) and arachidonic acid (ARA) are most abundant fatty acids in the brain. The decrease in the ratio of DHA to ARA in the brain is associated with normal and abnormal aging, such as Alzheimer's disease. In this study, we examine whether and how DHA and ARA affect biogenesis and function of extracellular vesicles (EVs) derived from astrocytes. EVs were collected from DHA or ARA-treated primary astrocytes and purified using a 100kDa MW-cutoff column. Cells were subjected to structural characterizations, including cell stiffness and membrane-cytoskeleton connectivity using atomic force microscopy, and surface area and actin polymerization using fluorescence microscopy. To examine the anti-neuroinflammatory effect of EVs, immortalized microglia (BV2 cells) were pretreated with astrocyte-derived EVs for 1 h followed by treatment with 100 ng/ml LPS for 24 h. Culture medium and cell lysis were collected to quantify the levels of TNFα and iNOS, phospho-p65 and p65. Both DHA and ARA increased the production and average size of astrocyte-derived EVs. Such increases in EV size and production could be associated with increased cell surface area, actin polymerization, and membrane-cytoskeleton connectivity after treatment with DHA or ARA. Treating astrocytes with DHA or ARA enhanced the anti-neuroinflammatory effect of their EVs on microglia stimulated by LPS. Information derived from this study should provide new insights into the function of astrocytes, and increasing production and function of EVs from astrocytes as 'nanotheranostics'.

Disclosures: X. Geng: None. **J. Shin:** None. **J.C. Lee:** None.

Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

Location: SDCC Halls B-H

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Program #/Poster #: 202.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NRF Grant NRF-2019R1C1C1011390
KBRI Grant 22-BR-02-03
KBRI Grant 22-BR-03-02

Title: The effect of obesity and HDL concentration through high-fat diet on the pathological characteristics of Alzheimer's disease

Authors: *M. CHOI, D. KIM, Y.-J. YOUN, Y. JEONG;
Korea Brain Res. Inst., Korea Brain Res. Inst., Daegu, Korea, Republic of

Abstract: Alzheimer's disease (AD) is a representative disease of dementia. The typical pathological features are accumulation of amyloid plaques in the brain and reactivity of glial cells such as astrocytes and microglia. Clinically, the development of AD and obesity are known to be correlated. In this study, we analyzed the changes of AD pathological characteristics in 5XFAD mice, an AD model after obesity induction through high-fat diet (HFD). Interestingly, after 16 weeks of HFD, HFD group was divided into two groups, HFD-Low and HFD-High depend on the body weight change only in transgenic group. This phenomenon was more dramatic in female mice. In the indirect calorimetry analysis, the energy expenditure of TG-Normal Diet (ND) group was increased compare with WT-ND group, whereas the level of TG-HFD high group was decreased compare with TG-ND group in Light-off time. Surprisingly, unlike clinical data, there was no difference in the blood level of LDL between the ND and HFD group, but rather the level of HDL was increased in HFD group. Interestingly, the serum concentration of APOA1 increased in both WT and TG HFD group. The reactivity of astrocytes and microglia in the dentate gyrus of hippocampus and the fornix of hypothalamus in 5XFAD mice was decreased in TG-HFD high group. The accumulation of amyloid plaques in the DG(dentate gyrus) region of the hippocampus was also significantly deceased in TG-HFD high group. In the mouse general behavior analysis, mice activity such as total distance and rearing was increased and repetitive behavior (e.g., circling grooming) was decreased in TG-HFD high group compared to TG-ND group. Finally, the working memory was significantly decreased in the TG-ND group. However, there was no difference in working memory between TG-HFD and WT-HFD groups. These results suggest that an increase of HDL level with specially APOA1 level in serum alleviates the pathological features of AD and would be a new potential therapeutic strategy for AD treatment.

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Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

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Program #/Poster #: 202.12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: PAPIIT Grant IN217921
PAIP Grant 5000-9179

Title: Involvement of carbonic anhydrase in the antinociceptive effect of Diosmin and Hesperidin in a model of plantar edema in wistar rats

Authors: A. MUÑIZ-ALQUICIRA[1], R. B. GARCIA[3], A. G. MARTINEZ[4], *A. CARBALLO-VILLALOBOS[2];
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Abstract: Currently, pain and inflammatory diseases represent a serious health problem of growing importance; therefore, there is a need to look for therapeutic alternatives for this condition, since the drugs currently used present important limitations due to the fact that they are associated with adverse effects; one of these alternatives are flavonoids. Diosmin and hesperidin are flavonoids widely used in the clinic in the treatment of venous insufficiency; however, there is new research suggesting that they can intervene in the modulation of some common mechanisms in pain and inflammation, which makes them good candidates for the treatment of diseases with these characteristics; Some of these mechanisms are the decrease of pro-inflammatory cytokines and the inhibition of carbonic anhydrase which plays an important role in the reversible catalysis of CO_2 hydration to form HCO^{3-} and H^{+}; this being a new approach to design agents for the treatment of this condition. The objetive of this work was to evaluate the anti-inflammatory and analgesic effect of the flavonoids Diosmin and Hesperidin; besides corroborating their inhibitory efficacy on carbonic anhydrase. We worked with the flavonoids diosmin and hesperidin in addition to acetazolamide and sulfonamide as antagonists to investigate the involvement of carbonic anhydrase in their action mechanism of action. For the evaluation, an induction of plantar edema was performed using the 1% formalin model using Wistar male rats (n=6 for 12 groups, 180-250 g of body weight); subsequently, the pain response was evaluated by counting the licking time and the number of shakes of the individuals during the first 5 min (phase 1) consecutive to the induction and 20 min later (phase 2), inside methacrylate cylinders. For the evaluation of inflammation, measurements were performed with the thread model across the width of the plantar area of both the damaged and healthy tracer limb. We observed that both flavonoids have an anti-inflammatory effect; in the formalin model mainly in (phase 2); on the other hand when they are administered jointly with the antagonists we see that the effect of diosmin and hesperidin is reversed both in the formalin model. This suggests the participation of carbon anhydrase in the anti-inflammatory mechanism of action of these flavonoids as well as their usefulness in the treatment of pain and inflammation.

Disclosures: A. Muñiz-Alquicira: None. **R.B. Garcia:** None. **A.G. Martinez:** None. **A. Carballo-Villalobos:** None.

Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 202.13

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: KMDF_PR_20200901_0088
NRF2020R1I1A1A0105509612

Title: Neuroprotective effects of $GABA_B$ receptor agonist on secondary brain damage after Traumatic brain injury in Mice

Authors: *J. PARK[1], Y. KIM[2], J. BAEK[1], J. PARK[1], C. KONG[1], Y. SEO[1], J. CHANG[1], W. CHANG[1];
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Abstract: Traumatic brain injury(TBI) is reported as a major cause of death and disability in worldwide. TBI not only causes physical damage to brain tissue from a direct primary impact, but also continuous processes such as oxidative stress, excitotoxicity, and neuroinflammation. These prolonged secondary injuries can induce behavioral impairments related with motor and cognition. Particularly, chronic neurological dysfunction can arise from neuro-inflammatory responses such as persistent glial cell activation. Baclofen, $GABA_B$ receptor agonist, has been explored for its neuroprotective effects in diverse disease animal models involving inflammatory pathways. The purpose of this study was to confirm neurological recovery by reducing TBI induced activated glial cells after baclofen treatment. 10weeks male C57BL/6 mice (20-25g) were made to moderate-severe controlled cortical impact(CCI) mice model. Baclofen was dissolved in saline and was administrated with intraperitoneal (i.p.) injection. Daily injections were performed from 24hours after CCI modeling to 3weeks. After the injections were completed, an additional observation period was given to test neurological severity scores for 3weeks. Compared with TBI group, modified neurological severity scores were decreased in baclofen groups. In histological results, the number of TUNEL positive cells was reduced in baclofen groups in cortex region. Iba-1 which represents microglia activated in peri-lesion area in TBI group whereas reduced in baclofen groups. In addition, the relative band intensity of IL-1 beta, one of the main proinflammatory cytokines, decreased in baclofen treatment groups in compared with TBI group. GFAP, a marker of astrocyte, was also significantly down regulated in the peri-contusion cortex of baclofen injection groups in both of immunohistochemistry and western blot. Baclofen has been emerged as a therapeutic drug for neurodegenerative diseases. Baclofen improved motor deficits induced by TBI and also reduced apopotic cells in peri-lesion cortex. Moreover, it was confirmed that baclofen down regulated activated glial cells and

proinflammatory cytokine in cortex region. Baclofen might be able to regulate secondary injury induced by post traumatic brain injury. However, in order to support neuroprotective effect, further analysis is required. Additionally, more research is needed on the detailed intervention mechanism of baclofen.

Disclosures: **J. Park:** None. **Y. Kim:** None. **J. Baek:** None. **J. Park:** None. **C. Kong:** None. **Y. Seo:** None. **J. Chang:** None. **W. Chang:** None.

Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: the Basic Science Research Program (2019R1I1A2A01060115) through the National research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning
the Korea Health Technology R&D Project (HI21C0572) through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare, Republic of Korea

Title: Role of substance P (SP)-neurokinin 1 (NK1) signal on the development and maintenance of chronic osteoarthritis pain in rat models.

Authors: *M. KWON[1,2], H. YOO[1,2], T. KIM[1], D. NAM[1], J. KIM[1,2,3,4];
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Abstract: Osteoarthritis (OA) is a progressive degenerative joint disease and a major cause of pain and disability with reducing the quality of life in individuals. Substance P (SP), one of neuropeptides binding to neurokinin 1 (NK1) receptor, not only plays a major role in exacerbating acute inflammation, but also is responsible for neurogenic inflammation in the pathological progression of OA. In the present study, we investigated the role of SP-NK1 signal in development and maintenance of chronic pain following OA in rat models by using NK1 antagonist.GR 82334, one of NK1 receptor antagonists, or saline were intra-articularly and intrathecally administered at acute phase (before and 1 day after OA) and chronic phase (14 days after OA) in monosodium iodoacetate (MIA, 4 mg/50 μl)-induced OA rats. Behavioral tests for inflammation and pain were performed before and after NK1 antagonist administration in OA rats. Safranin O fast green (SOFG) staining was used to evaluate the difference of histological change in the knee joint. The expression of SP and calcitonin gene related-peptide (CGRP) in L3-5 segment of spinal cord was quantified through immunohistochemistry. GR 82334 injected

in acute phase of OA decreased knee bending score and knee joint diameter ratio, and delayed the decrease of PWT compared to control. Also, the structure collapse of knee joint related to degree of inflammation was less, and the expression of SP and CGRP in spinal cord was significantly decreased by GR 82334 injection in acute phase. GR 82334 injected in chronic phase of OA increased PWT in both intra-articular and intrathecal injection, for 1 and 2 days respectively, and there was no effect in any other behaviors after GR 82334 injection in chronic phase.The present data showed that blocking SP-NK1 signal by NK1 antagonist at acute phase of OA alleviated the acute inflammation and delayed the development of pain, and blocking SP-NK1 signal at chronic phase of OA had pain relief effect. These results suggest that SP-NK1 signal might participate in the overall pathological process from acute inflammation to development and maintenance of chronic pain related to central sensitization following OA, which means that SP-NK1 signal would be a clinical key for complete treatment of OA.

Disclosures: **M. Kwon:** None. **H. Yoo:** None. **T. Kim:** None. **D. Nam:** None. **J. Kim:** None.

Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

Location: SDCC Halls B-H

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Program #/Poster #: 202.15

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01 DE022757
Veterans Affairs Merit Award 5I01BX000638

Title: Estrogen receptor-1 mediates pain induced by myelin basic protein fragments

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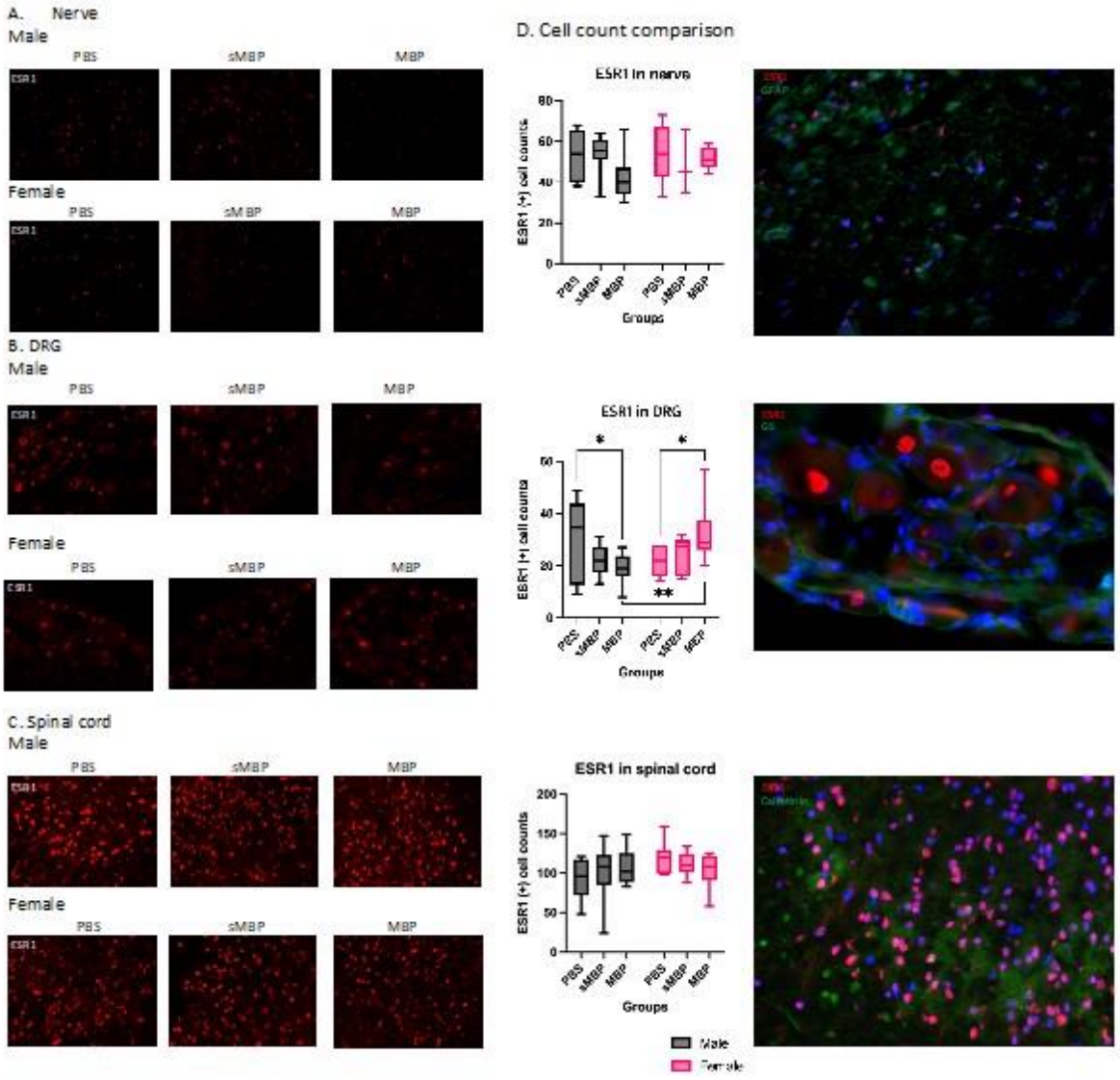
Abstract: There is increasing evidence for sexual dimorphism in mechanisms of neuropathic pain, including female-prevalent mechanical hypersensitivity induced by the pathogenic 84-104 fragment of myelin basic protein (MBP84-104). The present study aims to assess patterns of estrogen receptor (ESR)1 (α) gene expression in male and female mice after unilateral sciatic nerve intraneurial (IN) injection of MBP84-104 or scrambled (sMBP) peptides, or PBS vehicle. At day 7 after IN, ESR1 levels were assessed by RNAseq and bioinformatics analyses, immunofluorescence, and western blotting in sciatic nerve, lumbar (L)4-5 dorsal root ganglia (DRG), and spinal cord. We detected high baseline ESR1 levels in the nerve, DRG and spinal cord of both sexes, localized largely in DRG neurons and spinal interneurons. IN MBP84-104 induced sex-specific and segmental effects on ESR1 expression and/or predicted signaling activity as compared to sMBP or PBS. Most notably, MBP84-104 injection selectively induced

ESR1 mRNA and/or protein expression levels in female relative to male and/or suppressed in male relative to female spinal cord and/or DRG. To assess the functional significance of ESR1 in MBP84-104-induced pain, mechanical hypersensitivity in female mice was confirmed at day 7 after IN MBP84-104 by von Frey testing, followed by a bolus intrathecal administration of a selective estrogen receptor modulator tamoxifen or vehicle. Tamoxifen therapy caused significant reduction in MBP84-104-induced mechanical hypersensitivity. We conclude that activity of sensory neuronal ESR1 mediates neuropathic pain induced by MBP84-104. The relative roles of ESR1 in female and male somatosensory nervous system remain to be investigated, particularly in the context of higher female incidence of chronic pain states and personalization of analgesic strategies.



Disclosures: **Y. Park:** None. **J. Dolkas:** None. **M.V. Jariwala:** None. **K.A. Eddinger:** None. **Y. Miller:** None. **A.V. Chernov:** None. **T.L. Yaksh:** None. **V. Shubayev:** None.

Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 202.16

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NS114891
NS122918

Title: Characterization of Neutrophils in a Sciatic Nerve, Peripheral Nerve Injury.

Authors: *B. BALOG, J. NIEMI, T. DISABATO, R. E. ZIGMOND;
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Abstract: Neutrophils are an integral part of the innate immune response and are involved in myelin clearance during Wallerian degeneration. Although *Ccr2* knockout (KO) mice contain decreased infiltrated macrophages in the distal sciatic nerve, they exhibit normal myelin clearance, which at least partially depends on the presence of neutrophils (Lindborg et al., J Neurosci, 2017). As neutrophils and macrophages share certain cellular markers, e.g., CD11b, myeloperoxidase (MPO), arginase 1 (Arg 1), distinguishing the two cell types clearly is essential. Wild type (WT) and *Ccr2* KO mice received a unilateral sciatic nerve transection with the contralateral nerve serving as a sham control. 1, 2, 3, or 7 days later, the sciatic nerves were harvested. Sections were stained for Ly6G or Ly6B.2 together with MPO to identify neutrophils, while Iba1 was used to identify macrophages. A blinded individual imaged the slides, counted the cells, and calculated the percent area stained. The results showed a rapid increase in neutrophils in both genotypes peaking at 1-3 d, which then resolved by 7 d. Both WT and *Ccr2* KO mice had more neutrophils (Ly6G+MPO+ cells or Ly6B.2+MPO+ cells) present at 2-3 d in the distal nerve compared to the sham. Moreover, *Ccr2* KO mice had significantly more Ly6G+MPO+ cells than WT mice at 2-3 d. There was no difference between the two genotypes in the Ly6B.2 percent area stained at the injury site itself. Neutrophils have been divided into N1 pro-inflammatory cells and N2 anti-inflammatory that are Arg1+. No Ly6B.2 cells were co-labeled with Arg1 at 2 d, suggesting that they might be N1 neutrophils. The small number of cells co-labeled for Ly6B.2 and Iba1 could represent macrophages that have phagocytosed dead neutrophils, which they are known to do. Currently, we are using *Cxcr2* KO mice to determine the role of this chemokine receptor in neutrophil trafficking. Neutrophil extracellular traps (NETs) are structures consisting of extruded DNA bound by cellular proteins including citrullinated histone H3, the latter of which can be used to identify these structures. In infected tissues, NETs can sequester microbes. NETs have also been reported in the central nervous system after injury, although their role there remains to be determined. We now report NETs in

the distal sciatic nerve after axotomy. Attempts will be made to determine their function by pharmacologically blocking NET formation.

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Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 202.17

Topic: F.04. Neuroimmunology

Support: NIH Grant 1R01NS116704-01

Title: The Fibroblast-Derived Protein Pi16 Controls Inflammatory Pain

Authors: *R. GARRITY[1], N. ARORA[1], R. TRINH[1], C. GAFFNEY[1], R. MAHLINGAM[1], C. HEIJNEN[2], A. KAVELAARS[1], A. J. SHEPHERD[1];
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Abstract: We previously showed that Pi16 (peptidase inhibitor 16), a protein of unknown function largely expressed by fibroblasts, controlled neuropathic pain in the spared nerve injury model. PI16-knockout ($PI16^{-/-}$) mice were protected from neuropathic pain and exhibited reduced endothelial barrier permeability and reduced leukocyte infiltration. This suggests that PI16 promotes pain by increasing the permeability of the blood-nerve barrier, facilitating leukocyte infiltration. We next wanted to determine the extent of PI16's contribution to a mechanistically distinct model: the intraplantar CFA model of inflammatory pain. Following CFA injection, mechanical sensitivity was assessed using von Frey hairs, thermal sensitivity using Hargreaves' test, and paw edema using a digimatic micrometer. Significantly reduced mechanical and thermal hypersensitivity was observed in $PI16^{-/-}$ mice versus wild-type controls, with both strains exhibiting equal amounts of paw edema. Partial protection against CFA-induced hypersensitivity was observed in global- and fibroblast-specific inducible PI16 knockout mice. Interestingly, although increased PI16 immunoreactivity was detected in the epineurium surrounding the sciatic nerves of CFA-injected mice, no alterations in blood-nerve barrier permeability or leukocyte infiltration could be detected. However, intrathecal delivery of a neutralizing PI16 antibody was able to phenocopy the blunted CFA response of the $PI16^{-/-}$ mouse, suggesting that the spinal cord/DRG may be an important site of PI16 function in inflammatory pain. Indeed, bulk RNA-sequencing showed an increase in gene transcripts in the PI16-KO associated with myeloid cell activity, suggesting that the protective effects of PI16 deletion in inflammatory pain may be due to increased infiltration of the DRG by pain-suppressing leukocytes. We further addressed this by demonstrating that intrathecal depletion of $CD206^{+}$ cells or neutralization of IL-10 were sufficient to block the protective effects of PI16 deletion. Altogether, these studies suggest that PI16 function alters infiltration and/or phenotype of leukocytes in the vicinity of the DRG and sustains inflammatory pain hypersensitivity.

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Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 202.18

Topic: F.04. Neuroimmunology

Title: The Role of C-X-C motif chemokine receptor 2 in Chemotherapy Induced Peripheral Neuropathy

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Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is a major drawback in the use of chemotherapeutic agents for patients with cancer. Even though diverse studies have identified a broad scale of molecules that might be related to CIPN, there has not yet been any difference of chemokine pathways between various chemotherapeutic agents, such as vincristine and oxaliplatin, which are some of the widely used. We confirmed that subsequent treatments (intraperitoneal injection, 7 days) of vincristine (0.1 mg/kg) and oxaliplatin (3 mg/kg) induced pain, using von-Frey behavioral test. Next, the alterations of chemokine factors in DRG and spinal cord of CIPN models were examined with mRNA expression of CXCR2, CXCL1. To evaluate the involvement of CXCR2 in CIPN, we treated reparixin, the CXCR1/2 inhibitor with vincristine or oxaliplatin. Also, Janus kinase 2 (Jak2), downstream of CXCR1/2 pathway was blocked by treating ruxolitinib with vincristine or oxaliplatin. The subsequent treatments of vincristine and oxaliplatin induced mechanical allodynia lasts more than 1 week from the 5th day. After the induction of mechanical allodynia by vincristine and oxaliplatin, the mRNA expression of CXCR2 increased in spinal cord and DRG. Reparixin and ruxolitinib blocked the oxaliplatin-induced allodynia, but not vincristine. This suggests that oxaliplatin-induced neuropathy is associated with CXCR2-related pathway while vincristine-induced neuropathy have CXCR2-independent pathway. Although similar mechanical allodynia occurs as one of the side effects of vincristine and oxaliplatin, the molecular mechanisms causing mechanical allodynia are different between vincristine and oxaliplatin. Thus, it is important to choose the adequate CIPN treatment for each specific chemotherapeutic agent.

Disclosures: **H. Cho:** None. **Y. Choi:** None. **Y. Han:** None. **S. Jung:** None.

Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 202.19

Topic: F.04. Neuroimmunology

Support: NIH Grant R01AR071431S01
NIH Grant R01AR071431

Title: Evaluating the role of parasympathetic drive on knee osteoarthritis in the male rat

Authors: *C. J. CRUZ[1], T. D. YEATER[1], K. D. ALLEN[1,2,3];
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Abstract: OBJECTIVE: The parasympathetic nervous system maintains peripheral immune homeostasis; therefore, low parasympathetic drive may exacerbate inflammation in the periphery, such as the osteoarthritic joint. Many diseases that are comorbid to osteoarthritis (OA) display low parasympathetic drive; thus, we tested the hypothesis that reducing parasympathetic drive via vagotomy will accelerate OA and OA-related symptoms in a rat model of knee OA. **METHODS:** Male Sprague Dawley (12 weeks old) rats were randomly assigned between OA or OA+vagotomy groups (n=9 per group). OA was induced by transecting the medial collateral ligament and the medial meniscus, whereas vagotomy was caused by transecting the left cervical vagus nerve. Gait and tactile sensitivity were assessed at 4, 8, and 12 weeks after surgery, with histology performed at week 12. Behaviors were statistically analyzed using a linear mixed effects model, where surgery, timepoint, and the interaction were assessed using Satterthwaite's method. Multiple comparisons of least squared means were assessed using a Tukey's HSD test, when indicated. **RESULTS:** Relative to baseline, peak vertical force in the OA limb dropped at week 4, 8, and 12 weeks for the OA+vagotomy group and at weeks 8 and 12 for the OA only group ($p<0.05$). Also relative to baseline, stance time in the OA limb decreased at week 4 in the OA+vagotomy group and increased at weeks 8 and 12 in both groups ($p<0.05$). Stance time imbalance (time spent on non-OA vs. OA limb) and temporal asymmetry also increased at week 4 in the OA+vagotomy group relative to baseline ($p<0.05$). While tactile sensitivity shifted lower for OA+vagotomy animals relative to OA-only animals, these shifts were not statistically significant. Lastly, histological changes showed comparable cartilage loss and bone remodeling in both groups. **CONCLUSION:** Relative to baseline, vagotomy accelerated drops in peak vertical force, caused greater stance time imbalance, and lowered stance time of the OA limb at earlier stages of OA. However, at mid- and late-stage OA, both groups showed reduced peak vertical force in the affected limb and greater stance time of the OA limb. While vagotomy animals appeared to be more sensitive throughout OA progression, these differences were not statistically significant at these sample sizes. Combined, these data suggest that low parasympathetic feedback may accelerate the onset of OA symptoms.

Disclosures: C.J. Cruz: None. **T.D. Yeater:** None. **K.D. Allen:** None.

Poster

202. Neuroinflammation: Pain, TBI, and Nerve Injury

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 202.20

Topic: B.09. Glial Mechanisms

Title: Mirna-146a-5p nanoparticles reduce mia-induced pain behaviors and sensory peripheral innervation

Authors: *H. PARK[1,3], J. SHIN[1,3], D. KIM[4], N. SHIN[1,3], H. SHIN[3], J. BEOM[5], J. KIM[2], D. KIM[1,3,4];
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Abstract: Osteoarthritis (OA) is a degenerative disease that worsens over time, often resulting in chronic pain. Following the progression of OA, a calcitonin gene-related peptide (CGRP)-positive sensory nerves innervate the synovial membrane, meniscus, and subchondral bone of the knee joint in rodents, deteriorating OA pain. Recent studies have suggested that sensory innervation for OA pain between cartilage and subchondral bone is considered as a key factor for disease progression. However, the understanding of the mechanism poorly remains. In this study, we performed to reduce OA pain by regulating the distribution of differentiated sensory nerves in the subchondral bone through miRNA-146a-5p encapsulated PLGA nanoparticles (miR146a-5p NPs) in a monoiodoacetate (MIA)-induced OA model. MIA induced OA pain behaviors were measured 10 days and confirmed that the CGRP+ sensory nerves are increased in subchondral bone with the upregulation of neuroninflammatory cytokines, including (TNF)-α and MMP13. Appling miR146a-5p NPs into the knee joint of the MIA model showed the significant reduction of OA pain relate mRNA genes, distribution of CGRP+ sensory nerves, and pain behavior. As joint nociceptors terminated in the dorsal horn of the spinal cord, we also found that the decrease of microglia activation in miR146a-5p NPs group compared to MIA-induced rats. Taken together, injection of miR-146a-5p NPs can effectively down-regulate sensory nerve innervation in subchondral bone by reducing OA pain. Therefore, it is suggested that modulation of sensory innervation may be a new target in OA pain.

Disclosures: H. Park: None. **J. Shin:** None. **D. Kim:** None. **N. Shin:** None. **H. Shin:** None. **J. Beom:** None. **J. Kim:** None. **D. Kim:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.01

Topic: C.08. Ischemia

Support: NIH grant NS090904
NIH grant NS117827
NIH grant HL142975

Title: Activated protein C analog protects pericyte-deficient mice from ischemic brain injury

Authors: *Y. WANG[1], K. KISLER[1], A. M. NIKOLAKOPOULOU[1], J. GRIFFIN[2], B. V. ZLOKOVIC[1];
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Abstract: The blood–brain barrier (BBB) limits the entry of neurotoxic blood-derived products into the brain. Pericytes play a key role in maintaining BBB. BBB integrity is disrupted during the early onset of stroke. However, the role of pericytes in the pathogenesis of stroke is still poorly understood. 3K3A-APC, a cell-signaling recombinant variant of activated protein C (APC), has shown benefits in preclinical models of ischemic stroke and has favorable safety profile and reduces hemorrhage in Phase 2 study in ischemic stroke patients. In the present study, we used PDGFRβ heterozygous knockout (*Pdgfrb+/-*) mice to investigate the effects of pericyte deficiency on ischemic brain injury using transient proximal middle cerebral artery occlusion (tMCAO) stroke model. Additionally, we investigated the effects of 3K3A-APC therapy in this model. Compared to controls, *Pdgfrb+/-* mice showed a 26% greater loss of cerebral blood flow (CBF) during early reperfusion, and 40-50% increase in the infarct and edema volumes and motor neurological score 24 h after tMCAo. These changes were accompanied by 50% increase in both immunoglobulin G and fibrinogen pericapillary deposits in the ischemic cortex 8 h after tMCAo indicating an accelerated BBB breakdown, and 35 and 55% greater losses of pericyte coverage and number of degenerating neurons 24 h after tMCAo, respectively. Treatment of *Pdgfrb+/-* mice with 3K3A-APC administered intravenously 10 min and 4 h after tMCAo normalized CBF during the early reperfusion phase and reduced infarct and edema volume and motor neurological score by 55-60%, with similar reductions in BBB breakdown and number of degenerating neurons. Our data suggest that pericyte deficiency results in greater brain injury, BBB breakdown, and neuronal degeneration in stroked mice and that 3K3A-APC protects the brain from accelerated injury caused by pericyte deficiency. These findings may have implications for treatment of ischemic brain injury in neurological conditions associated with pericyte loss such as those seen during normal aging and in neurodegenerative disorders such as Alzheimer's disease.

Disclosures: Y. Wang: None. **K. Kisler:** None. **A.M. Nikolakopoulou:** None. **J. Griffin:** None. **B.V. Zlokovic:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

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Program #/Poster #: 203.02

Topic: C.08. Ischemia

Support: AHA Grant 20PRE35090021
NIH/NINDS Grant 3R01NS034773

Title: Resveratrol preconditioning protects against ischemia-induced synaptic dysfunction and cofilin hyperactivation in the mouse hippocampal slice

Authors: *I. ESCOBAR, J. XU, C. JACKSON, M. A. PEREZ-PINZON;
Neurol., Univ. of Miami, Miami, FL

Abstract: Perturbations in synaptic function are major determinants of cognitive decline and occur early after cerebral ischemia (CI). As such, prophylactic strategies, namely resveratrol preconditioning (RPC), offer a promising means to prevent/mitigate synaptic impairments during the initial stages of injury. Like traditional ischemic preconditioning, RPC renders the brain resistant to subsequent, lethal ischemic insults; however, whether RPC can protect against CI-induced synaptic dysfunction remains unclear. In this study, we investigated the effects of RPC on synaptic function in an *ex vivo* model of ischemia. Acute hippocampal slices were prepared from 8-12 week old male mice—injected i.p. with a single dose of resveratrol (10 mg/kg) or vehicle 48 hours prior—and subjected to oxygen and glucose deprivation (OGD) to mimic ischemic conditions. Electrophysiological recordings and intracellular calcium measurements during/after OGD revealed an RPC-mediated delay to anoxic depolarization (mean ± SEM: 9.74 ± 0.69 vs 7.63 ± 0.47 minutes; n= 9-11 slices (N= 6-7)) and decrease in cytosolic calcium levels (two-way ANOVA, treatment effect: $F_{(1, 54)}$= 53.97; p< 0.0001; N= 5-6). Moreover, RPC prevented aberrant increases in synaptic transmission (two-way ANOVA, treatment effect: $F_{(1, 33)}$= 5.754; p= 0.0223) and rescued deficits in hippocampal long-term potentiation, measured 1 hour after OGD (two-way RM ANOVA, treatment effect: $F_{(1,15)}$= 15.76; p= 0.0012). These findings demonstrate that RPC attenuates mechanisms of excitotoxicity and preserves hippocampal synaptic function acutely after CI. To elucidate potential mechanisms underlying RPC-mediated preservation of synaptic function, we investigated the role of the activity-regulated cytoskeleton-associated protein, Arc, which was found to be upregulated in the hippocampal membrane fraction following RPC (1.00 ± 0.06 vs 1.27 ± 0.09; N= 5). Evidence suggests that Arc modulates the phosphorylation status of the actin-binding protein, cofilin, which becomes hyperactivated (dephosphorylated) during OGD. Under conditions of oxidative stress, cofilin hyperactivation promotes the formation of pathological cofilin-actin rods that lead to synaptic impairment. Remarkably, RPC significantly attenuated OGD-induced cofilin hyperactivation (two-way ANOVA, treatment effect: $F_{(1, 56)}$ = 29.74; p< 0.0001; N= 5-7), indicating a role for RPC in phospho-cofilin regulation. To this end, our study provides further insight into mechanisms underlying RPC-mediated neuroprotection against CI and implicates RPC as a potential therapeutic strategy to improve cognitive outcomes.

Disclosures: I. Escobar: None. **J. Xu:** None. **C. Jackson:** None. **M.A. Perez-Pinzon:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.03

Topic: C.08. Ischemia

Support: MSIT 2020M3E5D9080660

Title: Epigallocatechin gallate alleviates ischemic neuronal injury-induced reduction in the neuronal calcium sensor protein

Authors: *P.-O. KOH[1], D.-J. PARK[1], J.-B. KANG[1], M.-O. KIM[2];
[1]Dept. of Anatomy, Col. of Vet. Med., Gyeongsang Natl. Univ., Jinju, Korea, Republic of; [2]Div. of Life Sci. and Applied Life Science, Col. of Natural Sci., Gyeongsang Natl. Univ., Jinju, 52828, Korea, Republic of

Abstract: Epigallocatechin gallate (EGCG) is a polyphenolic component and has anti-oxidative and anti-inflammatory effects in neurons. Ischemic stroke is a neurological disease that causes irreversible disorders, increases the intracellular calcium concentration, and induces apoptosis. The regulation of intracellular calcium concentration is important to maintain the function of the nervous system. Hippocalcin is a neuronal calcium sensor that controls intracellular calcium concentration. We investigated whether EGCG treatment regulates the expression of hippocalcin in cerebral ischemia and glutamate-induced neuronal damage. We performed middle cerebral artery occlusion (MCAO) to induce cerebral ischemia. EGCG (50 mg/kg) or PBS was injected into the abdominal cavity just before MCAO surgery, and cerebral cortex tissue was collected 24 h after MCAO surgery. MCAO damage caused severe neurological deficits and infarctions, and decreased the expression of hippocalcin in the cerebral cortex. EGCG improved these deficits and alleviated the decrease in hippocalcin expression. In cultured hippocampal cells, EGCG dose-dependently prevented glutamate-induced cell death and intracellular calcium overload. Glutamate exposure reduced hippocalcin level, decreased Bcl-2 expression, and increased Bax expression. However, EGCG treatment restored these changes. EGCG attenuated the increase in caspase-3 and cleaved caspase-3 expressions caused by glutamate exposure. The alleviate effect of EGCG was more pronounced in non-transfection conditions than in transfection conditions of hippocalcin siRNA. These findings show that EGCG attenuates hippocalcin reduction and intracellular calcium overload, and prevents apoptosis through regulation of Bcl-2 family proteins and caspase-3. Thus, we suggest that EGCG has neuroprotective effects by regulating hippocalcin expression in ischemic brain damage and glutamate-exposed cells. "This research was supported by the Neurological Disorder Research Program of the National Research Foundation (NRF) funded by the korean government (MSIT) (2020M3E5D9080660)"

Disclosures: P. Koh: None. **D. Park:** None. **J. Kang:** None. **M. Kim:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.04

Topic: C.08. Ischemia

Support: DDFG (German Research Council; project ID 431549029–SFB 1451)

Title: Transcranial-direct-current-stimulation accelerates motor recovery after cortical infarction in mice: the interplay of structural cellular responses and functional recovery

Authors: H. L. WALTER[1], A. PIKHOVYCH[1], H. ENDEPOLS[2], S. ROTTHUES[1], J. BAERMANN[1], H. BACKES[3], M. HOEHN[4], D. WIEDERMANN[3], B. NEUMAIER[2], G. R. FINK[1], M. A. RUEGER[1], *M. SCHROETER[1];
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Abstract: ***Background:*** Transcranial direct current stimulation (tDCS) promotes recovery after stroke in humans. The underlying mechanisms, however, remain to be elucidated. Animal models suggest tDCS effects on neuroinflammation, stem cell proliferation, neurogenesis, and neural plasticity. ***Objective:*** In a longitudinal study, we employed tDCS in the subacute and chronic phase after experimental focal cerebral ischemia in mice to explore the relationship between functional recovery and cellular processes. ***Methods:*** Mice received photothrombosis in the right motor cortex, verified by Magnetic Resonance Imaging (MRI). A composite neuroscore quantified subsequent functional deficits. Mice received tDCS daily: five sessions from day 5 to 9 or ten sessions with additional tDCS from day 12 to 16. TDCS with anodal or cathodal polarity was compared to sham stimulation. Further imaging to assess proliferation and neuroinflammation was performed by immunohistochemistry at different time points and Positron Emission Tomography (PET) at the end of the observation time of 3 weeks. ***Results:*** Cathodal tDCS at 198 kC/m² (220 A/m^2) between days 5-9 accelerated functional recovery, increased neurogenesis, decreased microglial activation, and mitigated CD16/32-expression associated with M1-phenotype. Anodal tDCS exerted similar effects on neurogenesis and microglial polarization but not on recovery of function or microglial activation. TDCS on days 12-16 after stroke did not induce any further effects, suggesting that the therapeutic time window was closed. ***Conclusion:*** Overall, data suggest that non-invasive neuromodulation by tDCS impacts neurogenesis and microglial activation as critical cellular processes influencing functional recovery during the early phase of regeneration from focal cerebral ischemia.

Disclosures: H.L. Walter: None. **A. Pikhovych:** None. **H. Endepols:** None. **S. Rotthues:** None. **J. Baermann:** None. **H. Backes:** None. **M. Hoehn:** None. **D. Wiedermann:** None. **B. Neumaier:** None. **G.R. Fink:** None. **M.A. Rueger:** None. **M. Schroeter:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.05

Topic: C.08. Ischemia

Title: Ischemia induction in OPN KO optic nerve head led to increased ectopic optic nerve calcification

Authors: *B. RANGEL[1], K. KAUSHAL[1], L. M. LOURO[1], R. DALAL[1], A. M. SHARIATI[1], K. TOMA[2], X. DUAN[2], Y. J. LIAO[1];
[1]Stanford Univ., Palo Alto, CA; [2]Univ. of California San Francisco, San Francisco, CA

Abstract: Optic disc drusen (ODD) are calcified concretions at the optic nerve head that cause severely crowded anterior optic nerve compartment and optic neuropathy. ODD occur in about 2.0% of general population - an incidence similar to that of glaucoma, and sometimes in combination with retinal degeneration such as cone-rod dystrophy, Usher syndrome or pseudoxanthoma elasticum. ODD can progress in number and size over time, sometimes dramatically. The most common cause of vision loss in ODD is anterior ischemic optic neuropathy (NAION), the most common *acute* optic neuropathy in adults older than 50. In this study, we induced photochemical thrombosis in adult OPN (also known as SPP1 for secreted phosphoprotein 1) knockout mice (B6.129S6(Cg)-*Spp1*^{tm1Blh}/J) using a 577 nm PASCAL laser (200 μm spot size, 50 mW power, 500 ms duration, 15 spots). We assessed retinal and optic nerve in vivo using spectral-domain optical coherence tomography (OCT). Twenty-one days after injury, we collected blood, optic nerve and retinae and used immunohistochemistry to quantify the density of RBPMS$^+$ RGCs after chronic NAION. The optic nerves were prepared for paraffin sections and stained with Alizarin Red S stain to look for evidence of ectopic calcification. Naïve mice were used as controls. For total calcium quantification we used StanbioTotal Calcium LiquiColor. One day after NAION induction, OCT imaging revealed thickening (swelling) of the peripapillary inner retina ganglion cell complex (GCC) (Baseline: 74.5 ± 2.3 μm, n=10; Day 1 86.4 ± 9.9 μm, n=10, $p<0.01$). Three weeks after NAION, OCT imaging showed a significant thinning of the GCC (71.2 ± 6.1, n=9, $p<0.05$) indicating loss in RGCs, which was confirmed by a 42.1% decrease in RBPMS$^+$ cells (Naïve eyes: 3855 ± 333.3, n=5; NAION eyes: 2232 ± 1051.0, n=5, $p<0.01$). Alizarin Red S staining of 6 μm paraffin-embedded optic nerve sections shows ectopic calcium deposits ranging from 15 μm to 50 μm in diameter through the injured optic nerve. Quantification of total calcium content shows significant *increase* of calcium in the optic nerve of OPN knockout mice (Naïve: 6.4 ± 0.2, NAION 10.3 ± 1.7 nmol Ca/dL, n=5 $p<0.01$) and plasma (Naïve: 3.1 ± 0.3, NAION: 3.3 ± 0.1 nmol Ca/dL, n=8, $p<0.05$) of chronic NAION OPN KO mice compared to control but not in the retina (6.3 ± 1.0, NAION 6.4 ± 1.6, n=5). In conclusion, photochemical thrombosis mouse model of NAION in osteopontin knockout mice led to ectopic calcification of the optic nerve. This is the first in vivo mouse model of optic disc drusen. This model most closely mimics patients who develop optic disc drusen formation after NAION.

Disclosures: **B. Rangel:** None. **K. Kaushal:** None. **L.M. Louro:** None. **R. Dalal:** None. **A.M. Shariati:** None. **K. Toma:** None. **X. Duan:** None. **Y.J. Liao:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.06

Topic: C.08. Ischemia

Support: NIH R01NS114651
NIH UL1TR002645
PHS K12GM111726
NIH T32NS082145

Title: Reduced complexity of neocortical forelimb movement representations following ischemic injury in mice

Authors: *C. C. WOLSH, R. M. BROWN, II, A. R. BROWN, J. A. BOYCHUK;
Cell. and Integrative Physiol., UT Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: Stroke is a predominant source of disability and there is a need to understand neuronal processes underlying its pathophysiology and recovery. Here, motor cortex was tested as this structure is often damaged by stroke in a manner that results in upper motor impairments. Stroke injury was experimentally induced by photothrombotic occlusion of the distal middle cerebral artery (MCAo-Injury group) and control animals received sham-injury (Uninjured group). Skilled motor behavior was assessed pre- and post-injury using the single pellet reaching task (SPR). Following post-injury SPR training, long duration intracortical microstimulation (LD-ICMS; 500ms pulse trains) was systematically applied to test sites in motor cortex to determine the location of all complex (multi-joint) forelimb movements of this structure since these responses may represent fundamental components of active motor behaviors. Behaviorally, the MCAo-injury group exhibited significantly reduced post-injury reaching accuracy relative to the Uninjured group on the SPR task. Consistent with our recently published findings, we observed four distinct complex forelimb movement regions of motor cortex in mice during LD-ICMS (Advance, Retract, Elevate, Dig) as well as expression of simple (single joint) movement responses. The 4 complex regions expressed differential patterns of change following injury. Based on LD-ICMS testing, MCAo-injury reduced the overall area of motor cortex capable of producing complex forelimb movements. Regions of Advance, Retract, and Dig complex movement types were significantly decreased by MCAo (relative to the Uninjured group) whereas the region of Elevate was not significantly altered. The area of dual forelimb-hindlimb movements was also reduced by MCAo. Conversely, area of simple forelimb movements (single joint twitches), was significantly increased in the MCAo group relative to the Uninjured group. Ongoing experiments are using optical stimulation of layer 5 pyramidal cells that are retrogradely traced from cervical or lumbar spinal cord (i.e., to target forelimb or hindlimb motor neurons respectively). These efforts are being used to directly target projection-class specific populations of corticospinal cells during motor learning and motor recovery after ischemic damage.

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Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.07

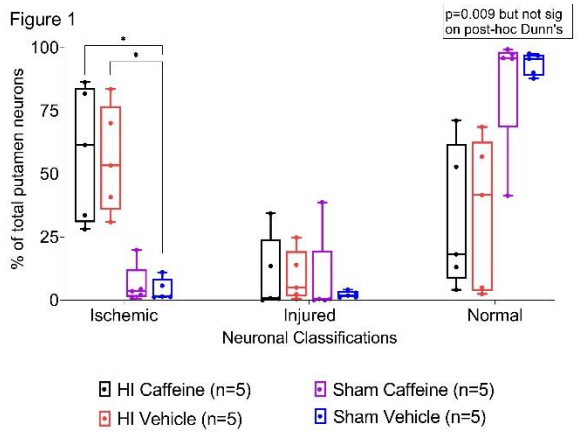
Topic: C.08. Ischemia

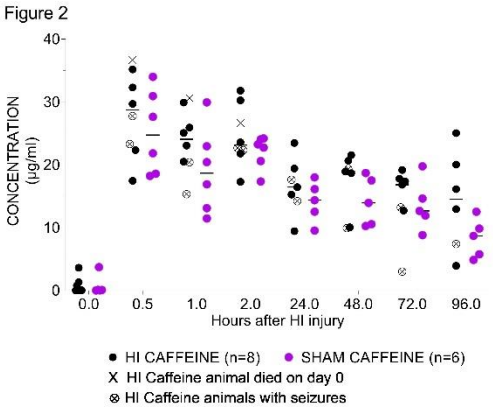
Support: Thrasher Research Fund Early Career Award
NIH NINDS grant R01 NS113921
NIH NINDS grant R01 NS107417

Title: Caffeine fails to protect the putamen in neonatal hypoxic-ischemic piglets

Authors: *M. W. CHEN, E. KULIKOWICZ, S. ADAMS, B. LESTER, J. K. LEE, L. J. MARTIN;
Johns Hopkins Sch. of Med., BALTIMORE, MD

Abstract: Neonatal hypoxic-ischemic encephalopathy (HIE) causes significant mortality and morbidity despite the use of therapeutic hypothermia. Supplemental therapies are needed. Caffeine decreases infarct size and reduces neuronal apoptosis in small animal models of brain injury. However, caffeine has unproven neuroprotective potential in a clinically relevant, large animal HIE model. We hypothesized that caffeine confers neuroprotection after hypoxia ischemia (HI) in a neonatal piglet model. METHODS: Neonatal piglets (2-3 days old) were randomized into 4 groups who received either HI or sham procedure with a multi-dose caffeine regimen or vehicle (saline). HI piglets received 45 min of hypoxia, 8 min of asphyxia and then resuscitation. Caffeine piglets received a 20 mg/kg intravenous (IV) bolus dose at 5 min post-HI followed by single doses of 10 mg/kg for 3 consecutive days. Animals were survived for 4 days. Neuropathological injury was analyzed by H&E staining. Within the putamen, ischemic-necrotic, injured (non-necrotic), and normal neurons were counted in 12 distinct 1000x microscopic fields and calculated as a percent of total neurons. Serum samples were collected at multiple time points and a commercial caffeine ELISA kit was used to determine serum caffeine levels. Data were analyzed by the Kruskal-Wallis ANOVA on ranks with post hoc Dunn's and 2-way RM ANOVA. RESULTS: HI caffeine (n=5) and HI vehicle (n=5) groups had significantly higher numbers of ischemic neurons compared to sham vehicle (n=5; p=0.003) (Figure 1). There were no significant differences between caffeine vs. vehicle within the HI or sham groups. Serum caffeine levels were significantly higher than pretreatment time and sustained at higher levels, but did not differ between the HI or sham groups (Figure 2). CONCLUSIONS: Caffeine did not protect against neuronal degeneration in the putamen of a large animal model of whole body HI despite sustained serum levels. Other brain regions warrant study. Comparable caffeine levels in HI and sham animals also suggest that a higher dose of caffeine may be needed for efficacy.





Disclosures: **M.W. Chen:** None. **E. Kulikowicz:** None. **S. Adams:** None. **B. Lester:** None. **J.K. Lee:** F. Consulting Fees (e.g., advisory boards); Dr. J Lee is a paid consultant for the United States Food and Drug Administration and for Edwards Life Sciences.. **L.J. Martin:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.08

Topic: C.08. Ischemia

Support: NIH NIBIB: R21EB024793
Roneet Carmell Memorial Endowment Fund

Title: Greater hyperperfusion immediately following resuscitation from cardiac arrest is associated with worse neurological outcome.

Authors: ***S. HAN**[1], A. BAZRAFKAN[2], M. RAFI[2], N. MAKI[2], S. DARA[2], A. LIU[2], J. MARTIN[2], Y. AKBARI[2,3,1];
[1]Anat. and Neurobio., [2]Neurol., [3]Beckman Laser Inst., Univ. of California-Irvine, Irvine, CA

Abstract: Introduction: Cardiac arrest (CA) affects millions in the US and many survivors have severe neurological deficits. Despite advancing treatments, there is still no effective treatment or guidelines on management to protect the brain. Shortly after resuscitation, there is a large spike in blood pressure (BP) and cerebral blood flow (CBF) called hyperperfusion, which lasts around 30 min, followed by hours of hypoperfusion, a state of low BP and CBF. Current American Heart Association guidelines only state that low BP during hypoperfusion phase should be avoided, but does not provide defined BP targets. Hyperperfusion is often missed due to it being transient and occurring early after resuscitation. However, this spike in BP and CBF may cause

reperfusion injury especially in the early stages when the blood-brain-barrier is still impaired. We investigated how the changes in BP and CBF during the highly dynamic first 30 min post-resuscitation may be related to injury and neurological outcome.
Methods: In 45 male Wistar rats, femoral BP and CBF was measured during CA and cardiopulmonary resuscitation (CPR). 32 rats underwent asphyxial CA without CBF monitoring, while 13 rats had CBF monitoring using laser speckle imaging. We quantified features of the BP and CBF curves during the hyperperfusion phase shortly post-CPR. We measured the time to reach the hyperperfusion peak, slope of the hyperperfusion curve, area under the curve of hyperperfusion for total hyperperfusion, and absolute value at the hyperperfusion peak. Neurological Deficit Score (NDS) was used as a behavioral outcome measure at 4hr and 24hr post-CPR. Fluorojade-B staining was done at 24hr post-CPR to assess for neuronal injury.
Results: In the non-CBF measured group, a higher hyperperfusion BP peak and faster time to peak were associated with worse 24hr NDS ($p<0.005$, $r=-0.53$, $p<0.01$, $r=-0.61$ respectively). Faster rise in BP during hyperperfusion and greater total hyperperfusion was associated with more neuronal injury in the thalamic reticular nucleus ($p<0.005$, $r=-0.68$, $p<0.05$, $r=-0.52$ respectively). In the CBF measured group, a higher hyperperfusion CBF peak correlated with worse 4hr NDS ($p<0.05$, $r=-0.68$).
Conclusion: In our preclinical model, we observed that a faster and greater hyperperfusion soon after resuscitation from CA was associated with more neuronal injury and worse neurological outcome. Too much perfusion shortly after resuscitation may potentially contribute to greater injury and so targeting an optimal BP after CA may reduce injury and improve outcome. These results may inform early hemodynamic interventions to improve neurological recovery in CA survivors.

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Poster

203. Mechanisms and Treatments for Ischemic Stroke I

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Program #/Poster #: 203.09

Topic: C.08. Ischemia

Support: NIH Grant F31NS124280 (GF)
NIH Grant R01NS120322 (TH)
NIH Grant R61NS123760 (CH)

Title: Digital neuropathology in translational models of cerebral ischemia using supervised machine learning

Authors: *G. M. FOGO[1], J. M. WIDER[2], E. GRULEY[2], J. MATHIEU[2], J. LIAO[2], K. J. EMAUS[1], C. H. HSU[2,3], R. W. NEUMAR[2], T. H. SANDERSON[2,4];

[1]Neurosci. Grad. Program, [2]Emergency Med., [3]Surgery, [4]Mol. and Integrative Physiol., Univ. of Michigan, Ann Arbor, MI

Abstract: Cerebral ischemic insults (i.e. stroke, cardiac arrest) continue to be leading causes of death and disability. There is a critical need for the development of therapeutics targeting neurological damage in these injuries. Preclinical trials of potential therapeutics require reliable animal models of injury and quantitative endpoints. A common issue for current translational efforts is the time consuming task of neuropathological assessment, a common primary outcome. Traditionally, these assessments are labor intensive and highly subjective. The meticulous nature of hand scoring/counting techniques in neuropathology creates a research bottleneck, thus slowing translational progress. To address this issue, we have generated a computational workflow that couples neuropathological staining and supervised machine learning for efficient and reliable quantification of neuronal cell death. To achieve this, we performed neuropathology in large animal models of cardiac arrest and neonatal hypoxic/ischemic encephalopathy (HIE) at short and long-term endpoints. Neonatal piglets were exposed to hypoxia/ischemia followed by a 14 day recovery period. Brain sections were stained with two common methods: cresyl violet and FluoroJade B. Cresyl violet images were taken from selectively vulnerable brain regions of the caudate nucleus, cingulate cortex, somatosensory cortex, along with ventral and dorsal hippocampus. Utilizing macros in ImageJ/FIJI and machine learning-based Weka segmentation, single cell segmentation was performed on cresyl violet stained tissue. From individual cells, measurements regarding size, morphology, staining intensity and distribution were captured. These variables served as inputs for trained random forest algorithms to classify cells as either alive or dead/degenerating. Classification outputs were then compiled to generate total cell counts and dead/live ratios for each brain region across injured and sham conditions. This methodology was further applied to an adult swine model of cardiac arrest with short term outcomes (24 hours). Our computational approach yielded neuropathological results that strongly correlated with FluoroJade staining, hand counting/classification, and qualitative scoring (1-5 scale) performed by experienced researchers. Here we present a rigorous and efficient computational workflow powered by supervised machine learning for the assessment of neuropathology in large animal models of cerebral ischemia. We believe further application of this methodology has the potential to greatly enhance the efficiency and reproducibility of neuroprotective preclinical trials.

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Poster

203. Mechanisms and Treatments for Ischemic Stroke I

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Program #/Poster #: 203.10

Topic: C.08. Ischemia

Support: NRF2017M3A9B4042583
NRF2021R1I1A1A0106129911

Title: Neurorestorative effects of conditioned medium of human pluripotent stem cell-derived neural precursor cells in ischemic stroke rat

Authors: *J. LEE[1], Y. LEE[2], M. KIM[3], H. KIM[4], H.-S. KIM[5];
[1]Res. Inst. of Hyperbaric Med. and Sci., Yonsei Univ. Wonju Col. of Med., Wonju, Korea, Republic of; [2]Dept. of Emergency Med., [3]Res. Inst. of Hyperbaric Med. and Sci., Yonsei Univ. Wonju Col. of Med., WONJU, Korea, Republic of; [4]Dept. of Emergency Med., Yonsei Univ. Wonju Severance Christian Hosp., WONJU, Korea, Republic of; [5]Dept. of Biomed. Sci., Catholic Kwandong Univ., Gangneung, Korea, Republic of

Abstract: Previous studies have shown that early therapeutic events of neural pre-cursor cells (NPCs) transplantation to animals with acute ischemic stroke readily protected neuronal cell damage and improved behavioral recovery through paracrine mechanisms. In this study, we tested the hypothesis that administration of conditioned medium from NPCs (NPC-CMs) could recapitulate the beneficial effects of cell transplantation. Rats with permanent middle cerebral artery occlusion (pMCAO) were randomly assigned to one of the following groups: PBS control, Vehicle (medium) controls, single (NPC-CM(S)) or multiple injection of NPC-CM(NPC-CM(M)) groups. A single intravenous injection of NPC-CM exhibited strong neuroregenerative potential to induce behavioral recovery, and multiple injections enhanced this activity further by suppressing inflammatory damage and inducing endogenous neurogenesis leading to histopathological and functional recovery. Proteome analysis of NPC-CM identified a number of proteins that are known to be associated with nervous system development, neurogenesis, and angiogenesis. In addition, transcriptome analysis revealed the importance of the inflammatory response during stroke recovery and some of the key hub genes in the interaction network were validated. Thus, our findings demonstrated that NPC-CM promoted functional recovery and reduced cerebral infract and inflammation with enhanced endogenous neurogenesis, and the results highlighted the potency of NPC-CM in stroke therapy.**Keywords**: conditioned medium, neural progenitor cells, ischemic stroke, proteome analysis, transcriptome analysis, neurogenesis, inflammation

Disclosures: **J. Lee:** None. **Y. Lee:** None. **M. Kim:** None. **H. Kim:** None. **H. Kim:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.11

Topic: C.08. Ischemia

Title: Protective effect of S allylcysteine administration to female rats after ischemia reperfusion injury

Authors: *M. ZENTENO-ANZURES, S. BAUTISTA-PEREZ, A. PEREZ-OLMOS, P. MALDONADO-JIMENEZ, A. ORTIZ-PLATA;
Natl. Inst. of Neurol. and Neurosurg., Ciudad de México, Mexico

Abstract: Stroke is a leading cause of death and disability worldwide of aged people. According to the cause stroke can be classified as hemorrhagic or ischemic; the second one is the more frequent and currently there is not a universal pharmacological treatment for it, the only pharmacological treatment approved is rTPA, with a therapeutic window of 4.5 hr in most cases. However, there are several compounds in preclinical studies that have shown protective properties, although in clinical trials fail, because this disease affects all population and preclinical studies only include male young animals without comorbidities. Between the possible treatments of stroke, S allylcysteine (SAC) has been used by its antioxidant and anti-inflammatory properties displayed after ischemic injury and other neurodegenerative diseases. Those effects had been studied with male rats and short periods of time after injury; however, the effect of SAC on neuroplasticity had not been proved.The objective of this work was to evaluate the effect of SAC administration on young female rats after 1 h of ischemia and 15 days of reperfusión, the ischemia was induced by the middle cerebral artery occlusion. Daily doses of SAC (100 mg/kg) were administered via intraperitoneal. Animals treated with SAC show physical improvement observed in the body weight, and fewer motor deficits compared to the ischemia group. SAC treated animals show a tendency of less memory impairment, anxiety and depression like behaviors. Also, SAC treatment diminishes the infarct area evaluated by Nissl, and hematoxylin and eosin stains, and increases the brain derived neurotrophic factor (BDNF) levels in the subventricular zone.In conclusion, SAC has a protective effect on female rats subjected to ischemia and 15 days of reperfusion, and this could be associated with the increase of the BDNF levels.

Disclosures: M. Zenteno-Anzures: None. **S. Bautista-Perez:** None. **A. Perez-Olmos:** None. **P. Maldonado-Jimenez:** None. **A. Ortiz-Plata:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.12

Topic: C.09.Stroke

Support: Department support

Title: Local field potential change during euthanasia

Authors: *J. TREJO, S. M. IZUAGBE, K. M. KILLIAN, Z. WANG, Y. B. PENG;
Dept. of Psychology, Univ. of Texas at Arlington, Arlington, TX

Abstract: Historically, the phenomenon of near-death experience (NDE) has raised many unanswered questions. In the present experiment, we utilize continuous local field potential

(LFP) during the euthanasia process to show the changes in power at designated frequency bands in four distinct regions of the brain from intracranially implanted electrodes: anterior cingulate cortex (ACC), thalamus (Po), trigeminal ganglion (TG), and the primary visual cortex (V1M). The LFP can be subdivided into five frequency bands: delta (0.1 - 3 Hz), theta (3 - 7 Hz), alpha (7 - 12 Hz), beta (12 - 30 Hz), and gamma (30 - 100 Hz). At the end of a migraine study, two groups of rats were euthanized: freely moving and anesthetized. In the freely moving group, after a one-week recovery from electrode implantation surgery, the animal was injected with nitroglycerin (NTG), a well-established migraine model. The LFP was recorded for 4.5 hours while animals were freely moving. CO_2 was then turned on after five minutes, with an additional 30 minutes of recording. In the anesthetized group, the LFP was recorded under isoflurane for 4.5 hours and euthanized immediately. The euthanasia process was the same in the freely moving group. The findings indicate that (1) in freely moving animals, a rebound spike in local field potential in all five frequency bands three minutes after CO_2 is turned on; (2) in the anesthetized group, a general decrease of power in all five frequency bands during the CO_2 euthanasia. This perhaps may be due to the rat being under continuous anesthesia for 4.5 hours, the smooth decrease of power in all frequency bands close to the bottom is significantly different from the pattern observed in the freely moving group in all Po, TG, and V1M. Previous studies have shown an overall increase in gamma activity during death. Overall, gamma activity was correlated to consciousness. While the neuronal activity might be too weak to generate a burst in the anesthetized group, the spiking at 2-3min observed in euthanasia in the freely moving group provides a potential explanation of the NDE phenomenon or terminal lucidity, as observed in some patients.

Disclosures: **J. Trejo:** None. **S.M. Izuagbe:** None. **K.M. Killian:** None. **Z. Wang:** None. **Y.B. Peng:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.13

Topic: C.09.Stroke

Support: Department of Psychology

Title: Phase amplitude coupling pattern change in local field potential during euthanasia

Authors: *K. M. KILLIAN, S. M. IZUAGBE, J. TREJO, Z. WANG, Y. B. PENG; Psychology, Univ. of Texas, Arlington, Arlington, TX

Abstract: This experiment utilizes phase amplitude coupling (PAC) using local field potential (LFP) signals during the euthanasia process to show the changes in power in four frequency bands at four regions of the brain from intracranially implanted electrodes: anterior cingulate cortex (ACC), thalamus (Po), trigeminal ganglion (TG), and the primary visual cortex (V1M).

Two groups of rats were present: freely moving and anesthetized. Both groups experienced 4.5-hour recordings from a different project and were euthanized at the end. After 5 minutes of baseline measurement CO_2 was turned on for the euthanasia process and an additional 30 minutes were recorded, with a total recording time of 35 minutes. PAC modulation index (MI) values were calculated and compared between two groups for each minute. PAC is divided into nine frequency band phases that span from 1 to 19, and frequency band amplitude spans from 1-99 Hz for every 2 Hz (i.e., 9 x 48 = 432 MIs for each minute). LFP data from these four channels were analyzed in PAC combinations (16 pairs). The findings indicate that (1) when comparing the freely moving group to the anesthetized group, the freely moving PAC MI values showed a delayed increase in MI values; (2) there were the most significant differences ($p < .05$) distributed from 7-12 minutes; (3) the PAC MI values were higher in the deceased rat when compared to the alive. However, there is no significant difference between the anesthetized and freely moving group after the animals were deceased (approx. >12 min). The underlying mechanisms that contribute to PAC change between the two states (alive vs. deceased) are unknown; however, the power analysis observed an increase of power in all frequency bands around 2-3 minutes after CO_2 was turned on during the euthanasia process. This may mean that the brain is somehow more active in the freely moving group when compared to anesthetized group. This may also contribute to the lower PAC values in the freely moving group. It is possible that when the animal is alive, the brain may become "de-synchronized" which could be a possible explanation for the PAC values being lower. When the rat is deceased (shows no neuronal activity) the brain may become "synchronized" again, brain death.

Disclosures: K.M. Killian: None. **S.M. Izuagbe:** None. **J. Trejo:** None. **Z. Wang:** None. **Y.B. Peng:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.14

Topic: C.09.Stroke

Support: Brazil Family Foundation
Bionics Queensland
CNNE Sustech-QBI
Alastair Rushworth Research Fund

Title: Effects of 40Hz non-invasive brain stimulation on neural and behavioural changes following photothrombotic ischemic stroke

Authors: *M. SAMANTZIS, L. TAINTON-HEAP, M. BALBI;
Queensland Brain Inst., Univ. of Queensland, Brisbane, Australia

Abstract: Stroke is a major cause of long term disability worldwide, however, current therapeutics are limited. Previous research in mice has demonstrated that optogenetic stimulation in the gamma frequency range, specifically at 40Hz, is beneficial for recovery post-stroke. However, this treatment option is highly invasive and not able to be easily translated to human patients. Thus, our project is investigating whether delivering non-invasive brain stimulation at 40Hz could be a potential therapeutic to restore neuronal dynamics and improve behaviour. In this study we used innovative automated home-cage technology to investigate the long-term neural and behavioural changes in mice following stroke. We performed simultaneous mesoscale brain imaging, behavioural recording, and brain stimulation. Baseline imaging was performed on awake mice over a period of three weeks, before inducing a photothrombotic stroke in the area between the primary motor cortex and somatosensory cortex. Motor impairments were assessed for three days prior to stroke, and seven days following stroke using the neurodeficit score and beam walking test. Cognitive function was assessed at day 28 post stroke using a novel object recognition test. Mice were imaged over a 30-day period following stroke and changes to DF/F, seed pixel correlations and functional nodes were investigated. Following stroke, our results show changes to both neuronal activity and connectivity, as well as motor and cognitive function. We also show that following brain stimulation there are altered patterns of neuronal activity and connectivity. These results suggest that non-invasive brain stimulation may be a potential therapeutic following stroke.

Disclosures: M. Samantzis: None. **L. Tainton-Heap:** None. **M. Balbi:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.15

Topic: C.09.Stroke

Support: Department of Veterans Affairs Merit Research Award I01BX002661
NIH Grant AA028175-01

Title: Transient focal ischemia in middle-aged mouse caused sleep disturbances similar to human stroke patients

Authors: *R. SHARMA[1], M. PARIKH[2], A. ZUNIGA[1], P. SAHOTA[3], M. M. THAKKAR[4]; [1]Truman VA Med. Center/University of Missouri, Columbia, MO; [2]Univ. of Missouri, Columbia, MO; [3]Neurol., Univ. of Missouri Columbia Sch. of Med., Columbia, MO; [4]Neurol., HSTMV Hospital/University of Missouri, Columbia, MO

Abstract: Background: Ischemic stroke (IS) is the fifth leading cause of mortality and highest contributor to disability annually. Approximately 15% of all IS occur in young adults, ages between 18 to 49 years, raising a major public health problems and long-term socioeconomic consequences. After IS, effective neuro-rehabilitation is a key factor in post-stroke recovery,

however, translational breakthroughs are long over-due. Sleep disturbances, frequently and chronically reported after IS, negatively impact recovery while accentuating the risk for stroke recurrence. Hence, to treat patients with IS, it is important that we understand how stroke affects sleep. In the present study, middle-aged C57BL/6J mice were used to examine the effect of middle cerebral artery occlusion (MCAO; widely used method to mimic IS in adult human population) on sleep-wakefulness. Our hypothesis: Middle-aged mice subjected to MCAO will display sleep disturbances similar to human stroke patient. Methods: To test our hypothesis, two surgeries were performed with a gap of one week. First, under sterile conditions and inhalation (isoflurane) anesthesia, stereotaxic surgery was performed to implant sleep recording electrodes and allowed animals to recover from surgical stress and habituate to a sleep recording setup. After one-week, focal cerebral ischemia was induced by middle cerebral artery occlusion (MCAO), via intraluminal technique, for 1h. In sham controls, similar surgery was performed except no occlusion was performed. Subsequently, animals were again hooked up to the recording set up after 12 h and initiated sleep recording for 10 days. Results: Our preliminary results showed that mice subjected to IS displayed altered sleep-wake rhythm such as insomnia-like symptoms during light (inactive) periods and daytime sleepiness during dark (active) periods, mimicking sleep among stroke patients. Conclusion: We believe that this is the first study to show the effects of IS on sleep-wakefulness in middle-aged mice. Understanding the mechanism of IS-induced sleep disturbances will help devise new and better therapeutic strategies for stroke recovery and rehabilitation.

Disclosures: R. Sharma: None. **M. Parikh:** None. **A. Zuniga:** None. **P. Sahota:** None. **M.M. Thakkar:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.16

Topic: C.09.Stroke

Support: NIH Grant T32 NS082145

Title: Mouse Skilled Forelimb Assay Modifications allow for High-Throughput Assessment of Motor Function after Stroke

Authors: *D. BETZ[1], A. BECKER[2], K. M. COTTER[3], A. M. SLOAN[4], A. M. STOWE[5], M. P. GOLDBERG[1];
[1]Neurol., UT Hlth. San Antonio, San Antonio, TX; [2]Behavioral Analysis, Univ. of North Texas, Denton, TX; [3]Neurol., Univ. of Kentucky Col. of Med., Lexington, KY; [4]Vulintus, Westminster, CO; [5]Univ. of Kentucky Med. Ctr., Lexington, KY

Abstract: Motor impairment is one of the most common long-term effects of stroke, making quantitative assays of rodent motor function critical to the field of stroke research. Modeling

motor function in mice additionally takes advantage of the wide availability of genetic and pharmacological tools. We have previously published an isometric pull task assay that features semi-automated training, automated quantification, measurable decline post-injury, and persistent deficits after focal, cortical stroke. This task can serve as a tool for assessing skilled forelimb function or rehabilitation following CNS injury. Here, we report modifications to our previously published protocol, namely the introduction of group training, standardized timelines and criteria, and modified adaptive training paradigms, that increase the throughput and efficacy of this test.Using a reward of peanut oil, mice are trained to reach and grasp a handle (isometric force transducer) that is positioned slightly outside of a pre-assembled operant chamber (Mototrak, Vulintus, Lafayette, CO). The Mototrak behavioral program records the number of attempts, force generated, success rate, and latency to maximal force based on feedback from the handle and an infrared beam sensor that detect reach behaviors. Our studies utilize adult male and female C57/Bl6 mice that receive unilateral stroke via the photothrombotic stroke model. First, mice underwent one week of group training where groups of 3-4 cagemate mice were trained to respond to the reinforcement apparatus, recognize peanut oil as a reinforcer, and reach for the isometric pull assembly (handle) with the appropriate paw. Then, mice were individually trained until they are able to perform 3 consecutive sessions with 50 pull attempts within 30 min and a success rate variance of < 15%, given a threshold for success of 20 grams. Once achieved, a three-day baseline measurement was obtained prior to stroke induction, and recovery was evaluated weekly post-injury. We found that this training paradigm significantly improved task rate, throughput, and baseline success rate measurement (nearly 100% pre-stroke in all mice). These results suggest that initial group training may be a strategy to improve training efficacy in mouse functional assays.

Disclosures: D. Betz: None. **A. Becker:** None. **K.M. Cotter:** None. **A.M. Sloan:** None. **A.M. Stowe:** None. **M.P. Goldberg:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.17

Topic: C.09.Stroke

Support: NS096246
NS125884

Title: The role of mitochondrial calcium uniporter (MCU) and MCUb in stroke

Authors: *T. NGUYEN, Z. LIN, G. WALTERS, N. DHANESHA, A. CHAUHAN, Y. USACHEV;
Univ. of Iowa, Iowa City, IA

Abstract: Stroke is the leading cause of death and long-term disability in the United States and worldwide. Current treatments for stroke have a narrow therapeutic window and currently there are no effective treatments to promote recovery following a stroke, suggesting the need for new effective therapeutic approaches. Following a stroke, mitochondrial dysfunction has shown to play a critical role in pathogenesis, and mitochondrial Ca^{2+} overload is implicated in neurotoxic processes including Ca^{2+} dependent excitotoxicity and neurodegeneration and can induce mitochondrial dysregulation. The mitochondrial calcium uniporter (MCU) is a highly conserved pore-forming subunit of the MCU complex that mediates Ca^{2+} uptake into the mitochondrial matrix. We found that deletion of MCU resulted in impaired Ca^{2+} uptake into mitochondria in neurons, inhibited mitochondrial Ca^{2+} dysregulation and excitotoxicity in brain mitochondria. MCUb is MCU paralog and is thought to function as a negative regulator of the MCU complex. We found that MCUb deletion increased mitochondria Ca^{2+} uptake in neurons. In vitro glutamate stimulation on cultured hippocampal neurons revealed that MCUb deletion increased glutamate-induced Ca^{2+} deregulation and excitotoxicity. To further determine the role of MCUb in ischemic stroke, we performed the Middle Cerebral Artery Occlusion (MCAO) in MCUb knockout mice. Interestingly, MCUb deletion increased stroke damage in male but not female mice. These results show that MCU and MCUb impose counteracting effects on neuronal Ca^{2+} and excitotoxicity and provides evidence of a critical role of mitochondria Ca^{2+} transport in stroke, which may lead to the development of new therapeutics that target MCU for treating this severe cerebrovascular disease.

Disclosures: T. Nguyen: None. **Z. Lin:** None. **G. Walters:** None. **N. Dhanesha:** None. **A. Chauhan:** None. **Y. Usachev:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.18

Topic: C.09.Stroke

Support: NRTS 2199 from the American Academy of Neurology (AAN; to WZ)
K08NS114165-01A1 from the National Institute of Neurological Disorders and Stroke (NINDS; to WZ)

Title: Contributions of Parvalbumin interneurons to peri-infarct circuit plasticity in the barrel cortex after photothrombotic stroke

Authors: *B. CAMPOS, B. VASQUEZ, W. ZEIGER;
UCLA, UCLA, Los Angeles, CA

Abstract: It has been widely hypothesized that adaptive plasticity of peri-infarct circuits contributes to functional recovery after stroke. Using photothrombotic (PT) strokes targeted to single barrels of the primary somatosensory barrel cortex (S1BF), we have previously found that

sensory-evoked activity from the whisker corresponding to the lesioned barrel is selectively reduced in layer 2/3 (L2/3) pyramidal neurons of surrounding barrels. Furthermore, we found a lack of functional remapping to spared peri-infarct circuits, even with forced-use rehabilitation. This suggests impaired adaptive plasticity in the peri-infarct cortex, but little is known about the mechanism. We hypothesize that Parvalbumin (PV) interneurons may be important regulators of plasticity post-stroke. To investigate the role PV cells in peri-infarct circuit remapping, we have been recording the activity of PV cells in the S1BF longitudinally using two-photon (2P) calcium imaging. First, we sought to define the normal spatial distribution of whisker responses in PV cells. Recording from L2/3 PV cells in the C1-whisker barrel of lightly anesthetized mice, we individually stimulated the C1, D1, B2 and E3 whiskers. We found that 39.80% of PV cells are responsive to C1 whisker stimulation, 14.06% to D1, only 1.82% to B2 and 1.02% to E3 whisker stimulation, closely mirroring the spatial distribution of whisker responses in L2/3 pyramidal cells. We next investigated PV cell responses during whisker trimming, a well-characterized paradigm for inducing plasticity in S1BF. We progressively trimmed whiskers until only the C1- and D1-, and finally only the D1-, whisker remained and recorded longitudinal changes in whisker-evoked responses of PV cells in the C1 barrel. We found that changes in PV cell responsivity were local, with reductions in the number of C1 responsive PV cells when the C1 whisker was spared (from 39.80% to 24.11%), but no significant change in responsivity of C1 barrel PV cells to the D1 whisker when the D1 whisker was spared. We are now applying these methods to investigate changes in PV cell activity in the peri-infarct cortex throughout recovery from stroke. Specifically, we induced PT strokes in the C1 barrel and imaged responses of PV cells to C1 and D2 whisker stimulation in the peri-infarct D2 barrel before and at 1, 2, 4, and 8 weeks post stroke. Although data analysis is not yet complete, these results will define changes in PV cell activity after stroke and contribute significantly to our understanding of the mechanisms regulating circuit plasticity in the peri-infarct cortex.

Disclosures: **B. Campos:** None. **B. Vasquez:** None. **W. Zeiger:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.19

Topic: C.09.Stroke

Support: NRTS 2199
NINDS Grant K08NS114165-01A1

Title: Longitudinal in-vivo imaging of Parvalbumin interneuron survival in the barrel cortex after photothrombotic stroke

Authors: *B. VASQUEZ, B. CAMPOS, M. EFTEKHARI, S. PANDA, W. ZEIGER; Neurol., UCLA, Los Angeles, CA

Abstract: Stroke is the fifth leading cause of death and a major cause of serious disability in the United States. Sensorimotor recovery is thought to be limited by GABAergic inhibition which curbs cortical excitability in the peri-infarct cortex. One type of interneuron, Parvalbumin (PV) interneurons, provide direct peri-somatic inhibition to excitatory neurons and play an important role in circuit plasticity in normal, healthy cortex. However, the effects of stroke on PV cells and their specific role in regulating circuit excitability after stroke and sensorimotor recovery remains unclear. Some studies have shown reduced numbers of PV cells by immunohistochemistry in peri-infarct cortex during recovery from stroke, but it is not known whether this reflects changes in PV cell survival or merely a reduction in parvalbumin protein expression. Here, we longitudinally assessed changes in the survival of PV cells for eight weeks after a photothrombotic (PT) ischemic stroke in the mouse barrel cortex (S1BF) using two-photon (2P) in vivo imaging of PV cells. First, we implanted a glass cranial window above the S1BF in 7-8-week-old PVCre:Ai9 double transgenic mice in which PV cells are labeled with the fluorescent protein tdTomato. We mapped the C1 barrel using intrinsic signal imaging and induced a PT stroke specifically targeting the C1 barrel. A second control group underwent sham PT strokes of the C1 barrel. We then used 2P microscopy to image and track all tdTomato-labeled PV cells up to 400 μm deep from a large region of the peri-infarct cortex rostral to the C1 barrel before stroke, and at 1, 2, 4, and 8 weeks after stroke. To quantify numbers of PV cells in the peri-infarct cortex over time in an unbiased fashion, we used the segmentation algorithm Cellpose and trained a customized cellular segmentation model using a subset of our data. A two-way ANOVA showed no significant effect ($p > 0.05$) of group, time, or group by time interaction in the number of PV cells in the peri-infarct cortex over eight weeks between the stroke (n=6) and sham control (n=7) groups. Our results demonstrate that after stroke and during recovery PV cell survival is largely static and there is not a delayed reduction in the number of PV cells in the peri-infarct cortex. In the future, we plan to investigate functional changes in peri-infarct PV cells to better define their roles in regulating cortical excitability and sensorimotor recovery after stroke.

Disclosures: **B. Vasquez:** None. **B. Campos:** None. **M. Eftekhari:** None. **S. Panda:** None. **W. Zeiger:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.20

Topic: C.09.Stroke

Support: AMED-CREST JP21gm1210010
JSPS KAKENHI 21K06386, 21H02820
Takeda Science Foundation
Uehara Memorial Foundation

Title: Functional connectivity is enhanced in the periinfarct sensorimotor cortex after ischemic stroke

Authors: *S. SAKAI, R. ISHIWATARI, Y. YOGIASHI, T. SHICHITA;
Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan

Abstract: Ischemic stroke causes neuronal cell death and destruction of neural circuits, resulting in impairment of motor, sensory and higher brain function. It has been thought that neurological functions are restored by the reorganization of neuronal circuits that compensate for the lost functions. Dendritic outgrowth and axon sprouting have been observed in the periinfarct area and the cortex contralateral to the injury region in animal stroke models. In human patients and animal models, it has been reported that connectivity among brain regions decreases immediately after stroke onset and is restored later. However, the detailed structure and functional significance of the neuronal circuits reorganized after stroke have not been elucidated. In this study, we analyzed the remodeling of functional connectivity among cortical areas after ischemic stroke in mice. Focal ischemic stroke was generated by photothrombosis in the hindlimb region of the primary somatosensory cortex. Wide-field calcium imaging of the entire cortex was performed in a mouse strain expressing GCaMP6f in excitatory neurons of the neocortex. Functional connectivity was calculated from the correlation of spontaneous activity under isoflurane anesthesia. In the periinfarct area, amplitude of spontaneous activity and response to hindlimb stimulation were reduced until 1 week after stroke onset and partially recovered 2 weeks later. Functional connectivity between somatosensory and motor cortices in the periinfarct area also decreased 1 week after stroke onset and was enhanced 3 weeks after stroke onset. Next, we examined whether the periinfarct area was important for the recovery of sensorimotor function by grid walk test. The results of the grid walk test showed that functional recovery occurred by 4 weeks after stroke onset. However, when the periinfarct somatosensory cortex was destroyed 8 weeks after stroke onset, the sensorimotor function declined again. These results indicate that the reorganized neuronal circuits in the periinfarct sensorimotor area would compensate for the lost neurological functions after ischemic stroke in the somatosensory cortex.

Disclosures: S. Sakai: None. **R. Ishiwatari:** None. **Y. Yogiashi:** None. **T. Shichita:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.21

Topic: C.09.Stroke

Support: NRF-2020R1A2C2008480
HI20C0408

Title: Amp-activated protein kinase (ampk) repression by the novel ampk inhibitor, 2g11, ameliorates global ischemic brain injury

Authors: *D. HONG[1], J.-W. EOM[2], A. KHO[1], S. LEE[1], B. KANG[1], S. LEE[1], Y.-H. KIM[2], J.-Y. KOH[3], B. CHOI[1], S. SUH[1];
[1]Hallym University, Col. of Med., Hallym University, Col. of Med., Chuncheon, Korea, Republic of; [2]Sejong Univ., Sejong Univ., Seoul, Korea, Republic of; [3]Univ. Ulsan Col. Med., Univ. Ulsan Col. Med., Seoul-City, Korea, Republic of

Abstract: Ischemic stroke causes severe brain pathologies and represents one of the greatest threats to the global health at large. Under certain conditions, such as an occlusion of blood vessels or a systemic reduction in blood flow due to a heart attack, insufficient supply of glucose, nutrients or oxygen can result in cerebral ischemia. These deleterious situations lead to activation of cell death cascades that evoke production of reactive oxygen species (ROS), excitotoxicity and ionic imbalance. AMP-activated protein kinase (AMPK) contains three subunits: the catalytic alpha subunit alpha, the regulatory beta subunit and the regulatory subunit gamma. Under physiological situations, phosphorylated AMPK is necessary for maintaining a positive energy balance and essential cellular processes such as glycolysis, gene transcription, glucose uptake and several other biological functions. However, brain injury-induced energy and metabolic stressors, like cerebral ischemia, increase AMPK phosphorylation. Highly phosphorylated AMPK contributes to excitotoxicity, oxidative and metabolic problems. As well, CNS injury-induced release of zinc in supra-physiological concentrations contributes to brain injury via mechanisms including ROS production, apoptotic cell death and DNA damage. For this reason, we hypothesized that regulating hyper-phosphorylated AMPK activity and excessive zinc release from synaptic vesicle is critical for protection against ischemic brain damage. Through compound C (a well-known AMPK inhibitor) structure-based virtual screening[1] , we identified a novel agent 2G11, which strikingly reduced zinc toxicity and AMPK activity when applied after GCI. In this study, we verified that 2G11 administration has neuroprotective effects via inhibition of AMPK activity and zinc translocation after global cerebral ischemia (GCI). In order to verify the effects of 2G11, we used a GCI animal disease model and oxygen glucose deprivation / reperfusion (OGD/R) in primary cultured hippocampal neurons. In this study, we confirmed that 2G11 treatment reduced AMPK hyper-phosphorylation, highly translocated zinc and finally neuronal death after ischemia. As a result, we have demonstrated that administration of 2G11 protected hippocampal neurons against GCI and OGD/R derived cellular damage. In conclusion, we propose that AMPK and it’s role in regulating synaptically released zinc may be a promising target to treat ischemic stroke.

Disclosures: D. Hong: None. **J. Eom:** None. **A. Kho:** None. **S. Lee:** None. **B. Kang:** None. **S. Lee:** None. **Y. Kim:** None. **J. Koh:** None. **B. Choi:** None. **S. Suh:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.22

Topic: C.09.Stroke

Title: Histological characteristics of ischemic stroke rat model according to behavioral experiment results

Authors: *H. JEONG, M. JEONG, G. KIM, S. YANG;
Incheon Natl. Univ., Incheon Natl. Univ., Incheon, Korea, Republic of

Abstract: Ischemic Stroke (IS) is a pathological symptom that arises from a brain infarction. The central cause of IS is the occlusion of arterial blood vessels. The location of an occluded blood vessel determines a neuropathological symptom associated with the brain area. In this study, we focused on the motor cortex that is closely involved in post-stroke motor impairment. To prepare an animal model that induces an infarction of the motor cortex, transient middle cerebral artery occlusion (tMCAO) was made in the rat. After that functional and anatomical outcomes were assessed using the modified Neurological Severity Score (mNSS) and measured infarct volumes, respectively. We found that mNSS is increased in damaged brain areas of the striatum and/or motor cortex. We are currently conducting experiments concerning the treatment of IS, especially the restoration of motor functions using a graphene-based multichannel array (MEA) as an electrotherapeutic method.

Disclosures: H. Jeong: None. **M. Jeong:** None. **G. Kim:** None. **S. Yang:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.23

Topic: C.09.Stroke

Support: NIH Grant R01NS093057

Title: Understanding mechanisms of stroke recovery through in vivo Ca^{2+} imaging of freely-behaving mice

Authors: *R. J. KOPCHOCK, III, T. CHIANG, H. CHEN, M. Y. CHENG, G. STEINBERG;
Neurosurg., Stanford Univ., Stanford, CA

Abstract: Stroke can lead to severe motor behavior deficits, but functional recovery may occur over time. Recovery outcome is attributed to neural circuit reorganization in areas adjacent and/or remote to the infarct. Understanding how these circuits are disrupted and reorganized is critical for the development of therapeutic strategies to augment stroke recovery. However, the specific cellular and circuit mechanisms facilitating post-stroke recovery remain unclear. In this study, we investigated changes in brain activity of the ipsilesional primary motor cortex (iM1) at cellular resolution using *in vivo* Ca^{2+} imaging in freely-behaving mice. To record mouse neuron activity, miniscopes were implanted into a GCaMP6 virus-infected region of the iM1. Following baseline activity recordings and behavior test measurements, strokes were induced via transient Middle Cerebral Artery occlusion (MCAo). Neuron activity and rotating beam behavior test

performance were then measured for 4 weeks to determine the longitudinal neuronal activity dynamics and their relation to stroke-induced deficits and recovery. Following stroke, there is a decrease in the number of active neurons detected, which then recovers gradually until returning to baseline around 2 weeks post-stroke. Neuronal firing rates are more variable after stroke, and there is a trend of reduction in the amplitude of these firing events, when compared to sham animals. Both neuron firing rate and amplitude in stroked animals recover to baseline after 2 weeks, which parallels the majority of the animals' recovery observed in the beam test behavior performance. Analysis of individual animal's neuron activity and behavior performance reveals a more detailed representation of how neuron activity dynamics are reflected in behavior changes. We observed different patterns of neuronal activity profile (number of detected neurons, neuron firing rate, and Ca^{2+} spike amplitude) between slow and fast recovered mice. Overall, this work provides a promising foundation for further investigation into the neural mechanisms underlying post-stroke deficits and recovery. By correlating behavior outcomes with changes in neuron activity, we can identify critical neuronal populations that mediate recovery after stroke.

Disclosures: R.J. Kopchock: None. **T. Chiang:** None. **H. Chen:** None. **M.Y. Cheng:** None. **G. Steinberg:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.24

Topic: C.09.Stroke

Support: PACEN Grant HC19C0028
NRF Grant 2022R1A2C1007948

Title: Protective effects of preconditioning with carbon dioxide inhalation against acute focal cerebral ischemia/reperfusion-induced brain injury in a rat middle cerebral artery occlusion model.

Authors: C. YOON[1], H. LEE[2], I. KWON[2], J. JUNG[1], Y. KIM[1,2], J. HEO[1,2], *H. NAM[1,2]; [1]Dept. of Neurol., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [2]Integrative Res. Ctr. for Cerebrovascular and Cardiovasc. Diseases, Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Stroke is a major cause of mortality and permanent neurological deficits all over the world. Despite its high incidence and death rate, clinical therapeutic options are still limited. The potential therapeutic target is to keep the ischemic penumbra alive and to prevent destruction of cerebral tissue. Ischemic preconditioning is a promising strategy to save the ischemic penumbra and reduce tissue damage. Carbon dioxide (CO_2) is the most potent vasodilator of cerebral vessels in the earth and CO_2 inhalation dramatically increases cerebral blood flow. However, it is not clear whether preconditioning treatment with CO_2 can effectively reduce cerebral tissue

damage and functional deficits. Hence, we evaluated the effects of preconditioning with CO_2 inhalation on focal ischemia-induced brain injury and neurological deficits in a rat ischemic stroke model. Adult male Wistar rats (270-320g, n=8) were inhaled with 20% CO_2 mixed gas (applied in 20% CO_2, 20% oxygen, and 60% nitrogen mixture) with 3 cycles of inhalation for 5 min followed by 10 min recovery (room air) before transient middle cerebral artery occlusion (tMCAO) for 90 min. To evaluate the neurological deficits, behavioral tests were blindly performed with the modified Neurological Severity Scores (mNSS), Longa and Garcia score at 24 h after tMCAO. In order to assess motor coordination and balance after stroke, we also performed the rotarod test and recorded the latency of rats to fall from the accelerating rotarod. We found that the mNSS was 0 in the sham and CO_2 pretreatment sham condition (n=5 per group). The CO_2 inhaled preconditioning tMCAO rats exhibited significantly lower mNSS compared to tMCAO rats (6±2.0 vs. 11±1.3, n=8, $p<0.0001$). Rats in both sham group and CO_2 pretreated sham group had the latencies in 300 sec. The latency was dramatically increased in CO_2 preconditioning tMCAO rats compared to tMCAO rats (99.5±53.5 sec vs. 27.5±8.7 sec, $p=0.0006$). Furthermore, preconditioning tMCAO rats with CO_2 showed significantly small infarction size compared to tMCAO rats (17.4±4.1% vs. 30.8±3.7%, $p<0.0001$). We demonstrated that CO_2 pretreatment plays an important role in reducing ischemic brain damage. In the sham animals, CO_2 inhalation did not show harmful effects and regional cerebral blood flow (rCBF) of CO_2 inhaled tMCAO group was increased (113.8%) as compared to tMCAO group (82.6%). Taken together, these findings suggest that preconditioning with CO_2 inhalation protects against ischemic brain injury and improves neurological outcomes without complication after acute ischemic stroke. Therefore, inhaled CO_2 preconditioning treatment may provide a novel therapeutic approach for ischemic stroke.

Disclosures: C. Yoon: None. **H. Lee:** None. **I. Kwon:** None. **J. Jung:** None. **Y. Kim:** None. **J. Heo:** None. **H. Nam:** None.

Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.25

Topic: C.09.Stroke

Support: PAPIIT-DGAPA IN207020
CONACYT A1-S-13219

Title: Spontaneous transient vasoconstriction at the beginning of reperfusion in experimental ischemic stroke

Authors: R. SANTANA-MARTINEZ, A. CARDENAS, J. TZOMPANTZI, ***L. B. TOVAR Y ROMO**;
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Abstract: Transient occlusion of the middle cerebral artery (tMCAo) by insertion of a monofilament through the internal carotid artery in rodents is arguably the most used in vivo model of ischemic stroke. Even though this model recapitulates most of the characteristics of ischemia and reperfusion phases of stroke, it harbors profound disadvantages in yielding reproducible infarct volumes across similar experimental conditions. It has been thoroughly discussed that such differences and high variance within experimental groups are due to biological disparities among experimental subjects that lie mainly in the anatomy of blood vessels. By monitoring blood perfusion levels with laser-Doppler flowmetry in the territory irrigated by the MCA in the rat brain, we observed that in 40% of animals, the reduced blood perfusion further plunges precisely at the moment of filament removal, indicating a component of vasoconstriction that is rapidly recovered in the following seconds to allow blood reperfusion. Animals that show this component have reproducible infarct volumes that better correlate with the percentage of decreased basal blood flow during tMCAo. We further examined this component in mice using in vivo two-photon microscopy to demonstrate blood flow dynamics at the reperfusion phase starting point. If stratified by the plunging component, data analyses of infarct volumes in animals subjected to tMCAo show a significantly reduced variability, contributing to a better understanding of the outcomes of experimental protocols employing this model and reducing the number of animals needed to draw significant conclusions. This work was supported by PAPIIT-DGAPA IN207020 and CONACYT A1-S-13219.

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Poster

203. Mechanisms and Treatments for Ischemic Stroke I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 203.26

Topic: C.08. Ischemia

Support: NIH Grant R01NS111021
K08 Grant NS088563
Department of Pediatrics R and D Grant
NIH Grant U54 HD090256

Title: Injury to the piriform-amygdalar area mediates risk taking behavior following perinatal hypoxic ischemic encephalopathy

Authors: T. SHEIKH, O. TAPARLI, E. BICKI, N. CAGATAY, M. HACKETT, S. YAPICI, N. AYCAN, N. DEVECI, B. OZAYDIN, P. FERRAZZANO, J. E. LEVINE, ***P. CENGIZ**; Univ. of Wisconsin, Madison, WI

Abstract: Background: Neonatal hypoxic ischemic (HI) encephalopathy may lead to cognitive disabilities, anxiety, risk taking behavior, learning and memory deficits in children. The severity

of injury to the limbic system that regulates emotion, behavior, long-term memory and olfaction may correlate closely with the outcomes of neonatal HI. We have previously shown that tyrosine kinase B (TrkB) mediated neuroprotection is estrogen receptor (ER) alpha dependent in the hippocampus and female biased following neonatal HI. Recently we have identified another region of the brain when injured increases the risk-taking behavior in females in early adulthood. To test the impact of piriform cortex and amygdalar area injury to anxiety and risk-taking behavior, elevated plus maze (EPM) was performed and MRIs were taken three months post-HI with and without TrkB agonist 7,8-dihydroxyflavone (7,8 DHF).**Methods:** HI was induced in postnatal (P) day 9 C57BL/6J mice by using Vannucci's HI model in mice. Mice were treated with 7,8 DHF (5mg/kg, i.p.) or vehicle control starting at 10 min post-HI for 7 days. At P 60+, mice underwent elevated plus maze (EPM) testing for 15 minutes. Five min segments of EPM video recordings were analyzed using AnyMaze software. Percent (%) time spent in the open arm is reported as mean ± SEM. ANOVA was used to analyze the EPM data. MRI was performed under anesthesia following EPM testing using a 4.7-tesla small animal MRI. Masks of piriform cortex were created using ITK-SNAP. Percent injury to the piriform cortex was calculated and correlated with each 5 min segment of the EPM test using linear regression. **Results:** Sham mice gradually spent less time in the open arm when they reached the last 5 min of the EPM test (10-15 min) ($p<0.05$). Female mice spent statistically significantly more time in the open arm in the 10-15 min segment of the test which was recovered by 7,8-DHF therapy ($p<0.05$) post-HI to the sham's level. Male mice demonstrated the complete opposite of female behavior post-HI and post-7,8-DHF therapy. The % time spent in the open arm positively correlated with the piriform cortex injury (R^2: 0,9, $p<0.001$). **Conclusion:** HI induces risk taking behavior in female mice which recovers with TrkB agonist therapy. Different pathways may be involved in neural circuitry regulating the male and female anxiety/risk taking behavior. However, our results suggest that injury to the limbic system, specifically to the piriform/amygdalar area, may be regulating the anxiety/risk taking behavior following neonatal HI.

Disclosures: **T. Sheikh:** A. Employment/Salary (full or part-time):; University of Wisconsin-Madison. **O. Taparli:** A. Employment/Salary (full or part-time):; University of Wisconsin-Madison. **E. Bicki:** A. Employment/Salary (full or part-time):; University of Wisconsin-Madison. **N. Cagatay:** None. **M. Hackett:** A. Employment/Salary (full or part-time):; University of Wisconsin-Madison. **S. Yapici:** A. Employment/Salary (full or part-time):; University of Wisconsin-Madison. **N. Aycan:** None. **N. Deveci:** None. **B. Ozaydin:** A. Employment/Salary (full or part-time):; University of Wisconsin-Madison. **P. Ferrazzano:** A. Employment/Salary (full or part-time):; University of Wisconsin-Madison. **J.E. Levine:** A. Employment/Salary (full or part-time):; University of Wisconsin-Madison. **P. Cengiz:** A. Employment/Salary (full or part-time):; University of Wisconsin-Madison.

Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 204.01

Topic: C.10. Brain Injury and Trauma

Support: NIH NS110609
Research Advisory Committee, Children's Hospital of Pittsburgh (COB)

Title: Combining α7 nicotinic acetylcholine receptor allosteric modulator and environmental enrichment improves sustained attention, cholinergic neurotransmission, and systemic inflammation after controlled cortical impact injury

Authors: *P. L. RENNERFELDT[1], R. A. REDDY[1], E. H. MOSCHONAS[1], N. S. RACE[1], T. RANELLONE[1], E. M. ANNAS[1], M. A. BERTOCCHI[1], J. P. CHENG[1], S. W. CARLSON[2], C. E. DIXON[2], A. E. KLINE[1], C. O. BONDI[1];
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Abstract: Traumatic brain injury (TBI) is a leading cause of cognitive disability worldwide. Post-TBI attentional impairments lack proven treatments and can result from cholinergic dysregulation, which suggests that pharmacological strategies that amplify activation of acetylcholine (ACh) receptors may ameliorate behavioral deficits. To more closely mimic the clinical setting, combining a pharmacological therapy with noninvasive rehabilitation (i.e., enriched environment, EE, housing) may prove to be an efficient approach for cognitive recovery. We predicted that chronic administration of NS-1738, a novel α7 nicotinic ACh receptor (α7-NAChR) type-I positive allosteric modulator (PAM), will improve sustained attention post-TBI, alone and in combination with EE. Blocking α7-NAChRs with methylycaconitine (MLA) will attenuate the beneficial effects of NS-1738, further confirming its mechanism of action. Adult male rats were trained in the 3-choice serial reaction time task (3-CSRT), reaching stable pre-injury baselines prior to moderate-severity right parietal controlled cortical impact (CCI) or sham injury. Rats were randomized to NS-1738 (3 mg/kg) or vehicle (1 mL/kg saline) starting post-injury day (PID) 1 and continued daily [subacute (7d); chronic (28d)]. The chronic paradigm co-investigated daily environmental enrichment (EE; 6h/d), and subgroups were also subjected to daily α7-NAChRs blockade via MLA (3 mg/kg) injections. 3-CSRT retrials occurred on PID 14-24. Medial prefrontal cortex (mPFC) Western blots assessed cholinergic markers [acetylcholinesterase (AChE), choline acetyltransferase (ChAT), and α7-NAChR]. Microarray analysis examined serum inflammatory gene expression. Statistical analysis utilized ANOVAs with Newman-Keuls post hoc tests. TBI rats exhibited impaired sustained attention versus shams ($p < 0.05$), which was improved by chronic NS-1738 ($p<0.05$) but not by subacute NS-1738 ($p > 0.05$) treatment. Moreover, NS-1738+EE rendered an additive effect on lowering omissions and improving inflammatory markers ($p < 0.05$) including TREM-1 (triggering receptor expressed on myeloid cells-1) and IL-1 RA (interleukin-1 receptor antagonist). TBI decreased mPFC ChAT and AChE ($p < 0.05$) with partial restoration by subacute NS-1738. TBI groups that received MLA demonstrated a reinstatement of performance deficits, as hypothesized. Our findings support benefits of α7-NAChR type-I PAM and/or EE treatment after experimental TBI on sustained attention, cholinergic neurotransmission, and systemic inflammation. Therefore, enhancing cholinergic transmission after TBI may be beneficial for neurobehavioral recovery.

Disclosures: P.L. Rennerfeldt: None. **R.A. Reddy:** None. **E.H. Moschonas:** None. **N.S. Race:** None. **T. Ranellone:** None. **E.M. Annas:** None. **M.A. Bertocchi:** None. **J.P. Cheng:** None. **S.W. Carlson:** None. **C.E. Dixon:** None. **A.E. Kline:** None. **C.O. Bondi:** None.

Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 204.02

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant NS110609
Research Advisory Committee Grant, Children's Hospital of Pittsburgh

Title: Psychosocial safety learning impairments and altered social preferences following parietal or frontal experimental TBI in rats

Authors: *N. S. RACE, M. BERTOCCHI, M. TOADER, E. H. MOSCHONAS, P. L. RENNERFELDT, J. P. CHENG, A. E. KLINE, C. O. BONDI;
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Abstract: Psychosocial function, defined as the cognitive integration of social and emotional behaviors, is often impaired following traumatic brain injury (TBI). Post-TBI psychosocial dysfunction negatively impacts quality of life and successful societal reintegration. Unfortunately, psychosocial dysfunction and associated post-TBI mental health disorders have little evidence supporting therapeutic interventions. Previous work found that blast TBI rats were unable to utilize a social safety signal (familiar partner rat) to overcome serial exposure to a highly penetrant anxiogenic stressor (bright light challenge) in a model of psychosocial safety learning. To investigate if the same paradigm could capture similar behavioral phenotypes in other TBI models, enhancing translational utility, we explored the social familiarity-induced anxiolysis (SoFiA) paradigm for the first time in rats exposed to controlled cortical impact injury (CCI). Adult male Sprague-Dawley rats (n = 7-11/group) were randomized and exposed to moderate severity right parietal CCI, right frontal CCI, or sham injury (craniectomy + anesthesia without impact). Assessing effects of sex and age is planned for future studies. After 14 day recovery, animals underwent serial SoFiA social interaction (SI) sessions with novel then familiar naïve partners under dim (non-anxiogenic) then bright (anxiogenic) lighting conditions for 7 consecutive days (recording SI time, scored by blinded investigators). Subsequently, social recognition/preference between two naïve partners (one novel, one familiar) was assessed under both lighting conditions (recording total and relative SI times). We observed profound, persistent significant reductions in SoFiA paradigm SI time ($p<0.05$, repeated measures ANOVA). The observed behavioral phenotype represents increased anxiety-like behavior unable to be safely coped with despite the presence of the social familiarity safety signal from the partner rats. Aberrant social preferences were also observed (reduced novelty seeking), but the ability to discriminate between novel and familiar partners was preserved. Psychosocial safety learning impairments in the SoFiA paradigm have now been observed in multiple TBI models, including for the first time in a CCI model in the present exploratory work. These data suggest the observed behavioral phenotype is robust and can be observed under numerous injury conditions, an important feature relevant to TBI clinical heterogeneity. SoFiA may represent a useful

preclinical paradigm for investigating the neurological underpinnings of and testing potential treatments for post-TBI psychosocial impairments.

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Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 204.03

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 5R01NS084967
NIH Grant 5R01NS121037

Title: A bridge to recovery: Amantadine prior to environmental enrichment hastens neurobehavior and cognition after brain trauma

Authors: *E. H. MOSCHONAS[1], R. A. BITTNER[2], V. J. VOZZELLA[2], C. J. BRENNAN[2], R. R. ELETI[2], J. P. CHENG[2], C. O. BONDI[1], A. E. KLINE[1];
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Abstract: Environmental enrichment (EE) ameliorates recovery after traumatic brain injury (TBI). EE has traditionally been provided immediately after TBI, but this approach does not always mimic clinical practice as rehabilitation is typically not initiated early after TBI because of injury limitations and the focus on life saving measures. Yet, treating TBI early may facilitate recovery. Hence, we sought to use amantadine (AMT) as a bridge therapy before commencing EE. It was hypothesized that bridging EE with AMT would result in better neurobehavioral benefits than AMT or EE alone. Isoflurane-anesthetized male rats received a cortical impact of moderate severity (2.8 mm deformation at 4 m/s) or sham injury and then housed in standard (STD) conditions for one week while they received a once daily i.p. administration of either AMT (10 mg/kg or 20 mg/kg) or saline vehicle (1 mL/kg) for 6 days (bridge). EE, which was presented in a manner to better imitate clinical rehabilitation (e.g., 6 h/day), began on day 7 for the AMT bridge and continuous EE groups. Motor (beam-walking) and cognition (acquisition of spatial learning and memory) were evaluated on days 7-11 and 14-19, respectively. Cortical lesion volume and hippocampal cell survival were quantified on day 21. Delayed and abbreviated EE, whether provided alone or in combination with AMT benefited both motor and cognition ($p<0.05$) vs. STD housing. The 20 mg/kg AMT bridge + EE group performed better than the EE alone group in the cognitive tests ($p<0.05$) but did not differ from EE alone or 10 mg/kg AMT bridge + EE in motor function ($p>0.05$). The added cognitive benefit of bridging EE with AMT (20 mg/kg) supports the hypothesis. These data also demonstrate that neither

immediate nor continuous EE is necessary to confer benefits after TBI, which replicate previous studies from our laboratory and validate this delayed-and-abbreviated paradigm as a clinically relevant model of neurorehabilitation.

Disclosures: E.H. Moschonas: None. **R.A. Bittner:** None. **V.J. Vozzella:** None. **C.J. Brennan:** None. **R.R. Eleti:** None. **J.P. Cheng:** None. **C.O. Bondi:** None. **A.E. Kline:** None.

Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

Location: SDCC Halls B-H

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Program #/Poster #: 204.04

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant NS084967 (AEK)
Research Advisory Committee, Children's Hospital of Pittsburgh of UPMC (COB)
María Zambrano Excellence Program from the Ministry of Science and Innovation and the University of Valladolid, Spain (VT)
Internationalization program of Junta de Castilla y Leon, Spain (CL-EI-2021 IBGM, VT)

Title: Environmental Enrichment Improves Traumatic Brain Injury-Induced Behavioral Phenotype and Associated Neurodegenerative Process

Authors: V. TAPIAS[1], E. H. MOSCHONAS[2], C. O. BONDI[2], V. J. VOZZELLA[2], I. N. COOPER[2], J. P. CHENG[2], N. LAJUD[3], *A. E. KLINE[2];
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Abstract: Traumatic brain injury (TBI) causes persistent cognitive impairment and neurodegeneration. Environmental enrichment (EE) refers to a housing condition that promotes sensory and social stimulation and improves cognition and motor performance but the underlying mechanisms responsible for such beneficial effects are not well defined. A reasonable explanation is that EE attenuates deleterious secondary sequelae like oxidative damage and neuroinflammation. In this study, anesthetized adult male rats received either a moderate-to-severe controlled cortical impact (CCI) or sham surgery and then were housed in either EE or standard conditions. The results showed a significant increase in protein nitration and oxidation of lipids, impaired cognition and motor performance, and augmented N-methyl-D-aspartate receptor subtype-1 (NMDAR1) levels. However, EE initiated 24 h after CCI resulted in reduced oxidative insult and microglial activation and significant improvement in beam-balance/walk performance and both spatial learning and memory. EE may exert a neuroprotective effect via sustained downregulation of NMDAR1.

Disclosures: **V. Tapias:** None. **E.H. Moschonas:** None. **C.O. Bondi:** None. **V.J. Vozzella:** None. **I.N. Cooper:** None. **J.P. Cheng:** None. **N. Lajud:** None. **A.E. Kline:** None.

Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 204.05

Topic: C.10. Brain Injury and Trauma

Support: NYS OPWDD Fellowship

Title: Characterizing the development of posttraumatic epileptogenesis in rats after controlled cortical impact injury

Authors: *A. MEJIA-BAUTISTA[1], D. S. LING[1], J. H. GOODMAN[2,1];
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Abstract: Posttraumatic epilepsy (PTE) can occur in up to 40% of patients who sustain a severe traumatic brain injury (TBI). Currently, there are no clinical treatments available that prevent the development of PTE. Our group has previously demonstrated that in the controlled cortical impact (CCI) model of TBI in rats, there is a progressive increase in evoked and spontaneous epileptiform activity in the weeks after injury, as measured in *ex vivo* neocortical slices. In the present study, we have begun to evaluate the development of seizures *in vivo*, using EEG recordings in freely moving rats. A severe CCI injury (2mm depth, 4 m/s) was induced in the somatosensory cortex of Sprague Dawley rats (P25-30). During the same surgery, a wireless EEG transmitter (Biopac Epoch) was implanted with one electrode placed in the cortex ipsilateral to the injury and the other electrode placed contralaterally. Due to the low incidence of seizure activity in rodent TBI models, a pentylenetetrazol (PTZ) challenge was used to assess epileptogenesis. Starting at two weeks post-CCI, rats were tested for seizure susceptibility once per week, using a single, subconvulsant dose of PTZ (25 mg/kg, i.p.). For each animal, baseline EEG and behavior were recorded for 30 minutes, at which time the PTZ was administered. Electrographic and behavioral seizure activity using a modified Racine scale was then observed for an additional 30 minutes. Sham-injured controls were tested in the same manner. PTZ-evoked seizure activity was observed on the injured side at 3-4 weeks post CCI injury accompanied by stage 1-2 behavioral seizures. Over the subsequent weeks, there was a progressive intensification of PTZ-evoked seizure events, with an increase in the duration and frequency of epileptiform burst discharges that were accompanied by increased behavioral seizure severity. By week 7 after CCI, spontaneous epileptiform activity was recorded ipsilateral to the injury site during the pre-PTZ monitoring period. Following injection of PTZ, the epileptiform activity began on the ipsilateral side, and it spread to the contralateral side. In subsequent PTZ challenges stage 5 behavioral seizures were observed. This approach has the

potential to help us characterize the posttraumatic epileptogenic process in a quantifiable manner, and test the efficacy of potential anti-epileptogenic therapies to prevent PTE.

Disclosures: A. Mejia-Bautista: None. **D.S. Ling:** None. **J.H. Goodman:** None.

Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 204.06

Topic: C.10. Brain Injury and Trauma

Title: The Impact of Dietary Polyphenols on Sequelae of Nervous System Insult

Authors: *T. TOGASHI[1], A. D. TROFIMOVA[2], W. L. HARDEMAN[2], M. MENDOZA[3], E. GIANG[2], R. E. HARTMAN[2];
[1]Psychology, Loma Linda Univ., Redlands, CA; [2]Psychology, Loma Linda Univ., Loma Linda, CA; [3]Psychology, Loma Linda Univ., LOMA LINDA, CA

Abstract: Traumatic brain injuries (TBIs) are a leading cause of death and disability, with more than 10 million reported globally each year. TBIs can induce long-lasting social and behavioral deficits, with some individuals at risk for symptoms as debilitating as constant suicidal ideation. In the case of severe or repeated injuries, there is an increased risk of neurodegeneration and death.Radiation exposure and TBI each have the potential to impair performance on cognitive and sensorimotor tasks, but the nature and degree of the interaction between these two nervous system insults is currently unclear. Furthermore, there are currently no proven pharmaceutical strategies to either protect against or repair nervous system damage initiated by irradiation and/or TBI. Here we show that a high-throughput *Drosophila* model of TBI induced climbing deficits, hypoactivity, and reduced lifespan, but that exposure to low doses of proton irradiation did not have significant effects on behavioral measures. Dietary supplementation with pomegranate polyphenols significantly increased lifespan regardless of TBI or irradiation status. These results suggest that dietary supplementation with polyphenols may increase the nervous system's resilience to insults such as TBI or irradiation. Furthermore, the data demonstrate a significant increase in longevity with dietary polyphenols regardless of injury status. These findings could be especially meaningful for populations at higher risk of possible injury and warrant further investigation into the use of polyphenols as a prophylactic treatment for possible neurodegenerative diseases. These findings also signify that this low-cost, high-throughput fruit fly model of TBI is effective at inducing behavior deficits and reduced lifespan. This methodology has produced replicable results across multiple experiments in our laboratory, suggesting that this is a useful platform with which to study varying outcomes and interventions related to nervous system injuries.

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Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

Location: SDCC Halls B-H

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Program #/Poster #: 204.07

Topic: C.10. Brain Injury and Trauma

Title: Neurodegeneration and inflammation attenuated by acetyl-L-carnitine in a C57BL/6J model of repetitive mild traumatic brain injury

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Abstract: Background: Repetitive mild traumatic brain injuries (mTBI) are a risk factor for the development of neurodegenerative disorders such as chronic traumatic encephalopathy (CTE), a disease with signature tau protein neuropathology and concomitant behavioural, motor control, and memory impairment. This exploratory study used a mouse model of repetitive mTBI to: (1) investigate the response of genes regulating neurodegeneration and neuroinflammation pathways, and (2) determine the efficacy of prophylactic acetyl L-carnitine (ALC) treatment in ameliorating the deleterious chronic changes induced by injury.
Methods: 12-week old male C57BL/6J mice were allocated to either a repetitive mTBI group with no ALC treatment, mTBI with ALC, no impact and ALC, or a control group involving repeated anesthetization without impact. 15 mTBIs were administered in 24 days using a modified weight drop apparatus previously shown to induce CTE-like pathology. ALC was administered as 600 mg/kg/day via subcutaneous injection, commencing 14 days prior to the first mTBI and continuing through the impact schedule. The prefrontal cortex and hippocampus were collected at 3 months post-injury and were examined by real-time RT-PCR for *Mapt, Tardbp, Gfap, and Ccl11* gene expression (n = 4 per group). To enable blinding conditions, samples were coded so that group names were not accessible to the investigators undertaking analysis.
Results: Analyses of gene expression in the prefrontal cortex showed elevated mRNA levels of *Tardbp* and *Ccl11* in the impact group that were reduced to baseline in the impact + ALC treatment group. In the hippocampus, gene expression of *Mapt, Tardbp and Gfap* was elevated in the impact group with levels normalised in the impact + ALC treatment group.
Conclusion: These data suggest that the cumulative effect of the impacts was sufficient to induce elevated neurodegenerative and inflammatory mRNA expression in key brain structures, with ALC protecting against chronic disruption. Previous models of neurodegeneration and injury incorporating ALC treatment have shown neuroprotection through anti-inflammatory effects. Future studies in our laboratory will examine an expanded panel of genes and proteins to determine if ALC mitigates damage inflicted in these various secondary neurodegenerative cascades following repetitive mTBI.

Disclosures: **M.I. Hiskens:** None. **A. Schneiders:** None. **K. Li:** None. **A. Fenning:** None.

Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

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Program #/Poster #: 204.08

Topic: C.10. Brain Injury and Trauma

Support: VA Grant 1BX004256
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NIH Grant T32AI132164

Title: Investigating the role of the complement system in cognitive decline after repetitive mild closed head brain injury

Authors: *K. MALLAH[1], D. BORUCKI[2], A. TOUTONJI[1], C. COUCH[3], D. HATCHELL[1], G. HARDIMAN[5], F. KOBEISSY[6], C. KRIEG[4], S. GUGLIETTA[1], S. TOMLINSON[1];
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Abstract: Repetitive mild closed head injury (rmCHI) results in the development of cognitive deficits and may lead to neurodegenerative diseases later in life. The underlying neuroimmune mechanisms linking rmCHI to cognitive decline are not well understood, and the role of the complement system in this context is unexplored. We developed a mouse model of rmCHI and compared pathophysiological and cognitive outcomes in animals subjected to 12 head impacts to non-injured animals. Immunofluorescence microscopy and flow cytometry was used to characterize local and peripheral immune cell recruitment after injury. Animals subjected to 12 hits exhibit worsened spatial learning on the Barnes Maze task as well as memory retention. Flow cytometry analysis revealed increased infiltration of innate and adaptive immune cells such as neutrophils. Immunofluorescence staining showed elevated complement expression in the hippocampus of the 12-hit group compared to non-injured animals. In a therapeutic approach, we treated animals with an injury site-targeted complement inhibitor, CR2Crry, which inhibits all activation pathways of complement at the central C3 cleavage step. CR2Crry treatment significantly improved memory retention on the Barnes maze compared to non-treated animals, which was coupled with a modification in the abundance of distinct microglial sub-populations as revealed by high dimensional analysis using CyTOF. This was associated with a reduction in several neurodegenerative associated pathways as shown by RNAseq and proteomics. In conclusion, we developed and characterized a closed head repetitive injury model and demonstrated a role for complement in cognitive decline post rmCHI. Targeting the complement system as a therapeutic approach in repetitive brain injuries requires further investigation.

Disclosures: **K. Mallah:** None. **D. Borucki:** None. **A. Toutonji:** None. **C. Couch:** None. **D. Hatchell:** None. **G. Hardiman:** None. **F. Kobeissy:** None. **C. Krieg:** None. **S. Guglietta:** None. **S. Tomlinson:** F. Consulting Fees (e.g., advisory boards); Consultant for Q32 Bio..

Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 204.09

Topic: C.10. Brain Injury and Trauma

Title: Diffuse traumatic axonal injury in mice: Sensory-motor, emotional and cognitive deficits

Authors: *H. SHAN[1,2], M. I. SCHEUBER[1,2], L. RODRÍGUEZ PERIS[1], M. E. SCHWAB[1,2]; [1]Univ. of Zurich, Schlieren, Switzerland; [2]Neurosci. center Zurich (ZNZ), UZH and ETH, Zurich, Switzerland

Abstract: Closed skull severe concussions are a frequent form of traumatic brain injury (TBI). Histologically, local small disruptions of blood vessels and fiber tracts are seen in different CNS regions, followed by inflammatory events including edema. To study potential regenerative effects of e.g. antibodies against the neurite growth inhibitory factor Nogo-A, we established a TBI model in adult mice with detailed quantitative read-outs of motor as well as complex brain functions. A metal rod (up to 110 g) was dropped vertically from up to 60 cm height through a guiding tube to the midline of the skull of adult C57BL/6J mice. The mice were supported by a sponge of intermediate softness with its head fixed laterally (modified Marmarou model). Motor ability and coordination were measured on the accelerating Rotarod (three parameters) and narrowing beam walk test, while emotional behavior/anxiety was assessed on the elevated plus maze (two arms open, two arms closed). Exploratory behavior and short-term object memory were tested using the novel object recognition test quantifying the object preference and distinction between lasting and novel object 6h after habituation. Sociability and social novelty were tested in a 3-chamber setup with paper balls and ‘stranger’ mice by measuring the sniffing time and determining the preference and discrimination between paper balls and mice, previously encountered mice and novel stranger mice, respectively. Finally, Barnes maze test (20 holes maze with escape box) was used to test spatial orientation, learning and long-term memory as well as the plasticity of memory (switch of escape box position). Lighter lesions led to only transitory deficits which recovered at 2-3 weeks. The more severe lesions produced long-term deficits in object recognition, social interaction and maze behavior, while locomotor behavior recovered to normal levels. Interestingly, some of the deficits were most pronounced only at 2-3 wks after injury. Histologically, GFAP was upregulated in local cortical areas 5 wks after surgery. In the next step, we are planning to apply anti-Nogo-A antibody to investigate whether inhibition of Nogo-A can enhance the functional recovery in specific behaviors and if enhanced axonal compensatory or regenerative sprouting can be induced in specific CNS regions after TBI.

Disclosures: **H. Shan:** None. **M.I. Scheuber:** None. **L. Rodríguez Peris:** None. **M.E. Schwab:** None.

Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 204.10

Topic: C.10. Brain Injury and Trauma

Support: the MN State SCI and TBI Research Program

Title: Characterization of Murine Repetitive Mild Traumatic Brain Injury Model.

Authors: *C. CHOI[1], R. C. ARMSTRONG[3], I. A. SCARISBRICK[1,2];
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Abstract: Repetitive mild traumatic brain injury (RmTBI) has emerged as a growing health care concern among athletes and military personnel. A mild TBI does not generate gross neuropathological changes, but the cumulative effect of mild injuries can result in substantial neurological impairment. Recent studies also report a negative association between RmTBI and earlier onset of neurodegenerative diseases. Despite its clinical significance, the molecular mechanisms underpinning human RmTBI pathology remain largely uncharacterized underscoring the need for development of an optimized animal model. Here, we characterized a murine model of RmTBI which is a modification of that recently reported (Yu et al., J Neurotrauma. 2017). There are several key features of the model. First, this is a closed-head model without scalp incision that can be completed in 5-10 min under isoflurane anesthesia allowing repeated impacts over a period of several days. Second, the model uses a modified stereotaxic frame to secure the head and the impactor tip can be positioned by stereotaxic coordinates to improve reproducibility. Unlike classic frame, rubber ear bars are used to permit restricted head movement. Finally, using the controlled impactor device, the impact parameters can be readily controlled (such as the impactor tip size, velocity, and dwell time), affording adjustable and reproducible RmTBI. In this study, 10 wk C57BL6/J male and female mice received a RmTBI using an Impact One impactor (Leica) onto the scalp approximately over bregma using a 3-mm-diameter tip (velocity: 4.0 m/sec; depth: 1.6 mm; dwell time: 200 ms) five times over 5 d (one impact/d). Sham mice underwent identical procedures without impact. There were no behavioral impairments immediately after impact, and skull fracture or intracranial bleeding were rare. RmTBI mice showed a significantly increased righting time (Sham 49.4 ± 3.4 s *vs* RmTBI 247.6 ± 2.7 s) and apnea time (Sham 0.0 ± 0.73s *vs* RmTBI 11.1 ± 0.6 s). Compared with the sham group, RmTBI group showed impaired social interaction (Sociability chamber) and reduced anxiety-related behavior (Zero-Maze), but no changes in short-term spatial working memory (Y-Maze, Noldus) at 4 wk after injury. Histological analyses revealed

mild astroglial and microglial activation in the cortex and corpus callosum. There is a need to further investigate axonal damage and demyelination in this model. These results suggest that our RmTBI model can reproduce clinically relevant behavioral changes, making it suitable for characterization of the neuropathological and behavioral consequences of RmTBI, and for screening potential therapeutic strategies for human disease.

Disclosures: C. Choi: None. **R.C. Armstrong:** None. **I.A. Scarisbrick:** None.

Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 204.11

Topic: C.10. Brain Injury and Trauma

Support: SERB, GOI; EMR/ 2017/000621
SERB, GOI; CRG/ 2020/004971
SERB, GOI; CRG/ 2021/008295
UGC, GOI; Senior research fellowship

Title: Gut microbiota is involved in epigenetic reprogramming in the amygdala: A role in repeated mild traumatic brain injury (rMTBI)-induced anxiety

Authors: *G. JADHAV, A. SAKHARKAR;
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Abstract: Anxiety is the most common hallmark of post-traumatic stress disorder (PTSD). Gut homeostasis maintains the intricate dialogue between host metabolism and brain function. Stress-induced gut dysbiosis is strongly linked with anxiety behaviors and vice versa. However, the underlying mechanisms of how gut-microbiota exerts its effects on the brain and behavior are largely unknown. We have previously reported that repeated mild traumatic brain injuries (rMTBI) cause gut dysbiosis owing to a reduction in the population of butyrate-producing bacterial families (Matharu et al., 2019, J Biosci 44:120). The current study addresses the question of whether rMTBI-induced gut dysbiosis impedes synaptic plasticity in the amygdala via epigenetic remodelling and manifests into anxiety behavior. Hence, a closed-head weight drop injury paradigm was employed to induce rMTBI in adult male Wistar rats (n=8) and anxiety behaviors were tested after 48 hours and 30 days. While fecal microbiota transplant (FMT) from a healthy donor (Naïve rats; 1ml intragastric, 10 doses on alternate days) and single strain probiotic - *Lactobacillus rhamnosus* GG (LGG; ATCC 53103) (1ml of 10^8 CFU/ml) were employed for gut probiosis, sodium butyrate (SB) treatment (500 mg/Kg) was used to correct the metabolite deficits caused due to dysbiosis. The epigenetic regulation of brain-derived neurotrophic factor (BDNF) expression in the amygdala of trauma-exposed rats was investigated. rMTBI provoked anxiety-like behavior and reduced BDNF mRNA and protein levels reflecting a loss in synaptic plasticity. Chromatin immunoprecipitation assays indicated hypoacetylation (H3-

K9ac) and higher binding of histone deacetylase 2 (HDAC2) at BDNF-IX promoter post rMTBI, whereas methylated DNA immunoprecipitation showed DNA hypermethylation (5mC). In addition, the co-operative function of DNA methylation and histone deacetylation was enhanced by higher MeCP2 binding, advocating the formation of a repressor complex. FMT, LGG, and SB normalized the rMTBI-induced anxiety, likely by dismantling the repressor complex at BDNF-IX promoter and thereby restoring BDNF levels in the amygdala. To decipher the substantial role of gut dysbiosis in neuro-epigenetic regulation of anxiety, FMT was offered to naïve recipients by using fecal microbiota of trauma-exposed rats. Interestingly, it elicited anxiety and lowered amygdaloid BDNF levels in naïve rats confirming the potential of gut-dysbiosis in driving stress-induced brain functions. In conclusion, gut microbiota may exert neuro-epigenetic effects and orchestrate the synaptic plasticity responsible for experience-dependent behavioural outcomes.

Disclosures: G. Jadhav: None. **A. Sakharkar:** None.

Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 204.12

Topic: C.10. Brain Injury and Trauma

Support: VA IK6BX005235
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The Neurosurgery Research and Education Foundation (NREF) Research Fellowship Grant

Title: P-selectin and Complement in the Pathological Sequelae of Germinal Matrix Hemorrhage

Authors: *D. HATCHELL, M. ALSHAREEF, T. VASAS, C. GUO, R. ESKANDARI, S. TOMLINSON;
Med. Univ. of South Carolina (MU Neurosci. Inst. - Grad., Charleston, SC

Abstract: Germinal matrix hemorrhage (GMH) is a devastating disease of pre-term infancy, with intraventricular hemorrhage resulting in post-hemorrhagic hydrocephalus (PHH), periventricular leukomalacia, and neurocognitive deficits. Our recent studies indicate a role for complement in the pathogenic sequelae of GMH. Here we demonstrate vascular expression of the adhesion molecule P-selectin after GMH, and investigate a strategy to specifically target complement inhibition to sites of P-selectin expression. We prepared two fusion proteins consisting of one of two anti-P-selectin single chain antibodies (scFv's) linked to the complement inhibitor Crry. One scFv targeting vehicle (2.12scFv) blocked the cell adhesion (PSGL-1) site of P-selectin, whereas the other (2.3scFv) bound P-selectin without blocking its function. Post-natal mice on day 4 (P4) were subjected to collagenase induced-GMH and treated with 2.3Psel-Crry, 2.12Psel-Crry, or vehicle. Histopathological and behavioral analyses were

performed at P14 and P45. Following GMH, 2.3Psel-Crry treatment resulted in reduced mortality, infarct size, and neurological deficits in adolescence, whereas 2.12Psel-Crry treatment resulted in worse outcomes. MRI revealed that 2.3Psel-Crry, but not 2.12Psel-Crry, reduced PHH development. Improved outcomes with 2.3Psel-Crry were accompanied by decreased inflammatory P-selectin expression, complement deposition, and microgliosis. While investigating the reason for this discrepancy in outcomes, we found that 2.12Psel-Crry, but not 2.3Psel-Crry, interfered with the coagulation cascade as determined by increased coagulation time and decreased platelet-leukocyte aggregation. This additional activity of 2.12Psel-Crry is likely due to its blockade of P-selectin function on platelets and provides an explanation for the worse outcome with 2.12Psel-Crry in this hemorrhagic condition. In conclusion, GMH induces expression of P-selectin, the targeting of which with a complement inhibitor protects against the pathogenic sequelae of GMH. The unexpected worsened outcomes with the 2.12Psel-Crry construct can be explained by its effect on interfering with the coagulation cascade, likely via inhibiting heterotypic platelet aggregation. Whereas the 2.3Psel-Crry construct has potential for protecting against the pathogenic sequelae of GMH, the 2.12Psel-Crry construct has potential for treatment of conditions that incorporate pathological thrombotic events, such as ischemic stroke.

Disclosures: D. Hatchell: None. **M. Alshareef:** None. **T. Vasas:** None. **C. Guo:** None. **R. Eskandari:** None. **S. Tomlinson:** None.

Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 204.13

Topic: C.10. Brain Injury and Trauma

Title: In vivo imaging system for cellular level visualization of the brain in a live mouse model

Authors: *S. HONG[1], A. KIM[2];
[1]KAIST, Daejeon, Korea, Republic of; [2]IVIM Technol., Daejeon, Korea, Republic of

Abstract: Many kinds of research have been conducted with *ex vivo* histological observation to investigate the central nervous system and the pathological mechanism in neuroinflammation. However, it could provide only static information at a single time point of harvest, which gave significant limitations in understanding the *in vivo* dynamically changing cellular behaviors in the central nervous system. Although fluorescence microscopy has been utilized to gain *in vivo* cellular visualization of live animal models in recent decades, cellular-level dynamic events such as cell-cell interaction and immune cell trafficking are still barely understood due to technical difficulties. In this work, by utilizing the video-rate laser-scanning confocal and two-photon mode convertible microscopy, we established the method for in vivo high-resolution cellular imaging window model, and we fabricated a stereotactic plate with a heating function for maintaining the conditions of a live animal model. With systemic fluorescent labeling and confocal imaging, the vasculature of the brain was clearly visualized at the cellular level. By

switching the imaging mode from confocal to two-photon, the collagen fibers were simultaneously observed from the same site in the brain. By using an ultrafast video-rate image acquisition system, rapidly flowing neutrophils in blood vessels of the cortex was observed. Finally, by using the photothrombosis-induced mouse model, we demonstrated repetitive cellular-level visualization of the same area in the brain cortex. For photothrombosis induction, the laser of which wavelength is 561nm was illuminated into the specific area in the cortex for 3-5 seconds after the retro-orbital injection of Rose Bengal. 4 days after the photothrombosis induction, the severely damaged vessels were observed in the photothrombosis-induced area in the cortex. To conclude, we expect the intravital imaging system could be an invaluable approach for understanding the dynamic cellular events of the central nervous system in pathological conditions.

Disclosures: S. Hong: None. **A. Kim:** None.

Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 204.14

Topic: C.10. Brain Injury and Trauma

Title: Sex-related differences of cognitive functions in college's soccer players with and without concussion history: A pilot study

Authors: *P. ACHARYA[1], G. ARGIANAS[1], E. PHILPOTT[1], H. GRAY[1], T. GEIRNAEIRT[1], M. DALECKI[2];
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Abstract: Previous studies provided some evidence that young athletes can show cognitive deficits after experiencing a concussion earlier in life, and female athletes seem to show more and longer-lasting symptoms than male athletes. However, it is not clear whether sex-related differences exist in cognitive functions among college soccer players. Thus, we aim to examine the effects of concussion history (CH) among college male and female soccer players on their attention capacity and executive functioning compared to team members with no history (NoH). We hypothesized potential sex-related differences in cognitive processing between CH and NoH players. Our preliminary data set included 16 athletes (mean age 19.1 yrs.), including 5 CH players (> 2.5 yrs. post-concussion, 2 females, 3 males) and 11 NoH players (6 females, 5 males). All players performed two cognitive tests on a laptop during the pre-season. i) A Stroop color-word executive function test: Four words (blue, green, red, and yellow) were presented on the screen for 96 trials. In 48 trials, the color and meaning of the word were the same, representing the congruent condition. The other 48 trials were the noncongruent condition where word color and meaning differed. Players were instructed to always select the text color, not the semantic meaning, with a matching key press. ii) A D2 sustained attention test involved nine-count sequences containing varying combinations of the letters d and p presented on the screen.

Each letter was framed with a different number of superscripts or subscripts of commas. Players had to press a 'D2' button when the letter d was surrounded by two commas and a 'Not D2' button otherwise. A new sequence of nine letters appeared once a previous sequence was finished. A block of sequences was terminated after 30 seconds, and 12 blocks were presented overall. ANOVAs were used to analyze response time (RT; milliseconds), error rate (ER; %), and sustained attention score (CS; D2 test only) in males and females with CH and NoH. For the Stroop test, there was a significant interaction of Sex * CH on RT ($p<0.05$) and no significant effects of Sex, CH, or Sex * CH on ER (all $p>0.05$). The post-hoc test showed greater RT for CH females (+29%) than NoH females ($p<0.05$), independent of the test condition, and no differences between CH and NoH males ($p>0.05$). For the D2 test, there were no significant Sex, CH, or Sex * CH effects found for CS, RT, or ER (all $p>0.05$). These preliminary data may suggest sex-related differences of cognitive functions processing speed in college soccer players with and without concussion history. More data collection is needed, and further testing during mid-and post-season is planned as well.

Disclosures: P. Acharya: None. **G. Argianas:** None. **E. Philpott:** None. **H. Gray:** None. **T. Geirnaeirt:** None. **M. Dalecki:** None.

Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 204.15

Topic: C.10. Brain Injury and Trauma

Support: NINDS R01-NS110905

Title: Linear analysis of interdependent data substantially inflates false positive rates for detecting effects of brain injury

Authors: M. FRANKOT[1], M. E. YOUNG[3], ***C. VONDER HAAR**[2];
[2]Ohio State Univ., [1]Ohio State Univ., Columbus, OH; [3]Kansas State Univ., Manhattan, KS

Abstract: Interdependent data outcomes are a feature of various measures used in the field of experimental traumatic brain injury (TBI). Relative abundance of transcripts (e.g., single cell or microbiome sequencing) and behaviors involving more than one outcome (e.g., MWM quadrants, forced-swim task, choice-based tasks) are among the most common examples. While independence of outcomes is a core assumption of linear models (e.g., ANOVA, linear regression), researchers commonly violate this assumption in their analyses of such data. To determine how problematic this violation is, we simulated data based on real data collected across 5 cohorts of rats performing a 4-choice decision-making task after TBI (N=109). We used Monte Carlo simulation methods to sample from populations with four different effect sizes (none, small, medium, large) at four different sample sizes (6, 10, 14, 20) and evaluated a linear regression model which (improperly) assumed independence of choice. This model resulted in

false positive rates exceeding 50% instead of the target 5%. Because of this issue, we evaluated other methods, including mixed effects regression (with separate intercept for each choice option), binomial mixed effects logistic regression with one reference choice, and Bayesian mixed effects multinomial logistic regression. Each of these drastically outperformed the interdependent linear model, with the Bayesian model providing slightly better sensitivity/specificity benefits than other models, but with a more conservative threshold for differences. Ultimately, labs need to consider the structure of their data and how their analyses are organized. Using linear models to analyze interdependent data impairs generalizability, particularly when stronger alternatives are available.

Disclosures: **M. Frankot:** None. **M.E. Young:** None. **C. Vonder Haar:** None.

Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 204.16

Topic: C.10. Brain Injury and Trauma

Support: NINDS R01-NS110905

Title: Effects of caloric restriction on the gut microbiome and rodent gambling task performance after traumatic brain injury

Authors: *R. SPEAS[1], N. M. BRESSLER[1], J. E. MCCLOSKEY[1], C. GRATZOL[2], M. FRANKOT[3], K. M. PECHACEK[1], K. M. MARTENS[1], C. VONDER HAAR[1];
[1]Neurosci., Ohio State Univ., Columbus, OH; [2]Neurosci., The Ohio State Univ., Columbus, OH; [3]Psychology, West Virginia Univ., Morgantown, WV

Abstract: Traumatic brain injuries (TBIs) increase risk for psychiatric disorders and subclinical symptoms, including deficits in impulsivity and risky decision-making. The gut microbiome is a particularly interesting candidate for the evolution of these deficits. Prior research in our laboratory has extensively used food-restricted rats to motivate responding, which may also influence the gut microbiome. In the current study, rats were trained on the Rodent Gambling Task (RGT), which measured decision-making via four choices, two high risk versus two low risk, but more optimal choices. To measure impulsivity, the rats were required to inhibit responding until given a light prompt. Motivation was measured by response latencies and omissions. Rats were randomly assigned to free-feeding or food-restricted. After behavioral baseline was determined, rats received a moderate-to-severe bilateral frontal controlled cortical impact injury. Fecal samples were collected at five timepoints: prior to receiving injury, and post injury at 1, 3, 7, and 30 days. Pre-injury, free-feeding increased omitted trials, reduced impulsivity, and led to fewer reinforcers. Post-injury, free-feeding significantly attenuated injury-related impairments in optimal choice and decreased trials, pellet collection, and premature responding. RNA was extracted from fecal samples, and bacterial diversity (alpha,

beta diversity) and composition (taxonomic abundance) will be reported at the poster. In conclusion, caloric restriction is useful to measure impulsivity, motivation, and decision-making. Ongoing studies are investigating the effects on the gut microbiome, and it is still to be determined whether these effects are drastic enough to warrant the loss of resolution in measuring impulsive behavior which accompanies free-feeding.

Disclosures: R. Speas: None. **N.M. Bressler:** None. **J.E. McCloskey:** None. **M. Frankot:** None. **K.M. Pechacek:** None. **K.M. Martens:** None. **C. Vonder Haar:** None.

Poster

204. Traumatic Brain Injury: Mechanistic Studies and Behavioral Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 204.17

Topic: C.10. Brain Injury and Trauma

Support: NINDS R01-NS110905

Title: The effects of receptor-specific dopamine modulation on gambling-like behavior following traumatic brain injury in rats

Authors: *N. M. BRESSLER, J. S. JORDAN, I. SATTAR, H. EBERLY, K. M. MARTENS, C. VONDER HAAR;
Ohio State Univ., Columbus, OH

Abstract: Traumatic brain injury (TBI) is a leading cause of disability worldwide and can lead to various cognitive deficits such as impulsivity and risky decision-making. Probabilistic decision-making is one of the many dopamine-mediated processes that is characteristically impaired after TBI. To better understand the role of dopamine in modulating post-TBI decision-making, an acute behavioral pharmacology approach was used to assess how agonism and antagonism of dopaminergic receptors (i.e., D1-like and D2-like) may influence behavior on the Rodent Gambling Task (RGT). The RGT is an operant task that recapitulates risky decision-making and impulsivity using operant conditioning chambers. Each trial of the RGT prompts the rat to select one of four options, each possessing a unique risk/reward profile, magnitude of reinforcement (sucrose pellets), and magnitude of punishment (time-out). Ultimately, there are two “safe” options, offering a lower magnitude of reinforcement but at a more optimal rate of reinforcement, and two “risky” options, offering a greater magnitude but at an overall detrimental rate. Prior to acquisition of the RGT, rats were given either a bilateral frontal controlled cortical impact injury (n=14) or a sham procedure (n=10) and were subsequently trained on the RGT for 6 weeks post-injury. Once rats achieved stable baseline on the RGT, four drugs were administered according to a counter-balanced Latin square design with one day of baseline, one day of testing, and one day of washout for each injection. The drugs administered via intraperitoneal injection were SKF-81297 (0.0, 0.01, 0.1, 0.3 mg/kg), quinpirole (0.0, 0.0125, 0.0375, 0.125mg/kg), SCH-23390 (0.0, 0.001, 0.003, 0.01 mg/kg), and eticlopride (0.0, 0.01,

0.03, 0.06 mg/kg) to agonize D1- and D2-like receptors, and antagonize D1- and D2-like receptors, respectively. TBI significantly decreased optimal decision-making but did not significantly alter omissions, a measure of motivation, or premature responses, a measure of impulsivity. Pharmacological challenges are ongoing and will be presented at the poster. The data from these challenge studies will elucidate changes to dopaminergic signaling in rat models of TBI as well as uncover potential therapeutic targets to ameliorate post-TBI impairments, namely risky decision-making and impulsivity.

Disclosures: **N.M. Bressler:** None. **J.S. Jordan:** None. **I. Sattar:** None. **H. Eberly:** None. **K.M. Martens:** None. **C. Vonder Haar:** None.

Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 205.01

Topic: C.11. Spinal Cord Injury and Plasticity

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Title: The alignment between data sharing mandates and domain-specific repositories. Examples of the Open Data Commons for Spinal Cord Injury (odc-sci.org) and Traumatic Brain Injury (odc-tbi.org)

Authors: *A. KELLER[1], J. C. GENSEL[2], A. TORRES-ESPIN[3], H. RADABAUGH[1], K. FOND[1], R. VAVREK[3], M. CHIU[4], J. HUIE[1], U. VISSER[5], J. L. BIXBY[5], V. P. LEMMON[5], J. S. GRETHE[4], M. E. MARTONE[4], A. R. FERGUSON[1], K. FOUAD[3];
[1]Univ. of California, San Francisco, San Francisco, CA; [2]Univ. of Kentucky Col. of Medicine,, Lexington, KY; [3]Univ. of Alberta, Edmonton, AB, Canada; [4]UCSD, San Diego, CA; [5]Univ. of Miami, Miami, FL

Abstract: Data sharing is becoming a fundamental activity of academic research. Multiple stakeholders have recently increased efforts to introduce solutions directed at improving data transparency in part to increase reproducibility, reduce waste, and increase data value. A significant portion of journals publishing biomedical research now requires the sharing of datasets underlying a paper's claims through data sharing repositories. Research communities are developing data sharing initiatives, and data sharing has become a scholarly field in its own right. In parallel, funding agencies (i.e. governmental and foundations) are increasingly aware of the value of research data, the importance of its reuse, and the need for policies to foster best data stewardship practices and reduce waste. This is in response to an international movement partly

promoted by the Organisation for Economic Co-operation and Development (OECD), an intergovernmental organization dedicated to promoting economic progress and world trade. In 2006, the OECD adopted the "Recommendation of the Council concerning Access to Research Data from Public Funding," a list of recommendations and declarations signed by the governments of 41 countries. In 2021, these recommendations were updated to incorporate further details on the relevant digital objects. Since then, research funding agencies have started a journey to generate recommendations and policies for data management and research data sharing. For example, the US National Institutes of Health (NIH) will begin mandating researchers and institutions it funds to make most, if not all, their data publicly available after January 2023 through the issue of its final Policy for Data Management and Sharing (DMS policy). Similarly, the Canadian Tri-Agency is in the process of incremental implementation of its policies on Digital Data Management.
The Open Data Commons (ODC) for Spinal Cord Injury (odc-sci.org) and Traumatic Brain Injury (odc-tbi.org) are two community-driven and domain-specific data sharing platforms designed to follow the FAIR (Findable, Accessible, Interoperable and Reusable) principles. These principles guide good data stewardship and sharing and have been endorsed by several stakeholders, including journals and funding agencies. Here we provide an overview of data management and sharing plans required for funding and how the ODCs provide platforms for compliance with these policies through FAIR sharing, initial data quality assurance, and citable digital object identifiers (DOIs).

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Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 205.02

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant NS088475
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Title: Developing data quality control and exploration tools for the Open Data Commons in Spinal Cord Injury (odc-sci.org) and Traumatic Brain Injury (odc-tbi.org)

Authors: *H. RADABAUGH[1,2], K. FOND[1], R. VAVREK LECERF[3], M. CHIU[4], A. KELLER[1], J. HUIE[1], J. C. GENSEL[5], U. VISSER[2], J. L. BIXBY[2], V. P. LEMMON[2], J. GRETHE[4], M. E. MARTONE[4], K. FOUAD[3], A. FERGUSON[1], A. TORRES-ESPIN[1,3];
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Abstract: The Open Data Commons (ODC) for Spinal Cord Injury (odc-sci.org) and Traumatic Brain Injury (odc-tbi.org) are two community-driven and domain-specific data sharing platforms designed to follow the FAIR (Findable, Accessible, Interoperable and Reusable) data principles. The FAIR principles provide guidance for the implementation and promotion of data sharing and reuse in a rigorous and reproducible manner. The ODC has established minimal data standards to ensure the sharing of FAIR data. All datasets are required to undergo quality control checks for ODC data specifications prior to uploading. To obtain a citable digital object identifier (DOI) for an uploaded dataset and release it for public access, the dataset must pass quality control checks performed by members of the ODC Data Team. Here, we present the development of open-source tools tailored to guide the ODC Data curation and administrative team and community users through data formatting compliance, quality checks, and initial exploration using code and a web-based graphical user interface (GUI) application. We have created prototypes of this tool in R and Python, including a UI, to allow users to perform two major tasks in data quality control: data formatting checks and data exploration. In this initial beta version of the tool, users can upload .csv files of a dataset as a tidy tabular structure (rows are observations, columns variables, the first row contains variable names) and a data dictionary following ODC specifications. Alternatively, the tool provides an automatic draft data dictionary with information extracted from the dataset. Once the files are available, the tool can automatically perform data checks and return a dynamic report in HTML format for exploratory analysis. The report provides interactive data summaries such as the amount of data missing, descriptive statistics, distributional histograms, and a correlation matrix between pairs of variables. This tool will facilitate FAIR data sharing through ODC and provide all users with the ability to perform a straightforward exploration of their data. Future work will be dedicated to user testing and ODC integration through the application programming interface (API). A beta version of the application can be tested at https://atpspin.shinyapps.io/ODC-SCI_data_check/

Disclosures: H. Radabaugh: None. **K. Fond:** None. **R. Vavrek Lecerf:** None. **M. Chiu:** None. **A. Keller:** None. **J. Huie:** None. **J.C. Gensel:** None. **U. Visser:** None. **J.L. Bixby:** None. **V.P. Lemmon:** None. **J. Grethe:** None. **M.E. Martone:** None. **K. Fouad:** None. **A. Ferguson:** None. **A. Torres-Espin:** None.

Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 205.03

Topic: C.11. Spinal Cord Injury and Plasticity

Support: U24NS122732
NS088475
NS106899
I01RX002245
I01RX002787

Title: The pan-neurotrauma (PANORAUMA) data commons

Authors: *J. HUIE[1], H. RADABAUGH[1], K. FOND[1], R. VAVREK LECERF[2], M. CHIU[3], A. KELLER[1], J. C. GENSEL[4], U. VISSER[5], J. L. BIXBY[5], V. P. LEMMON[5], J. GRETHE PH.D.[3], M. E. MARTONE[3], K. FOUAD[2], A. TORRES ESPÍN[1], A. R. FERGUSON[1];
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Abstract: Trauma to the central nervous system (CNS) affects more than 2.5 million people annually in the US, with an estimated economic cost of $80 billion in healthcare and loss of productivity. There are limited options to promote recovery after neurotrauma, partly because the precise pathophysiological processes impairing recovery remain poorly understood. This lack of knowledge is exacerbated by poor reproducibility of findings from animal models that limit therapeutic translation and reproducibility across species and into humans. Part of the problem is that neurotrauma is intrinsically complex, involving heterogeneous damage to the CNS, the body's most complex organ system. This results in a multifaceted trauma syndrome reflected across heterogeneous endpoints and multiple scales of analysis. This multi-scale heterogeneity makes traumatic brain injury (TBI) and spinal cord injury (SCI) difficult to understand using traditional analytical approaches that focus on single endpoints for testing therapeutic efficacy. Single endpoint testing provides a narrow window into the complex changes that describe SCI and TBI. Understanding these disorders involves managing and ingesting high-volume anatomical data, high-velocity physiology decision-support data, and a wide variety of functional/behavioral data then assessing correlations among these endpoints. In this sense, neurotrauma fundamentally entails a data management problem that involves the classic '3 Vs' of big data (volume, velocity, variety*)*. Of these, *variety* is perhaps the greatest data challenge in neurotrauma research for reproducibility in basic discovery, cross-species translation, and ultimately clinical implementation. The Open Data Commons (ODC) for Spinal Cord Injury (odc-sci.org) and Traumatic Brain Injury (odc-tbi.org) are two community-driven and domain-specific data sharing platforms designed to follow the FAIR (Findable, Accessible, Interoperable and Reusable) data principles. The National Institute of Neurological Disorders and Stroke (NINDS) awarded a Data Repositories Cooperative Agreement to establish a pan-neurotrauma(PANORAUMA) data commons that combines separate data assets to develop a pooled repository for preclinical discovery, reproducibility testing, and translational discovery both within and across neurotrauma types. Here we present the pan-neurotrauma data commons landscape and its integration to support data sharing for ongoing team science projects in neurotrauma.

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Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 205.04

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH SPARC OT2OD030537

Title: High-resolution magnetic resonance imaging of the human sacral spinal cord to support translational computational modeling for spinal cord stimulation therapies

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Abstract: Epidural spinal cord stimulation (SCS) is an attractive technique for treating lower urinary tract dysfunctions. SCS has shown great potential in controlling bladder function in animal experiments. However, we do not currently understand how to best optimize electrode design and stimulation paradigms to selectively control bladder functions in humans. For other neuromodulation applications, computational models have been an effective platform for designing these devices, but there are currently no models that accurately represent the complex human sacral cord anatomy. Here, we performed high-resolution magnetic resonance imaging (MRI) of the sacral cord for cadaveric spine samples and living humans, to build a realistic computational model of the human sacral cord. We acquired images of the T12-L2 vertebral levels from 6 cadaver specimens and 6 living humans and explored various MRI sequences and parameters to optimize image quality to specifically facilitate manual segmentation of white matter, gray matter, cerebrospinal fluid (CSF), epidural fat, and rootlets. For cadaver specimens we used a 3T (Siemens, Prisma) and a 9.4T (Bruker AV3 HD) scanner, and acquired 3D T2- and T2*-weighted images. 3T images were acquired using a flexible small coil at 300x300x1000 μm^3 voxel resolution while 9.4T images were acquired at 125 µm isotropic resolution using a radiofrequency coil. For living humans, we used a spine coil or a flexible small coil at a clinical 3T scanner, acquiring images in an axial orientation using a variety of sequences, including T2 SPACE Short Term Inversion Recovery, T2 SPACE ZOOMit, Multi Echo-Gradient Recalled Echo (GRE) T2*, T1 Fast Low-Angled Shot (FLASH), Magnetization Transfer (MT) FLASH and Proton Density (PD) SPACE. Voxel resolution was tested from 300x300x3000 μm^3 to 500x500x3000 μm^3 and scan time was 1.5 hours. For cadaver specimens, a GRE T2* (Repetition Time (TR)/Echo Time (TE)=20 ms/3.36 ms) sequence showed best contrast, allowing us to manually segment all relevant tissues. In living humans, motion artifacts due to movements and breathing reduced image quality, though we obtained sequences that enhanced the contrast and allowed manual segmentation of each tissue: PD SPACE (TR/TE=1700 ms/34 ms) for white matter and rootlets, MT FLASH (TR/TE=44 ms/2.76 ms) for gray matter, and T1 FLASH (TR/TE=20 ms/2.07 ms) for CSF. This study demonstrates the feasibility of high-resolution MRI

of the human sacral cord and will facilitate construction of realistic computational models for SCS.

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Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 205.05

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH SPARC OT2OD030537
NDSEG F-148887797

Title: An ultra-realistic model of spinal cord stimulation to control lower urinary tract function and optimize bladder neuroprosthetics

Authors: ***M. K. JANTZ**[1,2,6], X. FANG[1,3], A. DAMIANI[1,4], L. LIANG[1,2,6], C. GOPINATH[1,4], F. LIU[1,4], U. AGBOR[1,4], T. NEWTON[7], E. NEUFELD[7], A. FASSE[7], T. K. HITCHENS[5], L. E. FISHER[1,2,4,6], E. PIRONDINI[1,2,4], M. CAPOGROSSO[1,2,3], R. A. GAUNT[1,2,4,6];
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Abstract: Lower urinary tract (LUT) dysfunction is one of the major consequences of spinal cord injury (SCI), and improving bladder function is consistently rated as one of the top rehabilitation priorities for people living with SCI. One promising new technology to restore bladder function is epidural spinal cord stimulation (SCS), which we have previously shown can effectively recruit nerves innervating the LUT. However, for clinical translation efforts to accelerate, we need to determine effective stimulation lead locations and identify effective stimulation parameters. Here, we use a three-step computational model to simulate bladder function evoked by SCS. First, we generated a 3D finite element model of the sacral spinal cord using a high-resolution anatomical scan of a postmortem cat spine, spanning the L5 vertebra to the sacrum. We used diffusion tensor imaging and tractography to produce thousands of neural trajectories aligned to the finite element model to represent axons from the pelvic, pudendal, and sciatic nerves and assigned them realistic axon diameter distributions. Second, we used Sim4Life to simulate the electric potential values in the finite element model and determined the recruitment threshold of each neuron. Recruitment simulations were performed using high-resolution electrodes (0.29 x 1 mm) spanning the sacral cord and cauda equina. Third, we used

the estimated neural recruitment to drive a network model of spinal bladder reflexes to evaluate SCS-driven pressure changes. We conducted these simulations with multiple stimulation frequencies and bladder volumes.
Using our highly-realistic model, we identified stimulation locations and amplitudes that were selective for the pudendal and pelvic nerves that innervate the LUT, while minimizing sciatic nerve activity. Preliminary results show that the recruitment thresholds in the model and experimental data are very similar and that the electrode-to-electrode variations in selective recruitment of the pelvic and pudendal nerves mimics results seen in experimental data. The most caudal stimulation locations minimized sciatic nerve co-activation and improved pelvic nerve recruitment, further replicating experimental data. We studied how this neural activation affected bladder pressure simulated by the reflex model and successfully predicted excitatory and inhibitory bladder pressure changes resulting from different stimulation frequencies, reproducing observations from experiments in anesthetized cats. This model will allow us to further study and optimize strategies to improve bladder function after SCI and will also be provided as an open-source tool for the community.

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Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

Location: SDCC Halls B-H

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Program #/Poster #: 205.06

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH SPARC OT2OD030537
NDSEG F-148887797

Title: An open-source realistic model simulating the selectivity of the spinal pudendo-vesical reflex for predicting the neurostimulation effect on bladder control

Authors: *X. FANG[1,2], M. JANTZ[1,3,5], R. GAUNT[1,3,4,5], M. CAPOGROSSO[1,2,3,5];
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Abstract: Spinal cord stimulation (SCS) is a promising approach to treat lower urinary tract (LUT) dysfunction after spinal cord injury (SCI). Both pelvic and pudendal afferents have major roles in regulating spinal reflexes evoked by SCS, but experimentally, it is difficult to isolate and study their relative effects. As a result, computational models become useful tools to understand

the distinct effects of different reflex pathways and leverage this information to guide the design of selective neural interfaces. Here we developed a realistic computational model with python and NEURON to explore the different combinations of pudendal afferents and pelvic afferents recruitment and validate the model with experiment data to make predictions about bladder pressure change under specific SCS experiment conditions. The model is constructed by expanding a previous computational model of pudendo-vesical reflex for bladder control into a spiking neural network model with hundreds of cells. We modeled populations of pudendal and pelvic afferents as virtual Gaussian firing models that receive direct external input from SCS and bladder pressure. Excitatory and inhibitory interneurons were modeled as Integrate and Fire neurons while sacral parasympathetic nucleus (SPN) neurons used Hodgkin-Huxley membrane models including calcium channels that mimicked realistic firing rates. We then calculate and update the bladder pressure from firing rates of SPNs during simulation, and measure the effect of SCS on bladder control by comparing the pre- vs. post-stimulation bladder pressure.
The features of our model allowed us to explore the effect of stimulation parameters such as the stimulation amplitude and frequency to analyze the selective activation of pudendal vs. pelvic afferents. For selectivity analysis, we created a recruitment map and found that the pelvic recruitment significantly affects bladder pressure outputs when the recruitment of pelvic afferents is above 20%. In these conditions, the bladder pressure increment in response to SCS is not a monotonic function of the pudendal afferents recruitment and depends on the co-activation levels of pelvic afferents. These initial simulations reproduce data from experimental studies of SCS though further parameter exploration is needed. Our model is fully open-source and is now available on the o^2S^2PARC platform. It could be easily used by itself or combined with other studies of SCS or bladder function.

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Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

Location: SDCC Halls B-H

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Program #/Poster #: 205.07

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH SPARC OT2OD030537
NDSEG F-148887797

Title: An open-source high-resolution and anatomically realistic computational model of the cat lumbosacral spinal cord

Authors: *A. DAMIANI[1,2], L. LIANG[1,3,6], M. K. JANTZ[1,3,6], T. NEWTON[7], A. FASSE[7], L. FANG[1,2], U. AGBOR[1,2], M. DEL BROCCO[1,2], L. E. FISHER[1,3,2,6], M. CAPOGROSSO[1,3,4], E. NEUFELD[7], K. T. HITCHENS[5], E. PIRONDINI[1,2,3], R. GAUNT[1,3,2,6];

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Abstract: Lower urinary tract (LUT) dysfunction affects up to 20-40% of the population, with significant medical, psychological and social consequences. Recent studies in cats showed the potential of lumbosacral spinal cord stimulation (SCS) to control LUT functions. However, experiments alone cannot feasibly address all physiological questions. Anatomically accurate in-silico models may provide critical insights that would allow SCS to be optimized for LUT control. Common SCS models use simplified geometries that lack anatomical detail, which could affect neural activation and ultimately the translational benefits of model-based optimization. Here, we used a novel imaging and segmentation pipeline to develop an anatomically realistic computational model of the cat lumbosacral spinal cord, and compared it with a simplified model to characterize differences in simulation predictions.
The imaging pipeline consisted of a CT scan, a T2-weighted, a fat-selective, and a 19F MRI scan of *ex vivo* fixed L5-S3 cat spinal tissue, at 50µm resolution. This ensemble of acquisitions allowed automatic segmentation of vertebrae, gray matter, white matter, ventral/dorsal roots, epidural fat, and cerebrospinal fluid (CSF) with a convolutional neural network trained on a small set of manual segmentations. We also collected a diffusion weighted scan and used tractography to extract axon trajectories at each spinal level.
For the realistic model, we used all 700 slices with 7 automatically-segmented tissues to create a high-resolution 3D model in Sim4Life. We also added a 50µm thick dura around the CSF. Finally, we inserted axons into the model using tractography-extracted trajectories. For the simple model, we used only 11 slices without root tissue segmentation and generated the model by extrusion with solid modelling software. Axon trajectories were added manually. For both models, we used a structured finite element mesh, assigned conductivity values to each tissue region. In the complex model, we calculated anisotropic conductivity tensors for the white matter and roots. We simulated SCS using electrodes at multiple positions over the sacral cord and determined neural recruitment from the simulated potential. We found that the realistic model produced significantly different distributions of EM potential that we expect to affect neural recruitment, demonstrating the need for accurate models. Our modeling framework is implemented on o^{2}S^{2}PARC, an open-source interactive online simulation platform. This will provide open access for researchers to run simulations on our realistic lumbosacral cat spine model or generate their own model with our imaging pipeline.

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Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 205.08

Topic: C.11. Spinal Cord Injury and Plasticity

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Title: Fighting for Recovery on Multiple Fronts: The Past, Present and Future of Clinical Trials for Spinal Cord Injury

Authors: *V. A. DIETZ[1], N. ROBERTS[1], K. KNOX[1], S. MOORE[1], M. PITONAK[1], C. BARR[4], J. CENTO[4], S. LEININGER[4], K. NEW[4], P. NORWELL[4], M. RODREICK[4], C. GEOFFROY[2,3], A. STAMPAS[5], J. DULIN[3,1];
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Abstract: Through many decades of preclinical research, great progress has been achieved in understanding the complex nature of spinal cord injury (SCI). Preclinical research efforts have guided and shaped clinical trials, which are growing in number by the year. Currently, 1,149 clinical trials focused on improving outcomes after SCI are registered in the U.S. National Library of Medicine at ClinicalTrials.gov. We conducted a systematic analysis of these SCI clinical trials, using publicly accessible data downloaded from ClinicalTrials.gov. After extracting all available data for these trials, we categorized each trial according to the types of interventions being tested and the types of outcomes assessed. We then evaluated clinical trial characteristics, both globally and by year, in order to understand the areas of growth and change over time. With regard to clinical trial attributes, we found that most trials have low enrollment, only test single interventions, and have limited numbers of primary outcomes. Some gaps in reporting are apparent; for instance, 80% of clinical trials with “Completed” status do not have results posted, and the Phase of some trials is incorrectly classified as “Not applicable” despite testing a drug or biological compound. When analyzing trials based on types of interventions assessed, we identified the largest representation in trials testing rehab/training/exercise, neuromodulation, and behavioral modifications. Most overrepresented primary outcomes include motor function of the upper and lower extremities, safety, and pain. The most overrepresented secondary outcomes include quality of life and pain. Over the past 15 years, we identified increased representation of neuromodulation and rehab trials, and decreased representation of drug trials. Together, our work provides a comprehensive glimpse into the past, present, and future of SCI clinical trials, and suggests areas for improvement in clinical trial reporting.

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Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

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Topic: C.11. Spinal Cord Injury and Plasticity

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Title: Dynamic monitoring of wound healing in spinal cord using a clamp-type imaging window and optical coherence tomography

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Abstract: The continuous investigation of wound healing offers intuitive information to study and manage various injuries. It is essential to understand spinal cord (SC) when it has damage to the tight bundle of cells and nerves that sends and receives signals from the brain to and from the rest of the body. Although mouse model and optical imaging are indispensable elements in brain research, there are relatively few studies reported that describe the dynamic monitoring in SC. It is because that *in vivo* imaging of the spinal cord is certainly limited due to its movement as well as location surrounded by bone and dura mater. In this study, we introduced a new method for *in vivo* real-time and longitudinal imaging of the spinal cord using a novel spinal cord window chamber and optical coherence tomography (OCT). OCT has been introduced in biomedical imaging modality with various advantages including real-time, non-invasive, and deep tissue imaging. We utilized the OCT to observe three-dimensional structural and functional changes in a SC when incomplete spinal cord injury was recovered. Although its imaging capability is well suited to monitor SC including the wound healing process after injury, it has the challenge to apply to SC study *in vivo* and the same location because of the motion artifact as well as the lack of an optical imaging window. In order to enable dynamic and long-term imaging of mouse SC, we developed a minimally invasive intervertebral window. Our device is designed as a clamp-type integrated with a round-shaped imaging window, which is readily mounted at the vertebrae. Since our device is fabricated by 3D printing of biocompatible ABS filament and with PDMS material for imaging window, it also has high reproducibility and flexibility to build customized window reflecting the various shape of SC in short period of time. In our preliminary experiment, we successfully imaged the regional and volumetric structure of SC over 20 days, and confirmed that our method provides a reliable platform in various research aiming at interpretation of spinal cord physiology. We also quantified the injured area with home-built software for image processing and generated unique information, which cannot be obtained by conventional optical imaging *in vivo*. In particular, a ratio of gray and white matter was identified during the recovery phase which is crucial to investigate neural diseases such as multiple sclerosis disability. Through our experimental results, it is expected that a clamp-type window would be widely utilized with various optical imaging modalities including two-photon microscopy to elucidate the recovery process of the spinal cord with neurons and glial cells in future work.

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Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 205.10

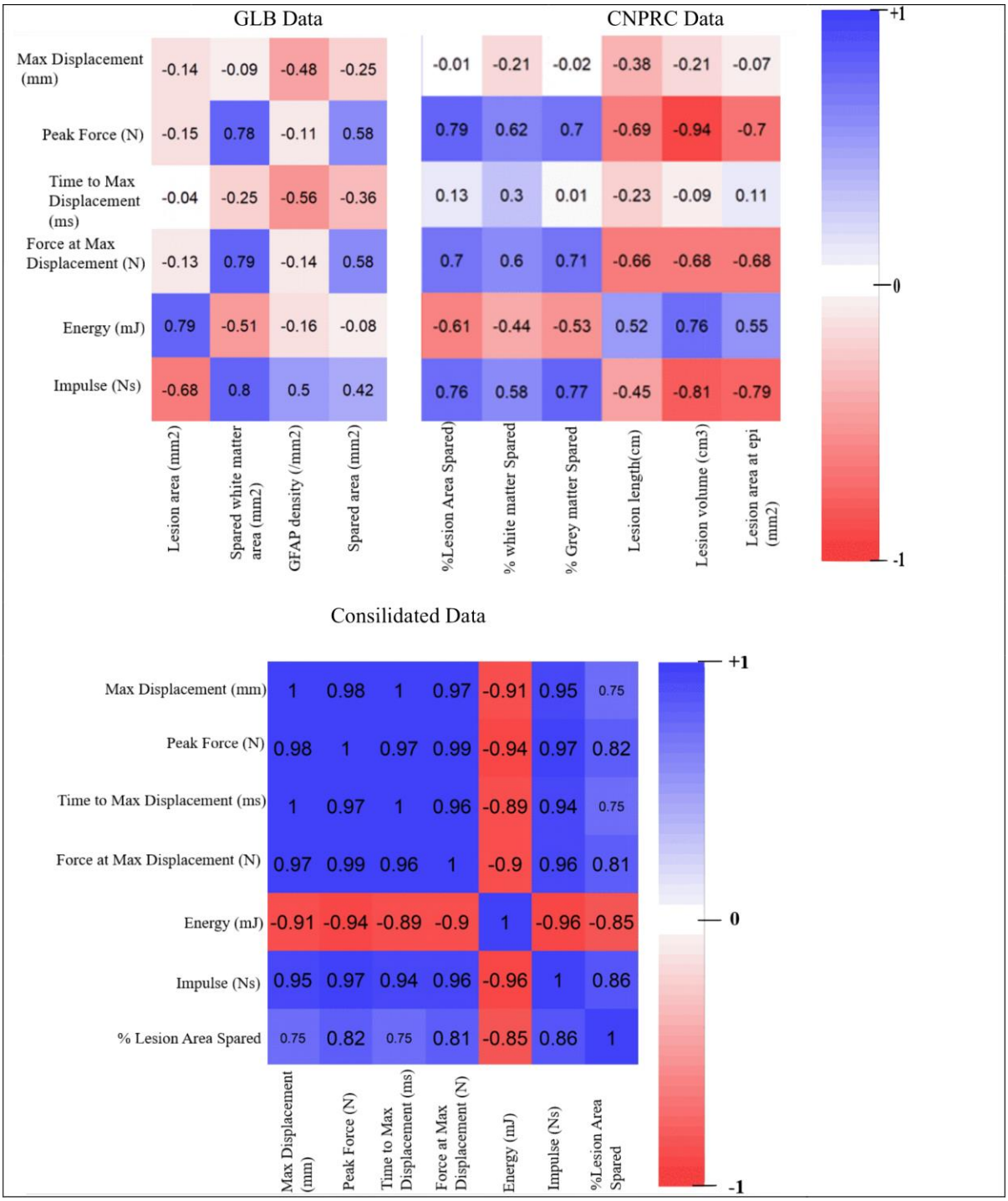
Topic: C.11. Spinal Cord Injury and Plasticity

Support: NSERC Discovery Grant-RGPIN – 2018 - 06382
ICORD seed

Title: Determining the Importance of Injury Biomechanics in the Interpretation of Non-Human Primate Experimental Spinal Cord Injury Models.

Authors: *F. KHORAMI[1], N. OBAID[1], J. LIU[2], Q. ZHU[2], C. J. SPARREY[1];
[1]Simon Fraser Univ., Surrey, BC, Canada; [2]Southern Med. Univ., Guangzhou, China

Abstract: Animal models of spinal cord injury (SCI) provide the foundation for developing and testing new treatments. However, different injury models and severity result in high variability that can make it difficult to compare outcomes across different labs. The objective of this study was to quantify the biomechanical variables which best correlate to injury outcomes in two distinct non-human primates (NHPs) SCI datasets.
Using experimental data from two separate studies, the California National Primate Research Center (CNPRC) and Guangdong Landau Biotechnology Co., Ltd (GLB), exposed 9 and 8 (7.5±1.6 and 7.7±1.5 years old respectively) male NHPs to contusion injuries. Missing data were imputed for PCA analysis and bivariate correlations were conducted for all pairwise comparisons between biomechanical variables and histological measures using R (R Core Team 2021). Impulse and energy variables are most highly correlated with all of the biomechanical variables and lesion measures (e.g. r = -0.85 and 0.86 for correlations between energy and impulse and % lesion area spared respectively) and are suggested to be recorded for more accurate animal models (Figure 1). The most relevant biomechanical variables in explaining the variability in dimension 1 included impulse, peak force, and energy in the CNPRC dataset and impulse and peak force in the GLB dataset.
In most SCI animal models, the injury biomechanics are assumed to be consistent and group assignment is random. However, we see much greater variability in large animal contusion injury models motivating the need to stratify group assignments using biomechanical data to ensure more balanced study groups. Findings from the study showed that impulse and energy variables were better determinants of injury histology as they take into account the time exposure of force and displacement and may better reflect the overall injury exposure.
Figure1: Pearson correlations between the biomechanical variables and injury parameters in GLB, CNPRC (top), and in a consolidated dataset (bottom).



Disclosures: **F. Khorami:** None. **N. Obaid:** None. **J. Liu:** None. **Q. Zhu:** None. **C.J. Sparrey:** None.

Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 205.11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: ISF Grant 590331

Title: Nano Topographical Modulation of Regenerating Neurons

Authors: *A. RICHTER-LEVIN[1], G. INDECH[1], N. IRONI[1], A. SHARONI[1], O. SHEFI[2]; [1]Bar Ilan Univ., Ramat Gan, Israel; [2]Fac. of Engin., Ramat-Gan, Israel

Abstract: Traditionally, axonal growth is considered to be guided by chemotaxic cues. More recently, it has been demonstrated that topographical cues can also serve as axonal guidance cues even of the nano scale. More than that, recent studies show that interactions of neurons with physical elements, are sufficient for maturation, triggering functional presynaptic buttons formation and other presynaptic features including vesicles and microtubular structures. By elucidating the various ways we can influence a regenerating neuron we can improve our ability to treat damaged nerve tissues. Specifically, in order to treat injured neurons, we aim to guide the regenerating axon to its proper target, and improve recovery by improving its growing rate. In this research we examine the impact of nano-topography on both growth direction and rate. We utilize cells of the leech nervous system, containing big, clearly identified neurons, which enable repeatedly examining the same cell. Following the preparation of nano-topographic cued surfaces by fabrication (photolithography), and developing a technique for exact cell positioning, we examined neurite growth of identified leech neurons, while systematically modifying nano-topographical cues. Furthermore, we employed long-time lapse imaging to assess the influence of nano-topography on rate of growth of regenerating neurons. First we examined which nano-topographical features (height [250nm vs 100nm] and shape [circle, line, zigzag]) are more effective. After establishing the most effective height (250nm) and shape (line) (250nm, line, $P < 0.0001$). Currently we are examining whether neurite's growth dynamics changes once encountering nano-topography. Finally, employing expansion microscopy, which enables us to differentiate between cells which were affected by the topography and those who did not, we now examine potential differences in nano-topography-induced molecular alterations between affected and non-affected cells. Our initial results reinforce the importance of nano-topographical influence in guiding regenerating neurons. Further examination may reveal associated molecular mechanisms.

Disclosures: **A. Richter-Levin:** None. **G. Indech:** None. **N. Ironi:** None. **A. Sharoni:** None. **O. Shefi:** None.

Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 205.12

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life

Title: Predicting multi-class trajectories of in-hospital routine laboratory values: application as dynamic biomarkers in spinal cord injury

Authors: *A. TORRES ESPÍN[1,3], D. FERNÁNDEZ[4], T. TRACK-SCI INVESTIGATORS[2]; [1]Univ. of California San Francisco, [2]Univ. of California San Francisco, San Francisco, CA; [3]Univ. of Alberta, Edmonton, AB, Canada; [4]Univ. Politècnica de Catalunya, Barcelona, Spain

Abstract: TRACK-SCI investigators (in alphabetic order): Beattie MS, Bresnahan JC, Dhall SS, DiGiorgio AM, Duong-Fernandez X, Ferguson AR, Hemmerle DD, Huie JR, Gonzalez K, Keller A, Kyritsis N, Manley GT, Pan JZ, Pascual LU, Saigal R, Singh V, Talbott JF, Weinstein P, Whetstone WD
Background: Early diagnosis and prognosis after acute traumatic spinal cord injury (SCI) is challenging due to inherent pathological complexities and population heterogeneity. Routinely collected data during standard patient care, such as laboratory values, can be used to assess underlying pathophysiological processes and drive biomarker discovery. After reviewing the state-of-art of blood-based biomarkers for SCI, we hypothesized that distinct temporal trends of blood markers can be modeled after SCI and that those would predict patient outcomes.
Methods: Using real-world data from available electronic health records, we assembled a big-data asset and modeled distinct laboratory values measured over time during the early hospitalization after acute spine trauma with or without SCI. We fitted trajectory growth mixture models (GMM) to determine distinct group trajectories on 20 blood markers commonly measured in these populations over time. The probability of group trajectory membership was used in a supervised learning task to predict patient outcomes.**Results:** Analyses of EHR show 2 and 3 non-linear heterogeneous temporal trajectories of blood electrolytes and hematology variables, respectively, after spine trauma and SCI. These trajectories are associated with different patient outcomes. In dynamic prediction experiments, the probability of belonging to a specific laboratory value trajectory is predictive of whether a patient would die in the hospital, a patient presented with an SCI, or SCI severity (motor complete vs. incomplete). In addition, using an external research dataset for the trajectory modeling from an observational study of level 1 trauma center data (TRACK-SCI), our results indicate that trajectory models derived from EHR can be generalizable.**Conclusions:** Routinely real-world data can be used to model blood markers' dynamic changes after SCI with prediction validity for clinical outcomes. Our work suggests that temporal blood trends are promising early predictors of SCI pathology. This work sets the base for further developing dynamic biomarkers in neurotrauma and other neurological conditions.

Disclosures: A. Torres Espín: None. **D. Fernández:** None. **T. TRACK-SCI Investigators:** None.

Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 205.13

Title: WITHDRAWN

Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 205.14

Topic: C.11. Spinal Cord Injury and Plasticity

Support: R01 NS121191
F32 HD107806

Title: In vivo assessment of cervical spinal cord injury progression using ultrasound imaging in a rodent model

Authors: *J. S. HARMON, A. A. ODARENKO, L. N. CATES, J. E. HYDE, M. F. BRUCE, Z. Z. KHAING;
Univ. of Washington, Univ. of Washington, Seattle, WA

Abstract: Traumatic spinal cord injury (SCI) is characterized by loss of blood flow at the injury epicenter and substantial hypoperfusion in the penumbral zone, resulting in sustained ischemia and cell death. This secondary injury, arising from hemorrhage, edema, and associated increased intraspinal pressure (ISP), is well-known but poorly characterized *in vivo*. To visualize and quantify injury progression during the acute to subacute phase post-SCI, we utilized B-mode (tissue anatomy) and contrast-enhanced ultrasound imaging (CEUS; microcirculatory hemodynamics). Epidural imaging was conducted following a three-level laminectomy (C4-C6) in female Long Evans rats. Ultrasound data were acquired at baseline, after unilateral contusion SCI (Infinite Horizon device; 150 kDyn), and at 4 hours post injury (hpi) or 24 hpi (N=8 each). CEUS acquisitions were conducted after an IV bolus of contrast agent (Definity, 0.1 mL, 0.2 mL saline flush). The spinal cord volume in a 3 mm rostrocaudal window, hematoma volume, rise time (i.e., vascular resistance), and area under the curve (AUC; i.e., blood volume) were calculated. For the 4 hpi cohort, significant increases in spinal cord volume (32.3±0.656, 35.6±1.01, 37.0±0.799 mm^3) and hematoma volume (9.89±0.829, 11.5±0.715 mm^3) were observed, indicating both progressive swelling and bleeding by 4 hpi ($p < 0.05$). A significant increase in rise time was observed in the contralateral gray matter (GM; 1.46±0.107, 1.75±0.111, 2.03±0.184 sec), indicating progressive increase in vascular resistance ($p < 0.05$). AUC decreased in both the contralateral and ipsilateral GM acutely following injury (-33.2% and -63.8% change from baseline, respectively); by 4 hpi, the contralateral GM began to recover (-8.78%) whereas the ipsilateral GM remained hypoperfused (-61.2%). The 24 hpi cohort

exhibited hyperemia in the contralateral GM (acute: -33.0% from baseline, 24 hpi: +27.0%) with a slight recovery in ipsilateral GM perfusion by 24 hpi (acute: -56.7%, 24 hpi: -42.8%). Vascular resistance (rise time) also began to decrease by 24 hpi in both the contralateral (acute: +24.3%, 24 hpi: +3.82%) and ipsilateral GM (acute: +61.6%, 24 hpi: +55.0%). However, while hemodynamic parameters improved to an extent, further bleeding was observed, resulting in a significantly larger hematoma volume by 24 hpi (acute: 10.7±0.792, 24 hpi: 15.3±1.03 mm^3; $p < 0.05$). Ongoing work will elucidate further changes in spinal hemodynamics by 72 hpi. Future work will investigate any sex-related differences in injury progression and will include either surgical (e.g., durotomy) or pharmacological intervention to mitigate secondary injury.

Disclosures: J.S. Harmon: None. **A.A. Odarenko:** None. **L.N. Cates:** None. **J.E. Hyde:** None. **M.F. Bruce:** None. **Z.Z. Khaing:** None.

Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 205.15

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Daniel and Ada Rice Foundation

Title: Identifying biomarkers for central neuropathic pain in rats with hyperalgesia and allodynia after spinal cord injury

Authors: *B. AVONTS, R. FESSLER, B. DAVID;
Rush Univ. Med. Ctr., Chicago, IL

Abstract: Central neuropathic pain (CNP) commonly develops in patients after spinal cord injury (SCI), causing debilitating symptoms and sensory abnormalities such as allodynia and hyperalgesia. CNP regularly presents itself around a year after the injury in humans, resulting from permanent cellular and anatomical changes. Previous scientific studies have demonstrated greater efficacy of treatments when delivered preemptively, but there is currently no biomarker to indicate which individuals are more susceptible to developing CNP. Thus, it is necessary to investigate the physiological processes contributing to sensory changes that develop over time in CNP. Here we assess heart rate, blood pressure, gait and cytokine levels as potential early biomarkers of CNP for the first eight weeks following injury. Using tail flick and von Frey tests we performed hierarchical clustering to determine the subpopulation of rats that developed allodynia or hyperalgesia. The tail flick test showed the subpopulation of hyperalgesic rats significantly different than the non-hyperalgesic rats at day 7 and days 21 through 56 post injury ($p<.05$). The von Frey test showed the subpopulation of allodynic rats significantly different than the non-allodynic rats at days 42 through 56 post injury ($p<.05$). There was no acute difference in blood pressure or heart rate in rats with hyperalgesia or allodynia. Animals were sacrificed on day 52 and the spinal cord injury epicenter was analyzed by flow cytometry for inflammatory

markers. There was a trend towards increased macrophage presence in rats with hyperalgesia compared to the non-hyperalgesic rats. We conclude further investigation may reveal acute changes in peripheral cytokines, mediating the immune response of macrophages at both the injury epicenter and regions of the cerebral cortex involved in processing of higher functions.

Disclosures: B. Avonts: None. **R. Fessler:** None. **B. David:** None.

Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 205.16

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Neuralink Corporation

Title: Motor cortex activity driven by attempted hand and limb motion in spinal cord injury patients measured using a 1.5T clinical MRI scanner

Authors: *A. R. WADE[1], J. E. O'DOHERTY[2], K. R. THOMSON[3], D. L. ADAMS[2]; [1]Psychology, Univ. of York, York, United Kingdom; [2]Neuralink Corp., Fremont, CA; [3]Biograph, San Mateo, CA

Abstract: There are over 250k people with spinal cord injury in the US. Many experience partial or complete paralysis that impedes their use of devices such as mobile phones and computers. Brain-computer interfaces can restore their ability to control electronic devices by decoding neural signals recorded from chronically implanted electrodes. To function optimally, the electrodes should be placed near neurons modulated by intended hand and arm movement. Primary motor cortex is often targeted, but the location, specificity, and responsivity of the underlying neuronal populations should guide precice electrode placement. We used a standard, clinical 1.5T MRI system (Siemens Espree) to measure the cortical blood-oxygenation level dependent signal in controls (N=30) and tetraplegic subjects (N=4) during attempted and imagined hand and arm movements. Subjects were cued to alternate motor tasks (fist clenching, finger tapping, or whole-arm motions) between left and right in a block-design fMRI paradigm (3s TR) at a resolution of 1.5 x 1.5 x 2.7 mm with 18 slices in an axial prescription covering the entire dorsal surface including motor cortex. Five repeats of the same functional task generated a total of ~20 minutes of data per subject. In the same session we also collected high-resolution whole-head T1 and T2-weighted anatomical data to aid in the localisation of responses. Overall, we measured robust responses in contralateral motor cortex during real (controls 30/30) and attempted (tetraplegic 3/4) tasks, including in a subject who was injured more than 30 years previously. We also observed responses in the ipsilateral motor cortex of all subjects. In contrast, imagined hand and arm movement in controls did not generate measurable signals in motor cortex. We conclude that people can induce hand-specific activity in motor cortex even after long periods of spinal cord injury, and that this activity can be measured at relatively high

resolution using widely-available 1.5T clinical scanners that are compatible with implants commonly found in this population.

Disclosures: **A.R. Wade:** F. Consulting Fees (e.g., advisory boards); Neuralink Corporation. **J.E. O'Doherty:** A. Employment/Salary (full or part-time):; Neuralink Corporation. F. Consulting Fees (e.g., advisory boards); Neuralink Corporation. **K.R. Thomson:** A. Employment/Salary (full or part-time):; Biograph. **D.L. Adams:** A. Employment/Salary (full or part-time):; Neuralink Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuralink Corporation.

Poster

205. Spinal Cord Injury: Data Commons, Modeling, Imaging, and Biomarkers

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 205.17

Topic: G.03. Motivation

Support: NSF DGE-1650116
The foundation of Hope
the NC TraCS Institute
The UNC Department of Psychiatry
The Burroughs Welcome Career Award
The Beckman Young Investigator Award
The Royster Fellowship

Title: Biometric Ocular Photometry (BOP): a tool to track arousal dynamics in awake animals

Authors: V. R. CURTIS[1], M. ORTIZ-JUZA[2], G. VELAZQUEZ-HERNANDEZ[3], *J. RODRIGUEZ-ROMAGUERA[4], N. C. PEGARD[5];
[2]Neurosci., [1]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [3]department of psychiatry, Inst. of Celullar Physiology, UNAM, Chapel Hill, NC; [4]Dept. of Psychiatry and Neurosci. Ctr., [5]Applied physical Sci., Univ. of North Carolina At Chapel Hill, Chapel Hill, NC

Abstract: Atypical arousal responses are key symptoms underlying many neuropsychiatric disorders that can be identified by observing rapid physiological read-outs such as pupillary dilation and constriction, heart rate, and respiratory cycles. Recording these biometric data while simultaneously monitoring neuronal dynamics in the brain is critically needed to reverse-engineer the neural circuit dynamics that regulate arousal responses. Yet, existing technologies that measure arousal biometric data are impractical. Systems that measure heart and respiration rates typically require the animal to be anesthetized or heavily restrained, and pupillometry techniques rely on bulky cameras that require post-processing. Together, slow and impractical biometric trackers are unfit to monitor arousal states in real-time and limit the ability to study the neural dynamic underlying arousal. To address these issues, we developed a new optical

technique, termed Biometric Ocular Photometry (BOP), that records multiple biometric markers of physiological arousal with a single device in ethologically relevant contexts. Our method combines an infrared light source and a photodetector to measure the diffused transmission of infrared light through the eye at kilohertz sampling rates. Since respiratory, cardiac, and pupillary dynamics modulate the transmission of infrared light, filtering the raw BOP data at frequency ranges that are typical for each physiological biometric returns simultaneous measurements of the pupil size (<1-2 Hz), respiratory rate (2-5 Hz), and heart rates (6-14 Hz) with minimal computation. We performed validation experiments showing that our BOP technology captures accurate biometric data. For this, we compared simultaneous recordings of BOP data and ground truth measurements of the fluctuations in pupil size (with a camera), heart rate (with electrocardiogram data), and respiratory cycles (with a pressure sensor under the chest). We further demonstrate that BOP can be implemented as a simple add-on in a *two-photon* microscope to track physiological arousal responses simultaneously with live imaging of neuron calcium dynamics in a subnucleus of the extended amygdala deep in the brain.

Disclosures: V.R. Curtis: None. **M. Ortiz-Juza:** None. **G. Velazquez-Hernandez:** None. **J. Rodriguez-Romaguera:** None. **N.C. Pegard:** None.

Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 206.01

Topic: D.01. Somatosensation

Support: NS 111643

Title: A comprehensive spinal circuit for mechanical itch

Authors: *D. ACTON, X. REN, M. D. GOULDING;
Mol. Neurobio. Lab., Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Itch functions to alert animals to chemical and biological agents in the external environment that can cause damage to the body. Two separate neuronal pathways that are activated by either mechanical or chemical stimuli convey itch information from the skin to the spinal cord, where it is integrated and modulated by dedicated microcircuits. Recent studies have identified multiple molecularly defined neuronal populations that form these circuits. Using a range of genetic, pharmacological, behavioral, and histological techniques, we have determined the relationships between cell types proposed to mediate mechanical itch so as to delineate a comprehensive pathway for mechanical itch transmission within the skin and spinal cord. Mechanical itch information has been shown to be transmitted from the skin to the spinal cord by sensory neurons expressing Toll-like receptor 5; these then synapse onto excitatory neurons within the dorsal horn that express Ucn3::Cre (Pan et al. 2019. Neuron 103, 1135-1149). Mechanical itch has also been shown to be transmitted within the dorsal horn by neurons

expressing the inhibitory neuropeptide Y (NPY) receptor Y1 (Acton et al. 2019. Cell Rep. 28, 625-639) and to be gated by NPY-expressing neurons (Bourane et al. 2015. Science 350, 550-554). Here, we show that endogenous NPY acts via Y1 receptors expressed by neurons downstream of Ucn3::Cre$^+$ neurons to gate mechanical itch, with Ucn3::Cre$^+$ neurons forming synapses onto Y1$^+$ neurons in lamina I. This spinal pathway for mechanical itch is distinct from the chemical itch transmission pathway that includes neurons that express gastrin-releasing peptide receptor (GRPR). Notwithstanding a report that GRPR$^+$ neurons also mediate mechanical itch (Chen et al. 2020. Nat. Commun. 11, 5074), we demonstrate that total ablation of the GRPR$^+$ population by intrathecal injection of 2 µg bombesin-saporin reduces chemical itch responses but fails to attenuate mechanical itch. These findings facilitate interpretation of recent studies of mechanical itch and allow us to hypothesize a complete spinal circuit for mechanical itch transmission that is parallel to and separate from the circuitry that transmits chemical itch.

Disclosures: D. Acton: None. **X. Ren:** None. **M.D. Goulding:** None.

Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 206.02

Topic: D.01. Somatosensation

Support: NIH grants R35NS111643
NIMH grants 1R01MH116203

Title: A novel spinoparabrachial pathway for mechanical itch

Authors: *X. REN[1,3], S. LIU[2,3], A. VIRLOGEUX[1], J. BRUSCH[1], S. J. KANG[2], S. HAN[2], M. GOULDING[1], D. ACTON[1];
[1]Mol. Neurosci. Lab., Salk Inst. for Biol. Studies, San Diego, CA; [2]Peptide Biol. Labs., Salk Inst. for Biol. Studies, La Jolla, CA; [3]Biol. Grad. Program, UCSD, San Diego, CA

Abstract: Itch is an unpleasant sensation that generates scratching, a conditional protective reflex behavior. Whereas scratching can be elicited via a simple spinal reflex pathway, itch incorporates sensory-discriminative, affective-motivational, and cognitive components, consistent with growing evidence that, in vivo, scratching is generated and modulated by supraspinal areas in a context-dependent manner. Scratching can be triggered by a range of cutaneous stimuli including light touch (mechanical itch) and chemical irritants such as histamine and chloroquine (chemical itch). Recent studies have identified distinct molecular and circuit mechanisms for the transmission and gating of mechanical and chemical itch information in the periphery and spinal cord; however, the supraspinal regions that facilitate context-dependent responses to itch have not been described in detail. Here, we show that the parabrachial nucleus (PBN) receives mechanical itch information from the spinal cord and is essential for eliciting protective scratching in response to tactile stimuli. Additionally, we have

identified and molecularly characterized the subsets of spinoparabrachial (SPB) projection neurons and PBN neurons that drive mechanical itch and alloknesis. We find that the ascending SPB neurons for the transmission of mechanical and chemical itch are segregated into discreet populations, which then converge in the PBN onto a common population of neurons that are required for scratching. Our results argue that the PBN plays an important role in the generation of protective scratching in response to light touch stimuli in awake behaving mice.

Disclosures: **X. Ren:** None. **S. Liu:** None. **A. Virlogeux:** None. **J. Brusch:** None. **S.J. Kang:** None. **S. Han:** None. **M. Goulding:** None. **D. Acton:** None.

Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 206.03

Topic: D.01. Somatosensation

Support: NIH R01AR074062
Stanley Glaser Foundation Research Award UM SJG 2019-18

Title: Itch-responsive neuronal projections from the lPBN to the amygdala play a role in itch but not pain

Authors: *D. PAVLENKO[1], H. ISHIDA[1], A. MARKAN[2], T. AKIYAMA[1];
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Abstract: Chronic and acute itch can not only cause significant discomfort in many people but can also decrease their quality of life and increase suicidal ideations. Currently, it is well known the parabrachial nucleus [PBN] receives sensory information, including itch, from the spinal cord and then relays it to multiple different brain regions including the central amygdala [CeA]. While previous research has shown a link between the lateral PBN [lPBN] and the CeA, the involvement of these projections in itch is relatively little studied. This research aims to elucidate the role of the itch-responsive neuronal projection from lPBN to CeA. To able to study this specific neural pathway, we are breeding JAX TRAP2 ($Fos^{2A\text{-}iCreER}$) mice bred with JAX Ai14 (Ai14(RCL-tdT)-D) mice and using the targeted recombination in active populations (TRAP2) system. Because the TRAP2xAi14 mice have a cre-ERT2 under *Fos* promotor, we are able to capture the itch-response neurons in the brain with a hydroxytamoxifen IP injection followed by a serotonin injection. While the cre is still present in the nucleus of the neurons, we inject an AAV containing channelrhodopsin-2 (ChR2) into the lPBN bilaterally. The opsin is only expressed in the previously captured neurons, and light fibers are implanted bilaterally just above the CeA. This allows us to focus on exclusively the itch-responsive neuronal projections from the lPBN to the CeA. Activation of the neurons with blue light led to a significant increase in spontaneous scratching over a 10-minute period. However, when combined with histamine, chloroquine, or serotonin injection, blue light activation did not increase the number of scratches

seen in a 30-minute period. Currently, we are hypothesizing that the pruritogen injection fully saturates the pathway, and therefore activating the pathway does not increase the amount of scratching. Additionally, we investigated the effect of blue light activation on mechanical and heat pain thresholds and found no change in pain perception. This led us to conclude that activating the itch response neuronal projections from the lPBN to the CeA does not play a role in pain.

Disclosures: **D. Pavlenko:** None. **H. Ishida:** None. **A. Markan:** None. **T. Akiyama:** None.

Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 206.04

Topic: D.01. Somatosensation

Support: UDEM-DIECI-04-2021

Title: Abnormalities in nociceptive perception of Ebf2 knockout mice in a cheek injection model

Authors: *M. B. D. CEPEDA-VARELA[1,2], D. FLORES-SALDÍVAR[1], G. MORENO LACALLE ZALDÍVAR[1], K. VILLARREAL-VALDÉS[1], A. C. MARTÍNEZ-TORRES[2], V. C. ZOMOSA-SIGNORET[1], R. VIDALTAMAYO[1];
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Abstract: Skin protects us from noxious elements: dirt, bacteria, virus, etc. and provides nociceptive information from such stimuli or from other potential threats, like injuries. These sensations can be interpreted in the brain, as pain or itch signals, depending on the diverse ligands that might be involved. The nociceptive system, together with the immune system, protects us from threats to the integrity of our skin. In pathological conditions, itch and/or pain, acute or chronic, can be disabling. In the last 30 years, efforts have been focused on studying the neuroimmune interactions between somatosensory systems and immune cells. Previously, our lab, found that neurons located in the principal sensory trigeminal nucleus and lamina II from the spinal cord express the transcription factor Early B cell Factor 2 (Ebf2), by analyzing brain and spinal cord cryosections from genetically engineered adult mice expressing the genetic reporter Tau-GFP under the control of the Ebf2 promoter (129SvEbf2/Tau-GFP mouse strain). Our results suggest that Ebf2 might be involved in the circuit formation or regulating the function of nociceptive neurons located in said regions. In this current work, we examined the nocifensive behaviors in wildtype (WT), heterozygous (HET) and knockout (KO), females and males, mice from the 129SvEbf2/Tau-GFP strain with a cheek injection model for simultaneous measurements of itch and pain responses to noxious stimuli, using either lipopolysaccharide (LPS) or histamine (HIS). Our results show a tendency in WT (n=3) and HET (n=3) mice treated with LPS; they present wiping bouts (painful behaviors) on the cheek injected, contrasting with

KO mice (n=3) which have minor wiping episodes and have the tendency to wipe more on the contralateral cheek. Interestingly, KO mice also have more scratching bouts on the injected cheek (related to itch) than WT mice when challenged with LPS. Furthermore, when analyzing the treated groups using HIS, a known pruritogen, in WT (n=2) and HET (n=2) mice, they scratch on the side of the injected cheek, whereas KO mice (n=2), tend to scratch the contralateral side, and have more wiping episodes on the ipsilateral cheek than the WT. Our results suggest that Ebf2 KO mice have two different abnormalities in nociceptive perception: first, they cannot discriminate the spatial location of a nociceptive stimuli, and second, they change the nociceptive modality perceived with respect to WT animals.

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Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 206.05

Topic: D.01. Somatosensation

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Title: Molecular determinants of mechanical itch sensitization in chronic itch

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Abstract: Chronic itch is associated with sensitization of the somatosensory nervous system. Recent studies have identified the neural circuits transmitting acute itch; however, the mechanisms by which itch transforms into a pathological state remain largely unknown. We have previously shown that Aβ low-threshold mechanoreceptors, together with spinal urocortin 3-positive (Ucn3+) excitatory interneurons and neuropeptide Y-positive (NPY+) inhibitory interneurons, form a microcircuit that transmits and gates acute mechanical itch. Here, using whole-cell patch clamp recordings, we observed increased excitability in spinal Ucn3+ neurons under chronic itch conditions. In contrast to Ucn3+ neurons, the excitability of spinal NPY+ neurons was largely reduced under chronic itch conditions. To explore the molecular mechanisms underlying sensitization of this microcircuit, we examined the mRNA expression levels of voltage-gated ion channels in recorded spinal Ucn3+ and NPY+ neurons by single-cell

qRT-PCR. We found that the expression levels of Nav1.6 and Cav2.3 channels were increased in spinal Ucn3+ neurons in chronic itch mice, while the expression level of SK3 channels was decreased. By contrast, the expression levels of Nav1.6 and BK channels were decreased in spinal NPY+ neurons in chronic itch mice. To determine the contribution of different ion channels in chronic itch sensitization, we then used a Markov Chain Monte Carlo method to parametrize a large number of biophysically distinct multi-compartment models of Ucn3+ and NPY+ neurons. These models included explicit representations of the ion channels that we found to be up- or down-regulated under chronic itch conditions. Our models demonstrated that changes in Nav1.6 conductance are predominantly responsible for the changes in excitability of both Ucn3+ and NPY+ neurons during chronic itch pathogenesis. Furthermore, when simulating microcircuits of our Ucn3+ and NPY+ models, we found that reduced Nav1.6 conductance in NPY+ models played a major role in opening the itch gate under chronic itch conditions. However, changing SK, BK, or R-type calcium channel conductance had negligible effects on the sensitization of this circuit. Therefore, our results suggest that Nav1.6 channels may play an essential role in mechanical itch sensitization. The findings presented here may open a new avenue for developing pharmaceutical strategies to treat chronic itch.

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Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

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Topic: D.02. Somatosensation – Pain

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Shriner's Hospital for Children

Title: Investigating the role of skin-nerve communication in models of atopic dermatitis

Authors: *A. BUNK[1], B. RUFF[3], J. HAHN[2], G. KHURANA HERSHEY[3], D. SUPP[2], S. DAVIDSON[1];
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Abstract: Chronic itch in atopic dermatitis (AD) remains a persistent, inadequately treated problem which exacerbates the disease and diminishes patient quality of life. Mounting evidence suggests that dysfunctions in skin-nerve communication are a major contributor to chronic itch in AD. In this study, we hypothesized that mediators secreted by atopic skin cause pruriceptive dorsal root ganglia (DRG) sensory neurons to become hypersensitized. To test this, we employed a mouse model of AD in which mice were treated with patches soaked in extract from the

fungus, *Aspergillus fumigatus,* or saline (negative control), over a period of 3 weeks. Using calcium imaging, we demonstrated that incubation in media supernatant taken from cultures of whole skin taken from the *A. fumigatus* extract-treated mice acutely sensitized DRG sensory neurons obtained from a naïve cohort of mice to both histamine and the non-histaminergic pruritogen, chloroquine, when compared to control. Similarly, we found that lower cervical and upper thoracic DRG sensory neurons taken directly from *A. fumigatus* extract-treated mice were innately sensitized to chloroquine, compared to control. Together, these results indicate that skin-secreted factors contribute to sensory neuron hypersensitivity in the *A. fumigatus* model. The chloroquine-specific sensitization observed in the second experiment is consistent with the non-histaminergic chronic itch that is prevalent in AD. These results warrant future study to identify the secreted factors responsible for the observed effects, how the identified factors cause their effects in neurons, and whether or not antagonism of these factors will ameliorate their effects. Ongoing investigations are focused on elucidating the specific contributions of keratinocytes, the primary cell type of the epidermis, to the observed effects using both 2D and 3D *in vitro* models of innervated skin. In our 2D model, primary human keratinocytes and mouse DRG sensory neurons are cocultured separated by axon-permeable microchannels in a silicone microfluidic device. Using this model, we can investigate how atopic keratinocytes affect sensory neuron chemical sensitivity as well as electrophysiological properties. In our 3D model, DRG sensory neurons are inoculated into the bottom of a layered engineered skin substitute. This model will enable us to study how atopic keratinocytes affect neurite growth and innervation density, and whether changes in innervation contribute to the disease phenotype. Presently we have demonstrated robust neuronal viability in this model as well as responsivity of neurons to stimulation with capsaicin.

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Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

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Program #/Poster #: 206.07

Topic: D.02. Somatosensation – Pain

Title: Effects of oxytocin on the responses of keratinocytes and spinal dorsal root ganglion neurons to ATP and mechanical stimulation

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Abstract: Mechanical stimuli on the peripheral tissue from the environment are received by keratinocytes, transmitted to the brain via dorsal root ganglion (DRG) neurons and finally recognized as pain. Previous studies using mice and rats have demonstrated that oxytocin (OT) has an analgesic effect by behavioral experiments (Antonio et al. 2021) . OT is a peptide

hormone mainly produced in hypothalamus. Recent studies suggest that OT has some analgesic effects on peripheral tissues, however, it has not been fully clarified whether OT can reduce pain sensation and the detailed molecular mechanism for OT production. Previous our study (Shindo et al., 2021) and other laboratory (Koizumi et al., 2004) have shown that ATP is an important transmitter in mechanical stimuli-related signaling in peripheral tissue. Therefore, in this study, we investigated the responses of keratinocytes and cultured DRG neurons to ATP and the effect of OT on them. Keratinocytes and DRG neurons were stained with Fluo4-AM, a fluorescent Ca^{2+} indicator, and their responses to various concentration of ATP, from 10^{-9} to 10^{-3} M, were measured using a confocal microscope. ATP induced transient Ca^{2+} response in both cell types. The ATP concentration that induced 50% response was 4.6×10^{-7} M in keratinocytes, and the pretreatment of OT increased it to 1.1×10^{-5} M. This indicates that OT reduced the sensitivity of keratinocyte to ATP. On the other hand, that was 3.9×10^{-7} M in DRG neurons, and OT decreased it to 3.8×10^{-8} M. The amplitudes of the Ca^{2+} response in DRG neurons were suppressed to 83% by the pretreatment of OT whereas it had few effects on that of keratinocytes. Next, Ca^{2+} responses of keratinocytes in response to mechanical stimulation and effect of OT on it was examined. Mechanical stimulation was applied during Ca^{2+} imaging by picking the cell surface of keratinocytes with a glass needle whose tip was rounded by heat. Mechanical stimulation evoked a transient Ca^{2+} response in the stimulated cell, which spreads to surrounding cells. Pretreatment with OT narrowed the area of keratinocytes showing Ca2+ response. It is probably because OT reduced the sensitivity of keratinocytes to ATP, which plays an important role in signal propagation among keratinocytes. From our results, OT has been found to reduce the sensitivity to ATP in peripheral tissues prior to reaching it to the brain by regulating the response of keratinocytes and DGR neurons in a variety of ways to reduce pain stimuli. We believe this can be applied in daily life in terms of optimizing how to maximize the pain-relieving effect of OT in peripheral tissues.

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Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

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Program #/Poster #: 206.08

Topic: D.02. Somatosensation – Pain

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Title: Keratinocyte PIEZO1 mediates touch sensation and touch allodynia

Authors: *A. MIKESELL, F. MOEHRING, K. SADLER, O. ISAEVA, C. MECCA, C. STUCKY;
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Abstract: Keratinocytes, which make up 95% of the cells in the epidermis, respond to mechanical stimulation and contribute to mechanosensation by signaling to sensory neurons in the skin. It is unknown if this keratinocyte-to-sensory neuron signaling is altered in pain conditions associated with tactile hypersensitivity. Identification of the mechanoreceptors that enable keratinocytes to respond to mechanical stimuli may lead to the development of topical therapeutics for chronic touch pain. Here, we tested the hypothesis that keratinocytes contribute mechanical pain following tissue injury. We first examined if keratinocytes contribute to injury related pain by using optogenetics to selectively inhibit the keratinocytes of epidermal-specific Archaerhodopsin expressing mice treated with paclitaxel. We found that keratinocyte inhibition partially reversed paclitaxel induced touch pain. We next sought to determine the keratinocyte mechanotransducers which enable keratinocytes to respond to mechanical stimuli. We focused on PIEZO1, a mechanotransduction channel expressed in the skin. We found that both mouse and human keratinocytes respond robustly to the PIEZO1 agonist Yoda1. We next found that mice with an epidermal deletion of PIEZO1 (PIEZO1cKO) were less responsive to a range of mechanical touch and pain assays. Using patch clamp electrophysiology, we found that keratinocytes isolated from PIEZO1cKO mice were less sensitive to mechanical stimulation compared to wildtype controls. Furthermore, PIEZO1cKO mice were partially protected against the development of mechanical pain induced by the chemotherapeutic paclitaxel. Lastly, we examined the full complement of pain conditions in which keratinocyte PIEZO1 is sensitized by isolating keratinocytes from models of mechanical pain, including spared nerve injury, diabetes, sickle cell disease, CFA, and CIPN. Using calcium imaging, we found that keratinocytes isolated from each injury condition were sensitized to activation by the PIEZO1 specific agonist Yoda1. Furthermore, primary human keratinocytes exposed to commonly used chemotherapeutics were sensitized to activation by Yoda1. Our data indicate that keratinocyte PIEZO1 mediates normal tactile sensation and may contribute to tactile pain following tissue injury.

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Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

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Topic: D.02. Somatosensation – Pain

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Title: Sickle cell disease hypersensitivity is mediated by TRPV4 in dorsal root ganglia neurons and keratinocytes

Authors: *V. L. EHLERS, K. E. SADLER, A. D. MENZEL, C. M. MECCA, C. L. STUCKY; Cell Biology, Neurobio. & Anat., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Patients living with sickle cell disease (SCD) experience pervasive and debilitating hypersensitivity to environmental stimuli. Tissue inflammation and neuropathy are major drivers of chronic pain in SCD, and current treatments for acute vaso-occlusive crises have limited effectiveness for treating persistent SCD pain. Thus, identification of novel peripheral targets that alleviate chronic pain in SCD is critical for improving patient quality of life. The nonselective cation channel transient receptor potential vanilloid 4 (TRPV4) is one candidate mechanism due to its role in normal somatosensation and allodynia following injury, and its known expression in peripheral tissues, including dorsal root ganglia (DRG) neurons and keratinocytes, making TRPV4 an ideal target. In the current experiments, we used evoked behavior assays, and *in vitro* calcium imaging and whole-cell patch clamp recordings to examine the hypothesis that TRPV4 mediates SCD hypersensitivity in DRG neurons and keratinocytes. We first demonstrate that SCD mice display complete and dose-dependent reversal of punctate mechanical allodynia when a TRPV4 antagonist is injected directly into the hindpaw. Functionally, DRG neurons from SCD mice display increased TRPV4-mediated calcium flux relative to wildtype (WT) mice, and TRPV4 blockade reduces the inward mechanical currents of these neurons. These effects are specific to small-diameter DRG neurons, many of which are nociceptors in rodents. While DRG neurons are traditionally thought to be the primary somatosensory detectors and transducers, increasing interest in non-neuronal cells, including keratinocytes, has spurred recent research on their role in somatosensation. Since keratinocytes compose much of the epidermis and are proximal to afferent nerve terminals, they likely play a role in peripheral somatosensation and hyperalgesia alongside DRG neurons. Thus, we also examined whether SCD hypersensitivity is driven by TRPV4 in these cells. Our data show that keratinocytes from SCD mice display robust TRPV4-mediated calcium flux relative to keratinocytes from WT mice. Together, these data suggest that TRPV4 plays an integral role in SCD hypersensitivity, both at the level of the DRG neuron and keratinocyte. Current work is investigating whether blocking TRPV4 in keratinocytes reduces mechanical currents, and whether TRPV4 is differentially expressed in DRG neurons and keratinocytes from SCD and WT mice.

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Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

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Program #/Poster #: 206.10

Topic: D.02. Somatosensation – Pain

Support: NINDS R21 NS121946

Title: Sting activation in microglia alleviates nerve injury-induced neuropathic pain

Authors: *A. S. PRUDENTE[1], A. S. ANDRIESSEN[2], S. LEE[1], C. F. COHEN[4], R.-R. JI[3], C. R. DONNELLY[3], T. BERTA[1];
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Abstract: Activation of the stimulator of interferon genes (STING) is a critical component of host innate immune defense. We recently reported the involvement of neuronal STING in pain regulation. However, STING is also expressed in microglia which can regulate microglia proliferation and neuroinflammation, two hallmarks of neuropathic pain. Thus, we hypothesized that STING activation in microglia can participate to alleviate nerve injury-induced neuropathic pain. We found that STING is upregulated in spinal proliferative microglia, but not in peripheral sensory neurons, in the spared nerve injury (SNI) mouse model of neuropathic pain. Repeated intrathecal (i.t.) injections of small molecule STING agonists (ADU-S100 and DMXAA, 35 nmol) reversed neuropathic pain behavior (i.e. mechanical allodynia) in male mice after SNI. However, the same treatment was ineffective in female mice, suggesting a sex dimorphism in STING signaling. Based on these data, we sought to determine the unique contribution of STING signaling in microglia to neuropathic pain, using Sting1$^{fx/fx}$; Tmeme119-Cre/ER^{T2} inducible conditional knockout (STING-cKO) mice. STING-cKO mice showed the specific elimination of STING expression in microglia, but not in peripheral sensory neurons. Notably, we found that the reversal of neuropathic pain behavior after SNI by STING agonists was abolished in these cKO mice compared to control wild-type (WT) mice. Abolishment of the behavioral effect of STING agonists was also observed in mice treated with neutralizing antibodies against IFN(Interferon)-β, suggesting the requirement of IFN signaling in microglia for the STING-mediated alleviation of neuropathic pain. In line with these data, activation of STING in microglia culture led to release of INFβ, and adoptive transfer of STING-activated microglia in WT mice was sufficient to alleviate neuropathic pain behavior after SNI. Neuropathic pain is difficult to treat and remains a major clinical challenge. Our data expand our mechanistic understanding of STING and microglial signaling in neuropathic pain, and thus may lead to new therapeutic treatments.

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Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

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Topic: D.02. Somatosensation – Pain

Support: NIH NS045594

Title: Sympathetic effect on regeneration and neuronal death in peripheral nervous system

Authors: *D. DE NARDIN LÜCKEMEYER, W. XIE, J. A. STRONG, J.-M. ZHANG; Univ. of Cincinnati, Univ. of Cincinnati, Cincinnati, OH

Abstract: Injury to the nervous system as consequence of traumatic injury or disease often causes a debilitating chronic pain syndrome which is refractory to available treatments. The consequence of neuronal damage is the disruption of the homeostasis environment and the peripheral nerve injury of sensory neurons can activate a regeneration program but can also lead to neuronal death. These conditions are thought to be exacerbated or maintained by sympathetic nervous system activity. Local sympathetic blockade or lesion is commonly used to treat pain conditions, however, the mechanism that underlies the maintenance or resolution of pain remains poorly understood. To better understand these mechanisms, we developed, in rats and mice, a localized microsympathectomy (mSYMPX), which consists of cutting the grey rami containing postganglionic sympathetic fibers as they enter the spinal nerves near the lumbar dorsal root ganglia (DRG). We reported that mSYMPX attenuated pain behaviors in several preclinical pain models (SNI, SNL, chemotherapy). In this study we investigated if the sympathetic interactions with sensory neurons are associated with regeneration and neuronal cell death. In these experiments, using male and female mice, propidium iodide (PI; label for dying cells) was intravenously injected and after 40 minutes, the mouse was sacrificed and the lumbar DRG removed and placed in our microscope chamber as an ex vivo live whole mount preparation. Few PI positive (PI+) neurons and scattered non-neuronal cells were observed in DRGs from naïve mice. The smaller non-neuronal cells were identified as resident macrophages expressing CX3CR1 using the $CCR2^{+/RFP}/CX3CR1^{+/GFP}$ dual-reporter mice. Surprisingly, in mice that had mSYMPX 2 days prior to the PI injection and DRG isolation, PI+ neurons were evident in the whole mount preparation as late as day 14. Furthermore, results from a proteome profiler mouse apoptosis array showed that some anti-apoptosis proteins are downregulated by mSYMPX and, in mice with nerve injury, pro-apoptotic proteins are upregulated. Thus, mSYMPX led to death of some sensory neurons in the adjacent DRG and altered pain sensitivity in animals with nerve injury.

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Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

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Title: The role of dermal fibroblasts in the initiation and resolution of TLR4-induced inflammatory pain

Authors: *M. E. LENERT, T. A. SZABO-PARDI, M. D. BURTON;
Univ. of Texas at Dallas, Richardson, TX

Abstract: The prominent role of non-neuronal cells driving pain states has gained much attention in recent years as pain affects over 100 million Americans annually. However, stromal cells, typically associated as supportive in nature, have not been well-studied in various pain states. Fibroblasts, a major stromal cell fraction, perform essential functions during inflammation, tissue remodeling, and wound healing; however, recent studies have suggested that fibroblasts play a role in mediating neuropathic pain. Toll-like receptor 4 (TLR4), a pattern-recognition receptor, is an essential component of the innate immune system and mediates cell-specific responses to pain states. Our study assessed the role of fibroblast specific TLR4 activation in inflammatory pain. We utilized a novel conditional genetic model that allowed for cell-specific expression of TLR4 on fibroblasts ($FSP1^{TLR4LoxTB}$) in a whole-body null or knockout background ($TLR4^{LoxTB}$). Mechanical sensitivity via von Frey filaments was performed after a local intraplantar injection in the hindpaw of ultrapure lipopolysaccharide (1µg LPS/20µL), a specific TLR4 agonist and both pain development and recovery were assessed in male and females. Plantar skin was collected at height of mechanical sensitivity (4-hours) post-LPS to assess fibroblast three-dimensional cell size and shape as a proxy of inflammation and pain using Imaris software. . All experiments were performed and analyzed by blinded experimenters. Male and female mice with fibroblast-specific expression of TLR4 develop mechanical sensitivity similarly to wild-type mice in response to LPS, whereas TLR4-null mice do not. We have discovered that dermal fibroblasts change morphology in response to LPS in vivo. In females, fibroblasts increase in cell volume and elongate compared to vehicle controls. Conversely, male fibroblasts have reduced cell volume and flatten out. Fibroblast activation via TLR4 is sufficient to induce inflammatory pain. Furthermore, morphology in activated fibroblasts in males and females indicate a potential sexual dimorphism in fibroblast activation states. Our current study is one of the first to show that direct activation of fibroblasts is sufficient to drive pain states and characterize morphological changes of activated fibroblasts in vivo.

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Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

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Title: B cells regulate initiation of neuropathic pain

Authors: *K. F. WILLCOX[1], M. J. LACAGNINA[1], C.-Y. LIN[1], M. V. CHAVEZ[1], J. T. LU[1], Y. A. ZUBERI[1], C. J. HEIJNEN[2], P. M. GRACE[1];
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Abstract: Background: Neuropathic pain, caused by injury to somatosensory nerves, is a chronic condition that can greatly reduce quality of life. Previous transcriptome data revealed increase in B cell-associated gene expression following chronic constriction injury (CCI) of the sciatic nerve. However, it is not known if B cells play a mechanistic role in neuropathic pain after peripheral nerve injury. Here, we investigate if B cells contribute to the development of neuropathic pain after CCI.
Methods: Subjects were adult male and female *Ighm*$^{-/-}$ knockout mice (muMT; lacking mature B cells), or C57BL/6J littermate (WT) mice. Neuropathic pain was induced via unilateral CCI of the sciatic nerve. B cells were isolated from spleens of WT mice using magnetic labelling and were adoptively transferred to muMT mice. B cells were depleted in WT mice via administration of anti-CD20 monoclonal antibody at the time of injury. Allodynia was quantified via paw withdrawal to innocuous punctate and dynamic stimuli, measured by von Frey filaments and paintbrush assay. Flow cytometry was performed to validate B cell reconstitution in muMT mice, and to characterize B cell phenotypes in WT mice after nerve injury. Spinal cord and dorsal root ganglia (DRG) were extracted from WT mice on day 14 post-CCI for immunohistochemistry.
Results: In both sexes, B cell deficient muMT mice were protected from punctate and dynamic allodynia after CCI, compared to WT littermates. Reconstituting muMT with B cells mice prior to injury resulted in normal development of allodynia after CCI. Depletion of B cells with anti-CD20 treatment prevented the development of CCI-induced mechanical allodynia. Following CCI, flow cytometry revealed B cell differentiation into antibody-secreting plasmablasts and plasma cells in the spleen and inguinal lymph nodes, and IHC showed increased IgG deposition in spinal cord and DRG.
Conclusion: These data reveal a pro-nociceptive role for B cells in CCI-induced neuropathic pain in both male and female mice. Modulating B cell function could be a potential therapeutic approach for neuropathic pain.

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Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

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Title: Macrophage/microglia-produced platelet-activating factor contribute to the development of neuropathic pain

Authors: *S. YAMAMOTO, T. HASHIDATE-YOSHIDA, T. SHIMIZU, H. SHINDOU; Lipid Life Sci., Natl. Ctr. for Global Hlth. and Med., Tokyo, Japan

Abstract: Neuropathic pain is characterized by debilitating chronic pain symptoms such as spontaneous pain, hyperalgesia, and allodynia, and is often caused by damage to the nervous system that results from cancer, chemotherapy, diabetes, and trauma. Platelet-activating factor (PAF) is a potent phospholipid mediator, which is involved in the pathology of neuropathic pain after peripheral nerve injury (PNI). However, the spatiotemporal changes of PAF levels after PNI remains unknown. Using immunohistochemistry, lipidomic analysis, behavioral test, and conditional knockout mice of the PAF biosynthetic enzyme LPCAT2 (lysophosphatidylcholine acyltransferase 2, also called LPLAT9), we examined which cell types express LPCAT2 and produce PAF in response to PNI in the dorsal root ganglion and spinal cord, and how PAF contributes to the development of neuropathic pain. We revealed that LPCAT2 was expressed in non-neuronal cells such as macrophages, and satellite glial cells in the dorsal root ganglion, and also expressed in microglia and oligodendrocytes in the spinal cord. After PNI, PAF levels were transiently increased on days 3 and 7, and the number of LPCAT2-expressing macrophages and microglia were also increased. In addition, the intrathecal injection of a PAF receptor antagonist (WEB2086) attenuated mechanical allodynia on day 7, but not day 14. Furthermore, we revealed that PNI-induced increase of PAF levels and mechanical allodynia were significantly attenuated in macrophages/microglia-specific LPCAT2 knockout mice (*Cx3cr1*^{CreERT2};*Lpcat2*$^{flox/flox}$). These results suggest that the transient increase of PAF derived from macrophages and microglia critically contributes to the development of neuropathic pain. Moreover, a blocking strategy of PAF signal during PAF biosynthesis by LPCAT2 (LPLAT9) is important to suppress neuropathic pain, and identification of a biomarker that reflects this period is needed as a companion drug.

Disclosures: S. Yamamoto: None. **T. Hashidate-Yoshida:** None. **T. Shimizu:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Ono Pharmaceutical Co., Ltd. **H. Shindou:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or

consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Ono Pharmaceutical Co., Ltd.

Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 206.15

Topic: D.02. Somatosensation – Pain

Support: US Department of Defense Grant 10669042
Lisa Dean Moseley Foundation Grant LDMF1709JC
Cleveland Clinic Anesthesiology Institute Interval Research Fund

Title: Enhancing fractalkine signaling attenuates neuroinflammation and neuropathic pain induced by chronic constriction nerve injury in mice

Authors: *Q. FAN, J. LI, F. LI, Y. YIN, J. CHENG;
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Abstract: Objective: Fractalkine (CX3CL1) signaling between neurons and $CX3CR1^{+}$ cells (microglia and macrophages) plays a critical role in neuroinflammation and neuropathic pain (NP). However, its roles in anti- vs. pro-neuroinflammation and anti- vs. pro-NP remains controversial despite of decades of research. To address this controversy, we investigated the impact of loss and gain of function of this signaling mechanism on neuroinflammation and NP by utilizing transgenic mice. Methods: Chronic constriction injury (CCI) was performed on wild-type (WT), $CX3CR1^{GFP/+}$, $CX3CR1^{GFP/GFP}$ and CX3CL1-Tg/$CX3CR1^{GFP/+}$ mice. Real-time PCR, immunohistochemical staining, and western blot were performed using specimens from the sciatic nerve, dorsal root ganglion (DRG), and spinal cord. Mechanical hyperalgesia was evaluated using Von-Frey Filament test in male and female WT and CX3CL1-Tg/$CX3CR1^{GFP/+}$ mice. Results: Comparing to sham control, $CX3CR1^{+}$ cells were morphologically activated in the sciatic nerve, DRG, and spinal cord of the ipsilateral side, evident at post-CCI Day 3, peaked in the second week, and persistent beyond 4 weeks. The $CX3CR1^{+}$ cell activation was significantly increased and prolonged in $CX3CR1^{GFP/GFP}$ mice, but dramatically reduced in full-length CX3CL1 over-expression mice. CCI induced rapid and dramatic increases in TNF-α, IL-1β and IL-6 transcription levels in the sciatic nerve and spinal cord of the ipsilateral side in WT mice compared to sham control. The increases were significantly and consistently higher in $CX3CR1^{GFP/GFP}$ mice, peaked within 3 days post-CCI and lasted for 8 weeks, but were reduced by more than 75% in the first 2 week and by more than 90% thereafter in CX3CL1-Tg mice. Full-length CX3CL1 and TGF-β contents declined after CCI in WT animals. In contrast, contents of full-length CX3CL1 and its C-terminal fragment, TGF-β family, and phosphorylated Smad2/3 proteins were significant increased in CX3CL1-Tg mice, indicating dramatically enhanced CX3CL1/TGF-β/Smad2/3 signaling. Importantly, CCI-induced mechanical hyperalgesia was significantly reduced in CX3CL1-Tg mice of both sexes. Conclusion: These

results support a new conceptual framework, in which CX3CL1 signaling pathways, through both the N-terminal and C-terminal fragments, play an anti-inflammatory and analgesic role after nerve injury. These pathways may represent new therapeutic targets for preventing and/or treating neuropathic pain.

Disclosures: Q. Fan: None. **J. Li:** None. **F. Li:** None. **Y. Yin:** None. **J. Cheng:** None.

Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 206.16

Topic: D.02. Somatosensation – Pain

Support: NIH DP2AI138239

Title: Activation of macrophages and GPR37 protects against systemic inflammation, sepsis, and pain following bacterial infection.

Authors: *S. BANG[1], C. R. DONNELLY[1], X. LUO[1], M. T. MORENO[2], X. TAO[1], Z. WANG[1], S. CHANDRA[1], A. V. BORTSOV[1], E. R. DERBYSHIRE[2], R.-R. JI[1,3,4];
[1]Dept. of Anesthesiol., Ctr. for Translational Pain Medicine, Duke Univ. Med. Ctr., Durham, NC; [2]Dept. of Chemistry,, Duke Univ., Durham, NC; [3]Dept. of Neurobio., , Duke Univ. Med. Ctr., Durham, NC; [4]Dept. of Cell Biol., Duke Univ. Med. Ctr., Durham, NC

Abstract: G-protein coupled receptor 37 (GPR37) is highly expressed in neurons and glial cells in the central nervous system. GPR37 is also called a parkin-associated endothelin-like receptor (PAELR) after being discovered to be a substrate of the E3 ubiquitin ligase parkin. GPR37 has been implicated in Parkinson's disease and Autism Spectrum Disorder. We recently found GPR37 expression in macrophages, which can promote macrophage phagocytosis and the resolution of inflammatory pain. The bacterium *Listeria monocytogenes* causes human listeriosis, a serious foodborne disease that can cause CNS infections known as neurological listeriosis. *Listeria* can survive intracellularly by evading the endo-phagosome response and infecting surrounding cells via neuronal connections. Bacterial infections can cause sepsis and severe pain. In this study, we show that GPR37 has a protective role in an animal model of systemic inflammation and pain caused by *Listeria* infections. When compared to wild mice, *Gpr37*-deficient mice had poor survival and severe hypothermia after intraperitoneal *Listeria* infection. Injection of listeria bacteria into the hind paw of wild-type mice produced robust mechanical allodynia for a week. Interestingly, we found that *Gpr37* knockout (KO) mice had delayed pain recovery compared to wild-type (WT) mice: mechanical pain failed to recover even two weeks after bacteria injection. Artesunate (ARU) is an anti-malaria drug. We found that ARU is an agonist of GPR37 and induces GPR37-dependent macrophage phagocytosis. In WT mice, ARU protects against *listeria*-induced systemic inflammation, but the protective effect of this anti-malaria drug is lost in *Gpr37* KO mice. Intraplantar injection of ARU was able to reduce listeria-

induced mechanical pain in WT mice but this analgesic effect was lost in KO mice. Furthermore, ARU prevented systemic inflammation by binding to GPR37 and increasing the autophagosome response for the clearance of *Listeria.* Adoptive transfer of ARU-primed WT macrophages but not KO macrophages, via I.PL application significantly alleviated infection-induced pain. Our findings show that the GPR37 protects against *Listeria* infection by macrophage activation. GPR37 agonists may help to relieve systemic inflammation and infection-induced pain.
Reference: Bang S, et al., Activation of GPR37 in macrophages confers protection against infection-induced sepsis and pain-like behaviour in mice. Nat Commun.2021 Mar 17;12(1):1704.
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Disclosures: S. Bang: None. **C.R. Donnelly:** None. **X. Luo:** None. **M.T. Moreno:** None. **X. Tao:** None. **Z. Wang:** None. **S. Chandra:** None. **A.V. Bortsov:** None. **E.R. Derbyshire:** None. **R. Ji:** None.

Poster

206. Itch Mechanisms and Non-Neuronal Cells and Pain

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 206.17

Topic: D.02. Somatosensation – Pain

Support: U.S. Army Medical Research and Materiel Command Grant W81XWH-19-1-0160
Rita Allen Foundation Award in Pain
University of Texas System Rising STARs Award
American Australian Association Sir Keith Murdoch Fellowship
National Institutes of Health R01 NS126252

Title: Antibody receptor signaling from spinal cord astrocytes promotes neuropathic pain

Authors: *M. J. LACAGNINA[1], K. F. WILLCOX[1], J. LI[1], C. J. HEIJNEN[2], P. M. GRACE[1]; [1]Labs. of Neuroimmunology, Dept. of Symptom Res., Univ. of Texas MD Anderson Cancer Ctr., Houston, TX; [2]Dept. of Psychological Sci., Rice Univ., Houston, TX

Abstract: Background: Neuropathic pain is a chronic and debilitating condition caused by injury to somatosensory nerves. Mechanical allodynia, a symptom of neuropathic pain in which normally innocuous touch is perceived as painful, is particularly resistant to existing pain treatments. Neuroinflammatory signaling arising from spinal cord glial cells are thought to be critical contributors to the development of allodynia, but it is unclear what mechanisms govern glial activation after nerve injury. Here, we demonstrate that Fc gamma receptor IIa (FcγRIIa), an immune receptor for immunoglobulin G (IgG) antibodies, contributes to the development of mechanical allodynia after neuropathic injury.
Methods: Subjects were adult male and female Sprague Dawley rats and *Fcgr*$^{-/-}$ knockout mice.

Neuropathic pain was induced by unilateral chronic constriction injury (CCI) of the sciatic nerve. Paw withdrawal to innocuous punctate and dynamic touch was measured by von Frey filaments and paintbrush assay. Gene and protein expression were measured in spinal cord dorsal horn and dorsal root ganglia. Viral-mediated CRISPR-Cas9 gene editing was used to reduce *Fcgr2a* expression in *Gfap*$^{+}$ spinal astrocytes *in vivo*. Astrocytes were purified by immunopanning and treated with IgG immune complexes (IgG-IC), the agonists for FcγRs, following siRNA knockdown of *Fcgr2a*.
Results: Genetically modified mice lacking FcγRs (*Fcgr*$^{-/-}$) are protected against mechanical allodynia after nerve injury, suggesting a role for FcγR activation in the development of neuropathic pain. Through a combination of *in situ* hybridization, cross-tissue gene expression analyses, and immunohistochemistry, we determined that the FcγR subtype FcγRIIa increases its expression in the ipsilateral spinal dorsal horn after injury, and its expression is primarily localized on spinal astrocytes. Employing a therapeutic CRISPR-Cas9 approach for cell-specific gene editing of *Fcgr2a*, we demonstrated that reducing *Fcgr2a* expression in *Gfap*$^{+}$ spinal astrocytes attenuates the development of mechanical allodynia after injury. Finally, activating FcγRs on astrocytes with IgG-IC results in production of pro-inflammatory mediators known to influence nociceptor hyperexcitability (including IL-1β, TNF, CCL2, and CXCL1), and this inflammatory response was blocked by siRNA knockdown of *Fcgr2a*.
Conclusion: These data suggest that FcγRIIa on spinal astrocytes may be activated following peripheral nerve injury to drive injury-induced tactile pain. These findings indicate that reducing autoantibody IgG signaling at glial FcγRs could be a novel therapeutic target to alleviate suffering from neuropathic pain.

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Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.01

Topic: D.02. Somatosensation – Pain

Support: Brain Research UK PhD Studentship 201819-25

Title: Investigating the potential role of anoctamin 1 in trigeminal CGRP signalling

Authors: *C. PALFREY, S. SHAH, N. GAMPER;
Univ. of Leeds, Leeds, United Kingdom

Abstract: In small-diameter (predominantly nociceptive) neurons of the dorsal root ganglia (DRG), the calcium-activated chloride channel anoctamin-1 (ANO1) confers amplification of nociception via preferential coupling of ANO1 to IP_3-sensitive Ca^{2+} release channels (IP_3R1). In these neurons, ANO1 activation induces Cl^- efflux and depolarization of the membrane potential,

likely to produce an excitatory effect. The trigeminal ganglia (TG) are the cranial somatosensory analogue of DRG; there is a large degree of functional similarity between the DRG and TG, however, there are also important differences with respect to pathological conditions that are unique to the trigeminal system, such as migraine and trigeminal neuralgia. Hence, it is necessary to investigate if ANO1-mediated signalling mechanisms are conserved in the TG. TG neurons express the canonical CGRP receptor, which is known to couple to IP_3-mediated Ca^{2+} release, supporting a framework to investigate ANO1 coupling to CGRP signalling in the TG. Immunofluorescent labelling suggests strong expression (95.9%) of ANO1 in the nociceptive population (somatic diameter < 35 μm) of rat TG neurons (28 day-old, both sexes) which co-localizes with both IP_3R1 and CGRP receptor proteins, RAMP1 and CLR. Proximity ligation assays (PLA) in rat TG neuron culture (6 day-old, both sexes) established < 40 nm proximity between ANO1 and IP_3R1 only in small-diameter (< 35 μm) neurons, supporting the hypothesized role for nociceptive ANO1-IP_3R1 coupling. PLA signal was detected between ANO1 and RAMP1 in TG neurons with somatic diameters ranging from 8.77 to 32.1 μm (n = 67, N = 7). Stimulation with 1 μM CGRP for 1 hour at 37°C (n = 41, N = 3) significantly increased the mean signal from 6.06 (SEM 1.09) puncta per cell to 12.0 ± 1.60 (p = 0.0020), suggesting a dynamic functional interaction between ANO1 and the CGRP receptor. These preliminary findings offer a promising insight into a possible role for ANO1 in CGRP signalling in the TG, presenting ANO1 as a potential therapeutic target for trigeminal pain disorders.

Disclosures: **C. Palfrey:** None. **S. Shah:** None. **N. Gamper:** None.

Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.02

Topic: D.02. Somatosensation – Pain

Support: DE026499
Intramural funds

Title: Mechanosensitive Ion Channels and TMJ nociception

Authors: *D. BEREITER, R. THOMPSON, F. AHMED, M. RAHMAN;
Univ. of Minnesota, Minneapolis, MN

Abstract: Temporomandibular joint disorders (TMD) are common conditions that present with pain in the temporomandibular joint (TMJ) and masticatory muscles and are dependent on estrogen status. Pain evoked by palpation of deep tissues overlying the TMJ and pain due to jaw opening are reliable diagnostic features of TMD; however, the basis for mechanical sensitivity in TMD is not well defined. To assess the mechanisms that drive jaw pain due to palpation or jaw movement in TMD, the contributions of the mechanosensitive ion channels, transient receptor potential vanilloid 4 (Trpv4) and Piezo2, were assessed in a rat model for TMJ inflammatory

hyperalgesia with minimal evidence of tissue damage. Single trigeminal ganglion (TG) neurons were recorded *in situ* in adult female ovariectomized rats (OvX) and in OvX rats treated with estradiol (OvXE). Intra-TMJ application of low dose (10μg/10μl) Complete Freund's Adjuvant (CFA) or vehicle was injected, and TG activity was recorded 10 days later without knowledge of prior treatment. CFA-treated OvX and OvXE rats displayed reduced head withdrawal thresholds to deep tissue palpation (-32 to -36%, $p<0.01$ vs sham) and decreased food intake at 10d post-CFA (-32 to -34%, $p<0.01$ vs sham). Jaw opening-evoked TG unit activity was increased in proportional to jaw open distance (4-14mm) and was significantly ($p<0.001$) greater in CFA-treated OvXE rats (216 ± 20 spikes/stim) vs OvX rats (128 ± 4 spikes/stim) and vs sham controls to maximum open distance. In OvXE rats (n=5-7 units/group), intra-TMJ injection of the Trpv4 antagonist, RN1734, reduced jaw open-evoked activity (-66 ± 4%, $p<0.001$ vs sham). Intra-TMJ injection of the Piezo2 antagonist, GsMTx4, also reduced activity (-61±4%, $p<0.01$ vs sham) to repeated jaw opening (14mm). CFA-treated rats displayed significant increases in Trpv4 protein levels for TMJ and TG tissue samples ($p<0.025$), while Piezo2 levels had smaller increases. To determine if loss of lubrication after CFA contributed to increased mechanical input, TMJ samples were assayed for lubricin, a proteoglycan product that is critical for normal joint function. Protein levels of lubricin were marginally reduced after CFA treatment. In conclusion, Trpv4 and Piezo2 activation contribute significantly to the transduction of mechanoreceptive signals that drive TG unit responses to jaw opening. High estrogen status is a significant factor that further enhances jaw movement-induced TG neural activity after TMJ inflammation.

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Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.03

Topic: D.02. Somatosensation – Pain

Support: NS109059
DE018661
DE023090

Title: Characterization of electrophysiological properties of Nav1.8-ChR2eYFP trigeminal ganglion neurons of mice

Authors: *S. GUPTA, J. GU, J. LING;
Dept. of Anesthesiol. and Perioperative Med., Univ. of Alabama, Birmingham, Birmingham, AL

Abstract: Nav1.8 channels are expressed in nociceptive trigeminal ganglion (TG) neurons and play a significant role in nociception as well as the development of trigeminal neuropathic pain. However, electrophysiological properties of Nav1.8-expressing TG neurons that innervate orofacial regions (V2 TG neurons) have not been fully characterized. In the present study, we

used *ex vivo* TG preparations made from Scn10a^{Cre}; Ai32 transgenic mice, and characterized electrophysiological properties of V2 TG neurons that express Nav1.8-ChR2eYFP (Nav1.8 TG neurons). Based on conduction velocity (CV) measured following electrical stimulation of infraorbital nerves, Nav1.8-TG neurons could be classified into C-fiber Nav1.8-TG neurons whose CV was < 1 m/s, Aδ-fiber Nav1.8-TG neurons whose CV was 1 to 10 m/s, and Nav1.8-Aβ-fiber TG neurons whose CV was > 10 m/s. C-fiber Nav1.8-TG neurons had small soma size of < 18 μm; Aδ-fiber Nav1.8-TG neurons had medium soma size of 18 to 33 μm; and Aβ-fiber Nav1.8-TG neurons had large soma size of > 33 μm. Passive membrane properties of these three classes of Nav1.8-TG neurons were characterized. C-fiber Nav1.8-TG neurons had significantly greater input resistance and lower membrane capacitance than Aδ-fiber Nav1.8-TG neurons and Aβ-fiber Nav1.8-TG neurons. C-fiber Nav1.8-TG neurons had more depolarized resting membrane potentials than Aδ-fiber Nav1.8-TG neurons and Aβ-fiber Nav1.8-TG neurons. For active membrane properties, AP amplitude and rheobase were significantly smaller in C-fiber Nav1.8-TG neurons than in Aδ-fiber Nav1.8-TG neurons and Aβ-fiber Nav1.8-TG neurons, and AP thresholds were significantly lower for Aδ-fiber Nav1.8-TG neurons than Aβ-fiber Nav1.8-TG neurons. AP widths were significantly broader in C-fiber Nav1.8-TG neurons than Aδ-fiber Nav1.8-TG neurons and Aβ-fiber Nav1.8-TG neurons. C-fiber Nav1.8-TG neurons had significantly greater AHP amplitude than Aβ-fiber Nav1.8-TG neurons, but no significant difference in AHP amplitude was observed between C-fiber Nav1.8-TG neurons and Aδ-fiber Nav1.8-TG neurons. Under the voltage-clamp configuration and in response to voltage steps, C-fiber Nav1.8-TG neurons showed smaller voltage-activated outward currents and more depolarized reversal potential than Aδ-fiber Nav1.8-TG neurons and Aβ-fiber Nav1.8-TG neurons. Taken together, C-fiber Nav1.8-TG neurons, Aδ-fiber Nav1.8-TG neurons, and Aβ-fiber Nav1.8-TG neurons display distinct electrophysiological properties.

Disclosures: S. Gupta: None. **J. Gu:** None. **J. Ling:** None.

Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.04

Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS109059
NIH Grant DE018661
NIH Grant DE023090

Title: Non-nociceptive and nociceptive-like trigeminal Aβ-afferent neurons of rats: mechanical and chemical sensitivity

Authors: *R. J. VADEN, J. G. GU;
Anesthesiol. and Perioperative Med., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: The significance of Aβ-afferent nociceptors in pain signaling is not understood and therefore often ignored. We recently identified a heterogeneous population of large-diameter somatosensory neurons located within the trigeminal ganglion (TG) that conduct in the Aβ velocity range. Here, we aim to determine the contribution of large-diameter TG neurons to non-nociceptive and nociceptive function. Experiments using ex vivo TG preparation and whole-cell patch-clamp of large-diameter neurons in adult male and female rats indicate the existence of five discrete subpopulations (I, II, III, IIIa, and IIIb) with distinct intrinsic electrophysiological properties, which respond differentially to mechanical somatic membrane displacement and focal application of sensory mediators 5-HT, ACh and ATP. Notably, action potential amplitude increases, kinetics slow, and the repolarizing dV/dt peak reduces significantly between types, progressively from I to IIIb. Analysis of current-voltage relationship indicates outward current weakens and inward current slows across types from I to IIIb. Additionally, injection of hyperpolarizing current reveals sag potential in I, II, III, and IIIa, that substantially reduces in IIIb. Mechanical somatic membrane displacement evokes graded, fast-, intermediate-, and slow-desensitizing inward currents in I, II, and III, respectively. However, IIIa and IIIb appear mechanically insensitive with minimal or no mechanically activated currents present. Inversely, focal application of 5-HT evokes large inward currents in IIIa and IIIb, that comparatively reduce in amplitude to near absence in I, II, and III. Similarly, ACh application elicits robust inward currents in IIIb, that significantly reduce in amplitude, but remain present in majority of I, II, III, and IIIa. Application of ATP evokes consistently small inward currents in I, and varied amplitude, yet unremarkable responses in II, III, IIIa, and IIIb. Together, these results provide evidence for the existence of distinguished large-diameter non-nociceptive and nociceptive-like neurons with functionally divergent mechanical and chemical sensitivity.

Disclosures: R.J. Vaden: None. **J.G. Gu:** None.

Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.05

Topic: D.01. Somatosensation

Support: DE029187-01S2

Title: Neuronal subclass specificity of mechano-gated current responses in trigeminal ganglion neurons innervating masseter muscle

Authors: K. A. LINDQUIST[1], A. N. AKOPIAN[2];
[1]Pharmacol., UT Hlth. Sci. Ctr. San Antonio, SAN ANTONIO, TX; [2]UT Hlth. Sci. Ctr., UT Hlth. Sci. Ctr., San Antonio, TX

Abstract: The field of mechano-sensation remains the final frontier of somatosensation research. We recently demonstrated that the masseter muscle is almost exclusively innervated by various

types of myelinated A-fibers. Using reporter mice to label specific subsets of sensory neurons, determined by our single-cell RNA sequencing data, in combination with back labeling with WGA, we seek to characterize adaptation properties of mechano-gated currents using whole-cell patch clamp electrophysiology. The term “adapting” has been used to describe the decay kinetics of mechano-gated currents. Current adaptation can be divided into rapid-adapting (RA), intermediate-adapting (IA), and slow-adapting (SA). We hypothesize patterns of threshold activation and current characteristics unique to neuronal subtypes. Using the current signature method, action potentials and outward currents are additionally measured to categorize neuronal responses. We demonstrate neuronal subclass specificity of mechano-gated current responses.

Disclosures: K.A. Lindquist: None. **A.N. Akopian:** None.

Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

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Program #/Poster #: 207.06

Topic: D.01. Somatosensation

Support: NRF-2019R1I1A1A01059697
NRF-2020R1A2C3008481

Title: Analgesic effect of Nav 1.7 channel inhibition in the trigeminal ganglion following allylisothiocyanate-induced pulpitis pain

Authors: *G. NAN[1,2], K. KIM[1], L. KIM[1,2], M. CHA[1], B. H. LEE[1,2];
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Abstract: Dental pulpitis causes abnormal pain signaling in the peripheral nervous system (PNS), resulting in ectopic persistent pain and hyperalgesia. However, the Nav1.7 expression related to the change of neuroactivity following pulpitis has not been investigated in the trigeminal ganglion (TG). The aim of our study was to whether the expression of Nav1.7 changes with allylisothiocyanate (AITC)-induced pulpitis in the PNS and the neuronal activities of the TGs can be affected by inhibition of Nav 1.7 channels. Acute pulpitis was induced through allylisothiocyanate (AITC) application to the rat maxillary molar tooth pulp. Three days after AITC application, abnormal pain behaviors were recorded, and the rats were euthanized to allow for immunohistochemical, optical imaging, and western blot analyses of the Nav1.7 expression in the TG. A significant increase in AITC-induced pain-like behaviors and histological evidence of pulpitis were observed. In addition, histological and western blot data showed that Nav1.7 expressions in the TGs were significantly higher in the AITC group than in the naive and saline group rats. Optical imaging showed that the AITC group showed higher neuronal activity after electrical stimulation of the TGs. Additionally, treatment of ProTxII, selective Nav1.7 blocker, on to the TGs in the AITC group effectively suppressed the hyperpolarized activity after

electrical stimulation. These findings indicate that the inhibition of the Nav1.7 channel could modulate nociceptive signal processing in the TG following pulp inflammation.

Disclosures: **G. Nan:** None. **K. Kim:** None. **L. Kim:** None. **M. Cha:** None. **B.H. Lee:** None.

Poster

207. Trigeminal Pain and Headache

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Program #/Poster #: 207.07

Topic: D.02. Somatosensation – Pain

Support: 5 T32 HL 7446-39

Title: Probing the fatty acid-derived metabolome associated with inflamed dental pulp tissue using high resolution mass spectrometry to further elucidate the role of essential fatty acids in inflammatory surgical pain

Authors: *G. M. SAMENUK[1], K. M. HARGREAVES[3], P. M. LOCOCO[4], M. TRAM[2], S. B. H. BACH[5];
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Abstract: Omega-3 and omega-6 fatty acids are major constituents of cellular membranes as well as precursors for many metabolic pathways in humans. The precursors for the essential omega-3 and omega-6 fatty acids are often metabolized by the same enzymes; however, they exhibit opposing biological activity (anti-inflammatory vs pro-inflammatory, respectively). This necessitates a need for a balanced ratio between the two because the precursors are only acquired through diet. Certain western-style diets can be disproportionately high in omega-6 fatty acids. This leads to an abundance of pro-inflammatory omega-6 fatty acids available to be metabolized, which can signal reliable predictors for inflammation. Yet, the resulting metabolites are not well classified, so in this study we aim to further our understanding of the inflammatory metabolome. In certain pain conditions, there is a correlation between increased omega-6 fatty acids in peripheral nerve tissue and increased allodynia and hyperalgesia. We aim to expand on these results to identify omega-6 fatty acid metabolites and the correlation to pain experienced in tooth extraction surgery due to systematic irreversible pulpitis (SIP). Lipids are extracted from human SIP dental pulp using a modified Bligh and Dyer method. Total pools of omega-3 and omega-6 fatty acids are quantified using a validated mass spectrometry-based method following base-catalyzed saponification. To explore and identify resulting metabolites, isotopically labelled omega fatty acids are spiked into homogenized dental pulp extracts. High-resolution mass spectrometry is used to identify the isotopically-labelled metabolites, as well as probe differences in the metabolome between SIP human dental pulp samples and controlled, third molar human dental pulp samples. Our findings show that more C_{13}-linoleic acid is metabolized in

inflammatory conditions, like SIP dental pulp extract, compared to controlled conditions. Additionally, correlations between parent omega fatty acid content are reported with respect to self-reported pain scores, and sex differences. This information provides the basis for exploratory research into the role of the fatty acid metabolome in pain and inflammation with the ultimate goal of pain-based biomarker discovery.

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Poster

207. Trigeminal Pain and Headache

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Title: Oral cancer cell mediators increase trigeminal nociceptor excitability and induce pain via PAR_2-TRPA1 axis

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Abstract: Oral squamous cell carcinoma (OSCC) patients experience debilitating mechanically-induced pain. OSCC cells secrete algogenic mediators that activate and sensitize adjacent axons and supporting cells of trigeminal ganglion (TG) sensory nerve fibers. Proteases released by cancer cells activate PAR_2, a G protein-coupled receptor with a central role in cancer pain and neurogenic inflammation. PAR_2 activation regulates downstream ion channel targets involved in mechanical pain, including TRPA1; the role of PAR_2-TRPA1 axis in oral cancer pain is not well understood. To investigate cancer cell mediators' effect on nociceptor excitability we exposed mice TG neurons to supernatant from human OSCC or matched control tissue (*i.e.,* same patient). Also, we injected supernatants into mice hindpaws to define PAR_2 role in oral cancer pain. OSCC patients (n=10) enrolled into the study reported a mean mechanical sensitivity score of 55±29 (0-100 scale) on the validated UCSF Oral Cancer Pain Questionnaire. TG neurons were pre-treated for 18 hours with OSCC or normal tissue supernatant. OSCC but not control tissue

supernatant reduced action potential (AP) rheobase ($p<0.001$, Student's t-test) and the effect was significantly inhibited in TG neurons from PAR_2-$Na_v1.8$ knock out (KO) mice (*F2rl1*$^{fl/fl}$*; Scn10a*^{Cre}). In behavioral assays, cancer but not normal tissue supernatant reduced mechanical and thermal withdrawal thresholds in wild-type (WT) but not PAR_2-$Na_v1.8$ mice ($p<0.01$, 2-way ANOVA). To test TRPA1 role in oral cancer nociception supernatant from the human oral cancer cell line (HSC-3) was inoculated into the periorbital region of *TRPA1* KO and WT mice. HSC-3 supernatant decreased facial withdrawal threshold to von Frey fiber stimulation in WT mice and the effect was completely inhibited in *TRPA1* KO mice ($p<0.001$, 2-way ANOVA; n=6). We tested the PAR_2 sensitization of TRPA1 role in the oral cancer pain setting with a two-bottle aversion. Mustard oil induced taste aversion was reduced in PAR_2-$Na_v1.8$ but not WT mice in the 4-Nitroquinoline 1-oxide induced oral cancer pain model, ($p<0.05$, 2-way ANOVA). A co-culture method was used to investigate HSC-3 supernatant's effect on *TRPA1* expression in Schwann cells (SC). In HSC-3 and SC co-culture *TRPA1* mRNA level was upregulated 5-fold with no effect on *F2RL1, S100B, RAMP1, CALCRL, TRPV1,* and *TRPV4* levels ($p<0.001$, 2-way ANOVA). Pre-treatment with hCGRP increased AITC-induced TRPA1 current amplitude in hSC ($p<0.05$ Student's t-test, n=6). We infer from these data that cancer cell mediators induce nociception by increasing the excitability of trigeminal nociceptors via PAR_2 and by modulating the expression and function of TRPA1.

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Poster

207. Trigeminal Pain and Headache

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Topic: D.02. Somatosensation – Pain

Support: PSC CUNY Grant 64683-00-52
NIH Grant 1R03NS126987-01

Title: Prrxl1 knockout mouse a model for chronic pain

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Abstract: It has been contended that most current models do not accurately represent the conditions of chronic pain in humans because they involve invasive procedures and are of relatively short durations. Here we introduce, the Prrxl1 knockout (KO) mouse, as a non-invasive model of chronic pain. In this mutant, somatotopic patterning is normal in spinal trigeminal nucleus (SpV), the start of the extralemniscal pathway, as well as the spinal caudalis nucleus

(SpVc). It is absent along the entire trigeminal lemniscal pathway from principal sensory nucleus (PrV) to cortex. Measurements using von Frey monofilaments revealed a previously observed pattern of *hypo*algesia in the spinal division of the animal which contrasts markedly with our novel finding of *hype*ralgesia in its cranial (trigeminal) division where Prrxl1 animals showed an increased facial withdrawal frequency compared to controls. In addition, many aspects of quality of life are disrupted in this animal. The mice exhibit prolonged and intensive bouts of grooming of the head and face as well as an avoidance of rough-textured surfaces suggestive of *allodynia*. On standard lab chow, the Prrxl1 KO shows a significant reduction in *both* the amount and the efficiency of its feeding behavior and a persistently reduced baseline body weight. However, given soft food over a 24-hour period, it eats significantly larger amounts than controls. Taken together, feeding behavior displayed by Prrxl1 KOs we believe is also indicative of pain. These conditions appear to be present in the animal immediately after weaning, and continue throughout its lifetime, so that the condition may be described as chronic. No such behavioral characteristics are evident in another trigeminal mutant (the Barrelless mouse: BRL) in which whisker patterning is present in PrV but poorly defined in thalamus and absent in cortex, suggesting that the condition in the Prrxl1 KO reflects a disruption of *both* somatosensory and nociceptive mechanisms at the brainstem level. Here we present data which suggest that this model may be highly informative with respect to *both* systems.

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Poster

207. Trigeminal Pain and Headache

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Topic: D.02. Somatosensation – Pain

Support: F31NS125993
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R01NS122784

Title: Development of an in-vivo Calcium imaging Preparation of the Trigeminal Ganglia in Rat

Authors: *J. GEDEON[1], J. B. PINEDA-FARIAS[1], R. F. SEKULA, Jr.[2], M. S. GOLD[1];
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Abstract: Jeremy GedeonSfN 2022 AbstractDevelopment of an *in-vivo* Calcium imaging Preparation of the Trigeminal Ganglia in Rat
Recent data indicates that an increase in axonal NaV1.1 enables action potential (AP) propagation in a subpopulation of axons in the trigeminal nerve following injury, and that selective block of this channel can attenuate chronic constriction injury (CCI)-induced mechanical hypersensitivity. The increase in axonal NaV1.1 is not associated with an increase in

protein or mRNA in the trigeminal ganglion (TG), suggesting this upregulation is due to stabilization, local translation, or increased trafficking. The goal of present study to begin to assess contribution of local translation as well as identify the afferent subpopulation(s) in which the increase occurs. To address this, RNAscope was first performed to investigate whether Nav1.1 mRNA is present along in the infraorbital nerve (ION). Quantitative RT-PCR of the ION with and without injury was then initiated to investigate changes in Nav1.1 mRNA expression. To enable analysis of afferent subpopulations in which NaV1.1 was upregulated, GCaMP6s expression in trigeminal ganglia was induced by AAV injection in neonatal rats. Results from this series of experiments confirms that mRNA is detectable in the ION, consistent with previous data reported by Korczeniewska et al (Eur. J Pain 2020, PMID: 32100907), though no apparent differences were observed with CCI-ION. Additionally, neonatal AAV9-GCaMP6s injection provided robust expression throughout the TG for *in-vivo* imaging. The absence of a detectable decrease in axonal NaV1.1 mRNA is consistent with an increase in mRNA trafficking to compensate for an increase in local translation. Ongoing experiments will be used to confirm these initial observations and optimize the TG preparation.

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Poster

207. Trigeminal Pain and Headache

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Topic: D.02. Somatosensation – Pain

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NEI Core grant EY08098

Title: Neurexin 3α has a role in varicella zoster-associated orofacial pain in male and proestrus but not diestrus female rats

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Abstract: Two important nuclei known to modulate orofacial pain signals are the parabrachial nucleus (PBN) and the amygdala. The PBN is a sensory relay hub that receives a multitude of sensory inputs, including taste, various aspects of autonomic control, and pain. The central amygdala (CeA) modulates affective motivational behavior and contains inhibitory GABAergic neurons, which project to and inhibit the lateral PBN. Our lab utilizes a postherpetic neuralgia (PHN) orofacial pain animal model where human varicella zoster virus (VZV) is injected into the whisker pad of rats, resulting in a herpes zoster-like (HZ; shingles) orofacial pain that persists for at least 3 months. In humans, the risk for developing HZ increases with immunosuppression,

advanced age, and the female sex. The most common complication following infection is PHN, which is observed with HZ of the orofacial region. Rodents having VZV-induced pain experience a hypersensitivity like human patients with PHN. Women report greater levels of HZ pain and like humans, female rats experience greater levels of VZV-induced pain. Interestingly, the VZV-induced pain is reduced in female rats during the proestrus phase of their estrous cycle. Previously, we reported that expression of neurexin 3α, a cell adhesion molecule that has an important role in GABA release, is increased in the CeA of proestrus female rats compared to diestrus rats. Therefore, we hypothesized that reduced VZV-associated orofacial pain in male and proestrus female rats is due to increased expression of NRXN3 in the CeA, which increases GABA release from the axon terminals in the PBN, thereby inhibiting ascending pain signals. To test this hypothesis, we used a combination of gene knockdown, fiber photometry, and affective pain behavior. Results indicate knockdown of NRXN3 in the CeA significantly increased VZV-induced orofacial pain in both male and proestrus female rats compared to diestrus rats. In addition, GABA release within the PBN was significantly inhibited following NRXN3 knockdown. In conclusion, VZV-induced orofacial pain is inhibited by NRXN3 within the CeA by increased GABA release within the PBN, thereby modulating ascending pain signals.

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Poster

207. Trigeminal Pain and Headache

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Program #/Poster #: 207.12

Topic: D.02. Somatosensation – Pain

Title: Assessment of Hippocampal Subfields and Amygdala Nuclei Volume in Trigeminal Neuralgia Patients After Pain Relief

Authors: *P. SRISAIKAEW[1], D. JORGENS[1], T. LATYPOV[2,1], M. R. WALKER[1], A. NOORANI[2], S. HANYCZ[1,4], W. WANG[1], M. HODAIE[3,1,2];
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Abstract: Objectives: Trigeminal neuralgia (TN) is a chronic neuropathic pain disorder and noted for its severe, shock-like pain in distributions of the trigeminal nerve branches. Over 70% of patients with chronic pain complain of an inability to focus as well as memory difficulties. One mechanistic avenue linking memory and pain may relate to limbic structures, which are known to be crucial in emotion and memory functioning. Emerging evidence suggests the hippocampus (HPC) and amygdala (AMG) are involved in nociceptive processing and pain modulation. Importantly, TN is highly amenable to surgical interventions, providing a unique opportunity to investigate the effect of pain relief. In this study, we aim to determine whether the

volumetric abnormalities of the HPC subfields and AMG nuclei in chronic pain patients normalize following successful surgical pain treatments.**Methods:** Anatomical T1-weighted images from 24 healthy controls and 20 TN patients (pre- and 6-months post-surgery) were collected and analyzed. All TN patients are treatment responders who reported >75% pain relief after successful surgery. FreeSurfer version 7.1.1 was used for HPC and AMG (including their subfields/nuclei) segmentation and evaluation of their volume. The HPC subfield segmentation included head, body, and tail. The AMG nuclei segmentation covers lateral (LA), basolateral (BA), accessory (Acc), central (CeA), medial (MA), and cortical (Cor) nuclei. The significant statistical level was set at $p<.05$.**Results:** The significant reduction in the volume of the bilateral whole HPC and its subfields ($p<.0001$) as well as the whole AMG and its nuclei ($p<.05$) were found when comparing both pre- and 6-months post-surgical TN timepoints with controls, after controlling for sex. The significant reversal of the bilateral whole HPC ($p<0.05$) and its subfields volume reductions (body: $p<0.05$ and tail: $p<.0001$) were found following successful surgery, but not in the head of the HPC. Interestingly, this was not true with the AMG; only the right BA volume was normalized after successful surgery ($p<.05$). However, there was no significant difference in both HPC and AMG volume compared by pain side.**Conclusions:** These findings suggest that the HPC and AMG subfields may not demonstrate similar normalization after successful pain. This may be related to the subjects' measures of pain-related fear and anxiety.

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Poster

207. Trigeminal Pain and Headache

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Program #/Poster #: 207.13

Topic: D.02. Somatosensation – Pain

Title: A characterization of a rat model of trigeminal neuropathic pain by chronic constriction Injury of infraorbital nerve and pharmacological improvement by gabapentin

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Abstract: Trigeminal neuralgia (TGN) is a neuropathic pain syndrome that is caused by the compression of the trigeminal nerve. TGN is the most common facial neuralgia and causes sudden pain attacks, usually in the lower part of the face, thereby substantially reducing the quality of life of patients. We replicated a rodent model of trigeminal neuropathic pain by chronic constriction injury (CCI) injury of surgical ligation of the infraorbital nerve (IoN). For model generation, the 8 to 10 week male Sprague Dawley rats (n=10 per group) were anesthetized and the facial skin between the eye and whiskers incised. The muscle peripheral fascia was separated to expose the infraorbital foramen and then the distal segment of the IoN

was ligated. For the sham group, the same surgical procedure was applied minus the nerve ligation. Behavioral responses after surgery were tested using the von Frey test and facial grooming observation. In the von Frey test, the rats were acclimatized in a dark, quiet room, and then placed in a custom cage. When the animal was not moving, von Frey filaments of a range of grades (2, 1, 0.4, 0.16, 0.02 g) were used to stimulate the vibrissa pad. Pain response to the mechanical stimulus was graded based on the intensity of the response. Facing grooming behavior was observed by recording the time spent face grooming for 10 minutes in a separate cage after acclimation. Rats that underwent CCI-IoN surgery showed increased response to mechanical stimuli from 7 days after surgery compared to the control and sham rats. The increased response score was maintained up to 3 weeks after surgery. The time spent face grooming was increased at 5 days after surgery compared to the control and sham rats, but this was attenuated by 4 weeks after surgery. Treatment with gabapentin significantly reduced the mechanical allodynia response and the time spent face grooming. In addition, the longitudinal effects of the CCI-IoN model for TGN and treatment with gabapentin on daily behavioral performance, including circadian rhythm, are currently evaluated in their home cage using our automated home cage analysis system. In conclusion, we showed that CCI induced by surgical ligation of IoN leads to increased pain response and behavioral changes. This model provides a platform for preclinical research on neuropathic pain.

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Poster

207. Trigeminal Pain and Headache

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Topic: D.02. Somatosensation – Pain

Title: Investigating the neuronal cell types in the trigeminal ganglion using Constellation Pharmacology

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Abstract: The trigeminal ganglion (TG) contains the soma of the peripheral nerves innervating the craniofacial region and transmit sensory and nociceptive signals using a wide variety of neurotransmitters. One of the neurotransmitters is a peptide called calcitonin gene-related peptide (CGRP) that signals pain under normal conditions but is found to be released in significant amounts in pathologies such as acute migraine and cluster headache attacks. Indeed, the antibodies for CGRP and its receptors are one of the treatment options for migraine. However, there is an unmet need for therapeutics to manage a range of clinical phenotypes of migraine and for different stages of the disease. For developing effective drug leads, we need to comprehensively classify and characterize different cell types that are involved in the relevant

circuitries. We will primarily focus on characterizing the cell types in the TG. Our goal is to characterize each cell type in the TG and track cell-type specific molecular changes that promote the progression of pathological headaches such as migraine. By chronicling the cell-specific molecular changes at different stages of the disease, we can identify stage-specific molecular targets and develop appropriate therapeutic interventions. To accomplish this goal, We employed Constellation pharmacology, a calcium imaging platform that uses diverse sets of selective pharmacological agents to elucidate cell-specific membrane macromolecules.Using the constellation pharmacology approach, we classified the TG neurons into 19 different cell types, broadly clustered into 4 main groups: (a) large diameter, mechanosensory neurons; (b) peptidergic nociceptors; (c) non-peptidergic nociceptors; and (d) small-diameter sensory neurons. We further classified CGRP expressing peptidergic nociceptors into 7 groups. One of the hallmarks of pain sensitive neurons is the expression of purinergic receptors that bind to adenosine triphosphate. We assessed the effects of CGRP on purinergic receptor expression in different cell types and found that CGRP elevated a unique subtype of purinergic receptors in 1 of the 7 subsets of peptidergic nociceptors. This effect was fully prevented by the treatment with CGRP antagonist $CGRP_{8-37}$. Interestingly, the calcium responses from the upregulated receptors displayed different kinetics (rapid desensitization), compared to the canonical responses. Our data suggests that a novel isoform of purinergic receptor, which is upregulated after CGRP treatment, can be a potential drug target for the management of pathological headaches.

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Poster

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Topic: D.02. Somatosensation – Pain

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Title: Contribution of parabrachial projecting corneal afferents to ocular pain

Authors: J. HAVELIN[1], S. DINSDALE[1], M. RICE[2], *I. D. MENG[3];
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Abstract: Primary afferent neurons innervating the cornea maintain ocular homeostasis through the regulation of tearing and blinking, and protect the eye from injury by evoking behavioral and sensory responses to noxious stimuli. Previous studies have demonstrated projections from corneal afferents to two distinct regions within the trigeminal brainstem nucleus, one located at the transition between Vi and Vc (Vi/Vc) and the other located further caudally at the transition between Vc and the first cervical vertebra (Vc/C1), that regulate tearing, blinking, and nociceptive responses, respectively. While a direct projection from the trigeminal ganglion (TG)

to lateral parabrachial nucleus (lPBN) has been described, it is currently unknown whether trigeminal afferents from the cornea contribute to this projection. This study identified lPBN projecting corneal afferents and determined their role in corneal pain-evoked responses in male and female C57B/6 mice. Fluorogold was applied to the corneal surface and DiI or the retrograde AAV pAAV-CAG-tdTomato injected into the lPBN. Dual labeled cell bodies within the TG were identified, indicating the presence of corneal innervating neurons that project directly to the lPBN. To demonstrate a direct association between the central terminals of Nav1.8-expressing primary afferent nociceptors and lPBN neurons activated by corneal stimulation, we utilized mice expressing the fluorescent reporter protein tdTomato or archaerhodopsin-T/eGFP (ArchT) in Nav1.8-Cre+ neurons. The TRPA1 agonist allyl isothiocyanate (AITC, 20%, 10 µl) was applied to the cornea 90 min prior to perfusion in anesthetized male and female Nav1.8-Cre;tdTomato and Nav1.8-Cre;ArchT mice. The neuronal activity marker c-Fos protein was immuno-labeled in vibratome-cut tissue. Primary afferent neurons identified by tdTomato or eGFP were found projecting to the lPBN in close proximity to Fos-positive neurons. The contribution of lPBN projecting corneal afferents to corneal hypertonic saline evoked eye wipe behavior was examined using double heterozygous Nav1.8-Cre;ArchT and Nav1.8-Cre;tdTomato (genotype control) mice. A wireless LED probe directed above the lPBN was implanted to allow for photic stimulation with ArchT activating light. Eye wipe behaviors were evoked with corneal application of hypertonic saline 5 and 7 days after LED implantation. ArchT activating light reduced eye wipe behaviors and palpebral opening compared to the light off control. The direct projection from TG corneal afferents to the lPBN may contribute to the heightened pain and anxiety experienced by patients with ocular pain.

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Poster

207. Trigeminal Pain and Headache

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Program #/Poster #: 207.16

Topic: D.02. Somatosensation – Pain

Title: Microstructural Properties in the Area of Neurovascular Conflict In Trigeminal Neuralgia

Authors: *D. JORGENS[1], P. S.-P. HUNG[1,3], P. SRISAIKAEW[1], T. LATYPOV[3,1], M. HODAIE[4,1,3,2];
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Abstract: Trigeminal neuralgia (TN) is one of the most frequently occurring forms of chronic neuropathic facial pain. Neurovascular conflicts (NVC) of the trigeminal nerve (CN V) by surrounding blood vessels have been theorized as a predominant factor for painful episodes. Interestingly, the presence of NVC is not always associated with the presence of chronic

orofacial pain. We hypothesize that the microstructural characterization of the NVC site on the CN V differs between symptomatic and asymptomatic sides.We test our hypothesis in a population of 37 TN patients (male: 12, female: 25, age: 58.3 ± 15.0) who present with image-based evidence of bilateral NVC and unilateral pain. The microstructural properties of CN V along tractography-derived streamlines between the root entry zone (REZ) and the trigeminal ganglion (TG) were assessed through scalar diffusion tensor measures including fractional anisotropy (FA), axial, radial and mean diffusivity (AD, RD, MD, respectively). We define five equidistant nerve sections between REZ and TG, including REZ, transition zone (TZ), mid-cisternal (mCS), distal cisternal, and peri-ganglionic segment. We compare the relative difference of averaged scalar measures in REZ with those in the four other nerve segments individually on the symptomatic and asymptomatic sides. Further, the averaged scalar measures in REZ are contrasted between both sides. In total, nine Wilcoxon signed-rank tests are performed independently per analyzed scalar measure and corrected for false discovery rate with Benjamini and Hochberg's method.The results showed significantly higher AD, RD, and MD on both the symptomatic ($p<0.001$: AD, RD, MD) and asymptomatic ($p<0.001$: AD, $p<0.01$: RD, MD) sides of CN V when compared to REZ. Interestingly, AD in mCS was significantly different on the symptomatic side ($p<0.05$), while this difference was not found for the asymptomatic side. All assessed scalar diffusion tensor measures remained statistically indistinguishable in the REZ comparing both sides.These findings suggest that the alteration of AD in TZ and mCS offers the potential for distinguishing between symptomatic and asymptomatic nerves in TN patients with bilateral NVC. This is consistent with reports of areas of focal demyelination in the trigeminal nerve, and their potential contribution to the expression of pain. Importantly, FA, which is an overall measure of microstructural changes may not be sufficiently sensitive to detect these small nerve-related changes.

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Poster

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Support: NIH grant R61 EY032468

Title: Prevalence and patterns of eye pain after refractive surgery in humans

Authors: *B. M. HARKNESS[1], D. M. HEGARTY[2], H. BEHRENS[2], W. D. CHAMBERLAIN[1], R. D. STUTZMAN[1], J. BETZ[3], M. PEREZ-BLANCO[3], A. GALOR[3], S. A. AICHER[4]; [1]Casey Eye Inst., Portland, OR; [2]Chem. Physiol. and Biochem., Oregon Hlth. ans Sci. Univ., Portland, OR; [3]Bascom Palmer Eye Inst., Univ. of Miami Hlth. Syst., Miami, FL; [4]Chem. Physiol. and Biochem., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: The cornea has the highest density of sensory nerves in the body, and most sensations evoked by corneal stimulation are painful. Corneal refractive surgeries including laser-assisted in situ keratomileusis (LASIK) and photorefractive keratectomy (PRK) cause injury to these nerves and can evoke both acute and persistent pain. We sought to measure pain levels before and after refractive surgery, and to identify risk factors for persistent pain. Study participants were patients seeking refractive surgery in eye clinics in Portland, OR and Miami, FL. Patients (n=117) included 45 males and 72 females, mean age= 34 [range 20 - 57], 74% self-identified as white, 36% Hispanic. Numeric Pain Scale (NRS) [0-10] was administered prior to surgery, one day after, and at 3- and 6-months postoperatively. Tear volume was measured by anesthetized Schirmer testing at baseline, 3 and 6 months. There was no difference in tear volume across time points and no correlation between pain score and tear volume at any time point. One-way ANOVA showed that pain scores were highest on Day 1 after surgery and also significantly elevated at 3 months, while pain scores at 6 months were similar to baseline. Linear regression analysis showed a significant correlation between Day 1 pain score and 3-month pain score, suggesting that acute pain after surgery is predictive of persistent pain at 3 months. We then measured the number of patients with eye pain, defined as NRS scores of 3 or higher, at each time point. Most (71%) had acute pain on Day 1 after surgery, regardless of surgery type. At 3 months after surgery, 21% of individuals had persistent pain, and 18% had pain at 6 months. Few patients (4%) had eye pain prior to surgery, and many in this group developed persistent pain. We analyzed pain score trends within the group experiencing eye pain at 3 months (n=25). Among these patients were subsets that had reported more severe acute pain (NRS > 6; n=16) or less severe acute pain (NRS 3 - 5; n=6) 1 day after surgery. A smaller group (n=3) had no baseline or acute post-surgical pain but developed pain by 3 months after surgery. Our findings suggest diverse time courses, and possibly different mechanisms, for the development of eye pain after refractive surgery. LASIK and PRK demonstrate overall excellent safety and patient satisfaction outcomes, yet acute & chronic symptoms and pain have been reported to occur. Characterization of pain profiles and correlated patient features may inform future surgical candidate screening protocols.

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Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.18

Topic: D.02. Somatosensation – Pain

Support: The McKnight Foundation
NIH Director's New Innovator Award
Lulu Merle Johnson Fellowship

Title: Brain-wide local field potential connectivity patterns underlying a migraine-related hypersensitivity network

Authors: *M. JOHNSON[1], B. HING[1], A. JIMENEZ[1], I. HULTMAN[2], S. SRIVASTAVA[2], R. HULTMAN[1];
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Abstract: Migraine is a complex neurological disorder encompassing many components of pain, sensation, and affect, making it a disorder of multisensory integration. Some of the most common migraine symptoms consist of hypersensitivity to various stimuli (ie visual, somatosensory, olfactory, and auditory), often evoking pain. Imaging studies in humans and preclinical studies in rodents have recently implicated individual brain regions as potentially important in migraine. These regions include parabrachial nucleus (PBN), thalamus (Po/MD/VPM), amygdala (BLA/CeA), and anterior cingulate cortex (ACC). However, one of the questions that remains unanswered is how these brain regions are functionally connected in such a way that contributes to migraine-related central nervous system symptoms. We utilized a multi-disciplinary, systems-based approach to study brain circuitry underlying central migraine-related symptoms in mouse models of migraine by investigating brain-wide connectivity of circuits implicated in migraine. Based on this approach, we studied complex preclinical evoked-migraine phenotypes (mechanical allodynia, spontaneous pain, and light aversion), represented by brain-wide local field potential activity to determine a migraine-related brain network. We utilized multi-site *in-vivo* microelectrode recordings to study brain connectivity in calcitonin gene-related peptide (CGRP) and nitroglycerin (NTG) mouse models of migraine. After inducing migraine in male and female CD1 and C57Bl/6J mice, we tested multisensory migraine-related behaviors in both implanted and unimplanted mice. During our studies, experimenters were blinded to treatment conditions and performed behavioral assays in replicates. We found that both implanted (n=13) and unimplanted (n=14) CD1 and C57Bl/6J mice exhibited mechanical allodynia and light aversion. Preliminarily, we found spectral power changes in Po and VPM in response to CGRP. Overall, our studies provide evidence for underlying central mechanisms of migraine-related symptoms, with an aim toward paving the way for individualized approaches to treating migraine.

Disclosures: M. Johnson: None. **B. Hing:** None. **A. Jimenez:** None. **I. Hultman:** None. **S. Srivastava:** None. **R. Hultman:** None.

Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.19

Topic: D.02. Somatosensation – Pain

Support: Rajyoga Meditation Research Foundation (RERF), India

Title: Alteration in grey matter volume of central pain processing areas of the brain among non-meditators, Rajyoga meditators, and patients with migraine disorder - A cross-sectional study

Authors: *R. M.G.[1], K. S. RAI[1], R. K V[2];
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Abstract: Alterations in grey matter volume (GMV) in central pain processing areas of the brain in patients with migraine (PM) have been reported in many studies. Also, it has been observed in cross-sectional and interventional studies that meditation has a beneficial effect on migraine. Till-date, no in-depth voxel-based morphometric studies to explore possible alterations in brain regions of PM in comparison to the same in Rajyoga meditators (RM) and non-meditators (NM)/controls have been reported. The aim of this cross-sectional study was to explore alterations in GMV of central pain-processing regions among NM, RM and PM. Study participants (n=108) included were age and handedness matched NM/controls, RM who were regular meditation practitioners (n=40/group), and PM (n=28). Equal numbers of male and female (n=20/group) participated in NM and RM groups, but 7 male and 21 female participants were included as per selection criteria, in PM group. There were 26 episodic and 2 chronic PM and most of them (75%) had onset of migraine during 20-40 years of age. All participants underwent MRI scans after obtaining ethical committee approval and informed consent. Structural MRI scans were preprocessed and analyzed using CAT12-r1987. The voxel-based morphometric method was applied with TFCE approach and results were reported significant if FWE corrected $p<0.05$. Results of whole-brain voxel-wise comparison of sMRI scans from NM and RM showed RM having significant increase in GMV in several pain processing regions including the right medial superior prefrontal cortex, right anterior orbital, right precentral, bilateral posterior cingulate, right supramarginal, right superior, middle and inferior temporal gyri, and right cerebellar crus1 that are involved in controlling cognitive, and emotional aspects of pain. Whereas, on comparing NM and PM it was observed that PM also showed a significant increase in GMV in fewer pain-processing regions including bilateral middle cingulate and paracingulate gyri. Additionally, RM was observed to have increased GMV in cerebellar crus1, on comparison of MRI scans from RM with PM. Significant increase in GMV in several specific central pain processing regions in RM and fewer pain-processing regions in PM identified in this study indicates that RM have integrative structural connectivity for better control over pain, particularly to overcome emotional and cognitive aspects of pain, whereas central control of pain is altered in PM and therefore poor. In conclusion, Rajyoga meditation practice in PM may be helpful to alleviate migraine, although more studies need to be done to identify the usefulness of RM as a complementary therapy for migraine.

Disclosures: R. M.g.: None. **K.S. Rai:** None. **R. K v:** None.

Poster

207. Trigeminal Pain and Headache

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.20

Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS118852

Title: Transient receptor potential melastatin 8 is required for nitroglycerin- and calcitonin gene-related peptide-induced migraine-like pain behaviors in mice

Authors: *C. WEI[1], B. KIM[2], D. D. MCKEMY[3];
[1]USC, [3]Neurobio., [2]USC, Los Angeles, CA

Abstract: Migraine is a complex neurovascular disorder that is one of the leading causes of disability and reduced quality of life. To address this complex disorder, several groups have performed genome-wide association studies identifying transient receptor potential melastatin 8 (TRPM8), a cold-sensitive cation channel, as a migraine susceptibility gene. Interestingly, migraine-associated TRPM8 single-nucleotide polymorphisms reside in noncoding regions, with those correlated with reduced migraine risk exhibiting lower TRPM8 expression and decreased cold sensitivity. However, the role of TRPM8 in migraine has yet to be defined. In this study, we aim to determine whether TRPM8 channels or neurons are required for migraine-like pain in inducible migraine models. We used nitroglycerin and calcitonin gene-related peptide to induce both acute and chronic migraine-like behavior. Mechanical allodynia and facial pain expression were recorded as measurements for evoked and spontaneous pain. We found that both TRPM8-null mice and mice with selective TRPM8 neuronal ablation were unable to develop acute or chronic migraine-like behavior. Moreover, the absence of migraine-like pain in mice lacking TRPM8 is not sexually dimorphic. Finally, we showed that inhibition of TRPM8 channels using a TRPM8 antagonist prevented acute but not established chronic migraine-like pain. These results are consistent with its association with migraine in genetic analyses and establish that TRPM8 channels are a component of the underlying mechanisms of migraine.

Disclosures: C. Wei: None. **B. Kim:** None. **D.D. McKemy:** None.

Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.21

Topic: D.02. Somatosensation – Pain

Title: Episodic migraine-like pain does not disrupt immobility-defined sleep duration in mice

Authors: *R. C. LILLO VIZIN, C. M. KOPRUSZINSKI, P. REDMAN, L. H. M. SOUZA, G. A. DRISKILL, S. RAHMAN, E. NAVRATILOVA, F. PORRECA;
Pharmacol., Univ. of Arizona, Tucson, AZ

Abstract: *Introduction:* Migraine is a neurological disorder that affects approximately 12% of the USA population and 15% globally, being three times more prevalent in women than in men. Migraines can be divided into episodic and chronic states and symptoms include heightened sensitivity to light, sound, and touch, throbbing head pain, allodynia, and nausea. Sleep disorders also have a high prevalence in the general population and migraine and sleep disorders are often comorbid. Clinical evidence shows that patients with migraine report sleep issues. However, the nature and direction of the association between migraine and sleep disorders remains unclear. Here, we aimed to evaluate the effect of episodic migraine-like pain on immobility-defined sleep, a non-invasive high-throughput method to predict sleep duration. *Methods:* Female and male C57BL6/J mice, 9-week-old were used. To validate immobility-defined sleep, mice EEG/EMG and video were simultaneously recorded. Immobile episodes, defined as lasting more than 40 s at 95% sensitivity detection, were analyzed from the video recordings using ANY-maze and compared to EEG/EMG recordings in naïve mice and in response to caffeine (20 mg/kg, i.p.) and doxepin (15 mg/kg, i.p.) treatments to induce wakefulness and sleep, respectively. To evaluate the effect of episodic migraine-like pain on immobility-defined sleep, mice were individually acclimated in sleep chambers. Then, baseline immobility-defined sleep was evaluated for 24 h followed by either a single injection of nitroglycerin (10 mg/kg, i.p.) or inflammatory mediators (5 μL, supradural, containing bradykinin, histamine, 5HT, PGE2, pH 5.0, in synthetic interstitial fluid); controls received either saline (10 mL/kg, i.p.) or synthetic interstitial fluid (5 μL, supradural). Treatment was performed at 7 AM (light phase) or 7 PM (dark phase) and sleep was evaluated for an additional 24 h post-treatment. In a separate group of mice, nitroglycerin- or inflammatory mediators-induced mechanical allodynia was evaluated in the periorbital and hind-paw regions using von-Frey filaments. *Results:* Immobility-defined sleep showed a remarkable correspondence with EEG/EMG-defined sleep, with a correlation of 94-99%. Nitroglycerin or dural inflammatory mediators induced robust mechanical allodynia consistent with migraine-like pain. However, these treatments did not affect the amount of sleep of mice in either the light or dark phases. *Conclusions:* Our study shows that immobility-defined sleep is a validated method to evaluate sleep duration in mouse migraine models. Further, episodic migraine-like pain does not disrupt the sleep duration of male or female mice.

Disclosures: R.C. Lillo Vizin: None. **C.M. Kopruszinski:** None. **P. Redman:** None. **L.H.M. Souza:** None. **G.A. Driskill:** None. **S. Rahman:** None. **E. Navratilova:** None. **F. Porreca:** None.

Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.22

Topic: D.02. Somatosensation – Pain

Support: Department support

Title: The Local Field Potential Change and Brain Coherence Alternations in Response to Migraine Headache Attacks

Authors: *Z. WANG, Q. LIN, Y. B. PENG;
Dept. of Psychology, Univ. of Texas at Arlington, Arlington, TX

Abstract: Migraine is a recurrent primary headache disorder with moderate to severe disability. However, the pathophysiology of migraine headaches remains poorly understood. Consequently, safe and effective therapies to alleviate migraine headaches are limited. Local field potential (LFP) recording, as a neurophysiological tool, has been widely utilized to investigate the combined neuronal activity. The ***purpose*** of this study was to determine differential LFP signatures and brain coherence alternations from the anterior cingulate cortex (ACC), the posterior nucleus of the thalamus (Po), the trigeminal ganglion (TG), and the primary visual cortex (V1M). The ***hypothesis*** was that various brain areas, which are involved in different orders of neurons in the trigeminovascular system (Po, TG, V1M) and pain processing (ACC), could show different response patterns/signatures with brain coherence changes to migraine attacks. In this study, we recorded LFP changes simultaneously from these four sites after nitroglycerin (NTG) or vehicle solution injection from both anesthetized and freely moving rats. Additionally, brain coherence was processed and light-aversive behavior measurements were implemented. The raw data of LFP was processed by power spectrum analysis in MATLAB. The powers (4 hours recording after injection) of delta (0.1-3 Hz), theta (3-7 Hz), alpha (7-12 Hz), beta (12-30 Hz), and gamma (30-100 Hz) bands were normalized by the average of the baseline (first 30 minutes). A mixed analysis of variance (mixed ANOVA) with LSD posthoc was used. The results indicated that significant elevations of LFP powers with various response patterns at the delta, theta, alpha, beta, and gamma bands following NTG injection were detected from both anesthetized (n = 12) and freely moving animals (n = 11), compared with the vehicle control group (n = 11, n = 10, respectively), $p < 0.05$. Remarkably, however, a surge of coherence alternations was exclusively observed from freely moving animals after NTG injection (n = 11) in contrast to the vehicle control group (n = 10), $p < 0.05$. Furthermore, the result of behavior test demonstrated that there was a significant increase in time spent in the dark box between the NTG group (M = 94.89%, SE = 0.015) and the vehicle control group (M = 75.55%, SE = 0.034), $p <$ 0.05. In conclusion, the multi-region LFP signatures and brain coherence alternations in response to migraine attacks were determined. Moreover, the results of behavior measurements in the freely moving group indicated NTG induced the phenomenon of photophobia in our study. All these findings offer novel insights into the interpretation of migraine mechanisms and related treatments.

Disclosures: Z. Wang: None. **Q. Lin:** None. **Y.B. Peng:** None.

Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.23

Topic: D.02. Somatosensation – Pain

Support: NAD Research Inc.

Title: Intranasal administration of nicotinamide adenine dinucleotide alleviates headaches associated with migraine headache pain and reduces adverse effects of anxiety disorders: A case report

Authors: *J. WHITE[1], A. PODESTA[2], G. A. DYESS[1], S. L. BROOM[3], R. F. MESTAYER, III[4];
[1]NAD research Inc., Springfield, LA; [2]Podesta Wellness, Tulane Clin. Fac., New Orleans, LA; [3]William Carey Univ., Waveland, MS; [4]Springfield Wellness Ctr., Springfield, LA

Abstract: **Introduction:** Research suggests that Nicotinamide Adenine Dinucleotide (NAD+) is effective in treating clinical conditions associated with disease and aging due to its vital role in healthy cell functioning. Clinicians around the U.S. developed intranasal (IN) NAD+ administration protocols directly on the sphenopalatine ganglion (SPG) for migraine/cluster headaches and symptoms associated with anxiety. **This case report describes a patient response to IN NAD+ experiencing symptoms associated with migraine headache, Agoraphobia, PTSD, and severe anxiety. Methods:** A 55-year-old male patient presented with disabling PTSD with paranoia, bipolar traits, and migraine/cluster headaches poorly controlled by traditional medications. Patient reported frequent panic attacks, debilitating headaches, anxiety with Agoraphobia, and history of attempted suicide. On 2/07/2020 the patient scored 27 on the Warwick-Edinburg Mental Well-being Scale (WEMWBS). Patient received NAD+ (0.375ml of 100mg/ml) and lidocaine (0.25ml of 20mg/1ml) into each nostril via sphenocatheter followed up by treatments at 1 and 4 weeks**.** Additional outcome measures were collected at 2m, 8m, and 1-year. **Findings:** After the first session, patient reported needing less migraine medication and improved sleep. After 3 days, patient reported less intense panic attacks during the day and cessation of nightly panic attacks. At 1 month, patient reported mild headaches and was able to taper off quetiapine and oxcarbazepine. Between 1 and 4-weeks, he reported 2 cluster headaches while no longer taking sumatriptan or requiring oxygen. On 3/15/2020, the patient scored 45 on the WEMWBS, showing significant improvement despite being on less medication. At 1-year follow-up, patient reported reoccurrence of nightmares, but not needing rescue medications or oxygen. **Discussion:** The mechanism of action for IN NAD+ application is unclear; however, data suggest that delivery directly on SPG facilitates the attenuation of migraine/cluster headaches and anxiety symptoms beyond medication therapy alone, with fewer side effects. NAD+ efficacy lessened over the extended break in treatment due to the COVID-19 pandemic, however significant dampening in symptoms was rapidly (< 4 days) observed, despite taking less medication. **Conclusion:** Data show a rapid and sustained treatment effect of decreased severity and alleviation of migraine pain and anxiety symptoms resulted in enhanced quality of life measures and less need for medications to manage symptoms. Further studies are warranted to empirically validate NAD+ as an alternative or augmenting treatment approach in a subset of clinical populations.

Disclosures: **J. White:** None. **A. Podesta:** None. **G.A. Dyess:** None. **S.L. Broom:** F. Consulting Fees (e.g., advisory boards); NAD Research Inc.. **R.F. Mestayer:** None.

Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.24

Topic: D.02. Somatosensation – Pain

Support: ANR - 'RARFRAIS' Project

Title: Functional brain and trigeminovascular changes in migraine using a new approach of neuroimaging: the functional ultrasound imaging

Authors: *L. DELAY, N. IALY RADIO, M. TANTER, S. PEZET;
Physics for Med. Lab., Physics for Med. Lab., Paris, France

Abstract: As a leading cause of disability worldwide, migraine is a neurovascular disorder characterized by headaches crisis and sensory hypersensitivities such as photophobia and/or allodynia. Migraine pathophysiology is complex and still partially understood. As a source of new findings, we used a novel brain neuroimaging modality that is particularly interesting for vascular diseases: the functional ultrasound imaging (fUSi). As an alternative to functional magnetic resonance imaging (fMRI), this modality of neuroimaging enables the measurement of cerebral blood volume with high sensitivity and spatio-temporal resolution (100 µm and 1 ms, respectively). Due to the vascular aspect of the pathophysiological alterations previously observed in migraine patients, this study aimed at deciphering i) the sequence of vascular dynamic changes on a whole brain, and ii) study changes of functional connectivity in different parts of the trigeminovascular system in a relevant animal model of migraine induced by systemic administrations of isosorbide dinitrate (ISDN), a nitric oxide donor. These results are crucial to understand the involvement of these modifications in migraine pain. Our results show that repetitive administrations of ISDN induce important alterations of the intrinsic connectivity within the pain matrix five days after the first injection associated with orofacial mechanical allodynia and a specific cerebral blood flow dynamic pattern during migraine crisis. This study adds new insights in migraine pathophysiology and proved the potential of fUS imaging as an important new technology to decipher nociceptive components of neurovascular diseases such as migraine.

Disclosures: L. Delay: None. **N. Ialy Radio:** None. **M. Tanter:** None. **S. Pezet:** None.

Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.25

Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS072204
NIH Grant NS104200
University of Texas System

Title: Peroxynitrite contributes to behavioral responses, increased trigeminal excitability, and changes in mitochondrial function in a preclinical model of migraine

Authors: *J. LACKOVIC, V. JEEVAKUMAR, M. D. BURTON, T. J. PRICE, G. DUSSOR; Neurosci., Univ. of Texas At Dallas, Richardson, TX

Abstract: Despite being one of the most disabling disorders worldwide, the underlying pathophysiology associated with migraine is still poorly understood. Nitric oxide (NO) donors have been well-documented as being one of the most consistent experimental triggers, in which 75% of migraineurs develop an attack within 6 hours of NO donor administration. Additionally, NO donor administration has been shown to only cause headaches and no other types of pain, strongly implicating NO in migraine. NO reacts with superoxide radicals to form peroxynitrite (PN), a molecule that sensitizes sensory neurons in preclinical pain models. Neutralizers of PN in the form of scavenger compounds and decomposition catalysts have demonstrated efficacy in preclinical pain models. Based on this, we wanted to test the hypothesis that the effects of NO on migraine are due to PN formation. Female and male mice were subjected to three consecutive days of restraint stress or to dural stimulation with the pro-inflammatory cytokine interleukin-6. Following resolution of the initial post-stimulus behavioral responses, animals were tested for hyperalgesic priming using a normally non-noxious dose of the NO donor sodium nitroprusside (SNP) or dural pH 7.0, respectively. We measured periorbital von Frey and grimace responses in both models and measured stress-induced changes in 3-nitrotyrosine (3-NT) expression (a marker for PN activity) and trigeminal ganglia (TG) mitochondrial function. Additionally, we recorded TG neuronal activity in response to the PN generator SIN-1. We then tested the effects of the PN decomposition catalysts, FeTMPyP and FeTPPS, or the PN scavenger MnTBAP against these behavioral, molecular, and neuronal changes. Neutralizing PN attenuated stress-induced periorbital hypersensitivity and priming to SNP, with no effect on priming to dural pH 7.0. These compounds also prevented stress-induced increases in 3-NT expression in both the TG and dura mater and attenuated TG neuronal hyperexcitability caused by SIN-1. Surprisingly, FeTMPyP attenuated changes in TG mitochondrial function caused by SNP in stressed males only. Together, these data strongly implicate PN in migraine mechanisms and underscore the therapeutic potential of targeting PN.

Disclosures: J. Lackovic: None. **V. Jeevakumar:** None. **M.D. Burton:** None. **T.J. Price:** None. **G. Dussor:** None.

Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.26

Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS104110

Title: The role of glucocorticoids in repetitive stress-induced migraine-like behaviors in mice

Authors: *Y.-Y. HU[1], R. SOUZA[1], A. N. AKOPIAN[2], C. K. MCINTYRE[3], G. O. DUSSOR[1]; [1]Neurosci., Univ. of Texas at Dallas, Richardson, TX; [2]Endodontics, UT Hlth. Sci. Ctr., San Antonio, TX; [3]Neurosci., Univ. Texas at Dallas, Richardson, TX

Abstract: Stress is one of the most common precipitating factors in migraine and it is identified as a trigger in nearly 70% of migraine patients. In many patients, the relief from stress is the trigger as attacks do not occur until the stressful event is over. However, how relief from stress acts as a migraine trigger is not well understood. The nervous system responds to stressors by activating the HPA axis and releasing glucocorticoids to change behaviors and adapt to environmental change. However, migraine headache may be an adverse consequence of this glucocorticoid response. We have shown previously that repeated restraint stress in mice evokes migraine-like behavioral responses as well as priming to the nitric oxide donor sodium nitroprusside (SNP). In the present study, we used CD-1 mice to investigate the effect of glucocorticoids on stress-induced migraine-like behaviors. We gave subcutaneous injections of metyrapone, an inhibitor of glucocorticoid synthesis, 40 minutes prior to each restraint stress exposure. Metyrapone attenuated both the initial restraint stress-induced migraine-like behaviors and priming to SNP in male and female mice. These results indicate that migraine-like behaviors in this model are dependent on normal synthesis of corticosterone (CORT). To test whether CORT injections alone can mimic restraint stress-induced migraine-like behaviors, we administered subcutaneous injections of CORT or vehicle for 3 consecutive days in the absence of restraint stress. Daily CORT injections led to migraine-like behaviors in female mice during the post-stress phase and SNP induced priming phase, although the pattern of response was different from restraint stress. However, male mice did not show any facial hypersensitivity after CORT injections. To further investigate whether the HPA axis is involved in this mechanism, we gave subcutaneous injections of α-MSH or ACTH post-stress at two time points and examined whether they can alleviate stress-induced hypersensitivity. α-MSH or ACTH injections post-stress attenuated both the initial restraint stress-induced migraine-like behaviors and priming to SNP in female mice. These data indicate that the HPA axis plays an important role in the behavioral responses to repeated stress. Together, these results reveal that glucocorticoids are critical for the development of migraine-like behavioral responses following repeated stress in mice. Better understanding of where, when, and how glucocorticoids influence the nervous system in the context of stress may provide important information for how stress contributes to migraine and may lead to new therapeutic approaches.

Disclosures: Y. Hu: None. **R. Souza:** None. **A.N. Akopian:** None. **C.K. McIntyre:** None. **G.O. Dussor:** None.

Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.27

Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS104200

Title: The role of ATP-sensitive potassium channels in a preclinical stress-induced migraine model

Authors: *H.-R. MEI, G. O. DUSSOR;
Neurosci., Univ. of Texas at Dallas, Richardson, TX

Abstract: Migraine is the second most disabling disease worldwide, affecting around 15% of people, but there is insufficient understanding of the mechanisms causing the disorder. Several recently published studies found that intravenous infusion of levcromakalim, a K_{ATP} channel opener, induced migraine attacks in migraine patients but not in healthy subjects. In order to investigate how and where these channels may contribute to migraine, the present study used a preclinical migraine model and pharmacological tools to probe for K_{ATP} channel function. We have shown previously that stress, the most commonly reported migraine trigger, induces facial hypersensitivity and priming to a low-dose of the NO donor sodium nitroprusside (SNP; 0.1 mg/kg). Von Frey filaments were used to measure periorbital mechanical thresholds and grimace scores were evaluated by observing mouse facial features. Injection of the K_{ATP} channel blocker glibenclamide (30 mg/kg) 1 hour before SNP significantly reversed the SNP responses on day 14 after stress. Repetitive exposure to stress also caused priming to subthreshold levcromakalim (1 mg/kg) on day 14; levcromakalim significantly reduced the facial withdrawal thresholds in previously stressed but not control mice. To determine whether K_{ATP} channels within the dura mater may contribute to these behavioral effects, we also tested responses to dural injection of stimuli. In naïve mice, dural injection of 2.98 μg or 29.8 μg SNP led to facial hypersensitivity, while no responses were induced by 0.298 μg SNP. Similarly, dural application of 0.3 μg or 1 μg levcromakalim, but not 0.1 μg resulted in facial hypersensitivity. However, repetitive-stress exposure caused priming to dural injection of subthreshold SNP (0.298 μg) or levcomakalim (0.1 μg). Co-injection of sumatriptan (0.01 μg) with levcromakalim (1 μg) on the dura effectively alleviated levcromakalim-induced migraine-like behavior. These findings are consistent with observations in humans where opening of K_{ATP} channels triggers migraine attacks and additionally implicate these channels in the downstream effects of nitric oxide donors. Importantly, they demonstrate that opening of K_{ATP} channels within the dura contributes to migraine-related behavior, suggesting that the dura is one location where these channels contribute to the disorder. Together, these data and future studies using this model may help to further investigate the potential of K_{ATP} channel blockers as novel migraine therapeutics.

Disclosures: H. Mei: None. **G.O. Dussor:** None.

Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.28

Topic: D.02. Somatosensation – Pain

Title: Inflammatory pain and increased involvement of purinergic signaling in patients with symptomatic pulpitis

Authors: *L. SEE, S.-M. LEE, B. KARABUCAK, C. H. MITCHELL;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Introduction: Identifying molecular contributions to the perception of inflammatory pain is central to the development of therapeutic strategies. Correlations between pain sensation and relevant markers in human patients provide insight into clinically relevant mechanisms. Purinergic signaling is closely associated with pain on multiple levels, and may be influenced by levels of both receptors and available agonist. While the ionotropic ATP receptors P2X2 and P2X3 are implicated in pain detection, the concentration of agonist extracellular ATP is correlated with expression of ectoATPase CD39, and hemichannel pannexin 1, a conduit for ATP release. This study probed the potential involvement of purinergic nociception in dental pain by comparing distributions of the aforementioned ATP receptors and markers in symptomatic and asymptomatic pulpitis.
Material and Methods: Patients were screened for extractions of sound teeth and teeth with moderate-to-severe caries with/without pain. Thirty six patients were consented, thirty nine teeth collected and divided into 3 groups (n=13/group) based on the clinical diagnosis: normal pulp (NP), asymptomatic irreversible pulpitis (AIP), symptomatic irreversible pulpitis (SIP). Hematoxyin-and-eosin staining was performed to confirm the histological diagnosis. Immunofluorescent staining (n=10) and Western blots (n=3) were performed for P2X2, P2X3, CD39, Pannexin-1, and neuronal marker PGP9.5. Parametric data were analyzed by ANOVA, nonparametric data analyzed by a Kruskall-Wallis test.
Results: Immunoreactivity of all markers were observed throughout the body of pulpal tissue. P2X3 and P2X2 were found on nerves fibers; whereas Pannexin-1 and CD39 were found accompanying nerve fibers, blood vessels, and other cell structures throughout the pulp. With inflammation, all markers were found clustered in larger bundles. Immunofluorescent analyses of area stained and Western blot analyses demonstrated the highest expression of all antibodies in SIP samples, with increased expression of CD39, P2X2 and Pannexin-1 in AIP compared to NP. There was a statistically significant increase in PGP9.5, P2X3, and CD39 expression in SIP compared to NP samples ($p<0.05$).
Conclusion: These data suggest increased expression of P2X3, P2X2, CD39 and Pannexin-1 in human pulpal samples from symptomatic teeth of patients describing pain. Further confirmation would support a model of purinergic involvement in pulpal nociception where purinergic receptors, P2X3 and P2X2/3, may be putative targets for emerging anti-nociceptive drugs targeting dental inflammatory pain.

Disclosures: L. See: None. **S. Lee:** None. **B. Karabucak:** None. **C.H. Mitchell:** None.

Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.29

Topic: D.02. Somatosensation – Pain

Support: NIH 1R01DE029202-01
NIH 3R01DE029202-01S1

Title: Attenuating Orofacial Pain States with TSP4 Domain-Specific Peptides in Trigeminal Nerve Injured Rats

Authors: *B. DOAN, K. GENARO, Y. GWAK, Z. LUO;
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Abstract: Thrombospondin-4 (TSP4) upregulation in the spinal cord following trigeminal nerve injury contributes to the development of neuropathic pain. We reported previously that interactions between the Epidermal Growth Factor (EGF)-Like domain of TSP4 and voltage-dependent calcium channel α2δ1 subunit ($Ca_v\alpha_2\delta_1$) plays a critical role in injury-induced aberrant excitatory synapse formation and neuropathic pain state development. We hypothesized that blocking TSP4/$Ca_v\alpha_2\delta_1$ interaction may serve as a target for orofacial neuropathic pain medications. We tested this hypothesis by examining the efficacy of TSP4 peptides with high binding affinity to $Ca_v\alpha_2\delta_1$ in reversing neuropathic pain states, which were induced in adult rats with chronic constriction injury (CCI) of peripheral branches of the trigeminal nerve. Behavioral responses to mechanical and cold stimulation were assessed with the orofacial operant test. Rats were trained to voluntarily contact their facial region to a mechanical (12 filaments) or a cold (4 ^{o}C) stimulation module in order to access sweetened milk as a positive reward. After CCI rats showed clear mechanical allodynia and thermal hyperalgesia, we examined the effects of three distinct TSP4 peptides, with high binding affinity to the $Ca_v\alpha_2\delta_1$ proteins, in blocking orofacial neuropathic pain states. Peptide one and Peptide two are from the EGF-Like domain of TSP4 and Peptide three is from the C-terminal domain of TSP4. All peptides were administered intra-cisterna magna. Rats were tested 4 hours, 7, 14 and 21 days after the injection. Data from this study supports the presence of chronic orofacial neuropathic pain states shown as reduced operant behavior to mechanical and cold thermal stimulation in the CCI group. Treatment with Peptide one alone diminished mechanical allodynia and thermal hyperalgesia briefly in the CCI rats. Similar treatment with Peptide two or the control peptide alone did not alter sensory responses in either the sham or CCI groups. The combined treatment of Peptide one and Peptide two (1 nmol each/20 μL) produced a longer-lasting, antinociceptive effect compared with Peptide one treatment alone in the CCI rats. The domain-specific peptide effects are likely mediated by blocking TSP4/$Ca_v\alpha_2\delta_1$ interaction. These findings support an important role of TSP4/$Ca_v\alpha_2\delta_1$ interaction in orofacial pain processing, and that combinational treatment with Peptide one and Peptide two may have synergistic antinociceptive effects. Thus, blocking TSP4/$Ca_v\alpha_2\delta_1$ interaction with TSP4 EGF-Like domain peptides may warrant further investigation as a new pharmacological approach for target-specific management of neuropathic pain states.

Disclosures: B. Doan: None. **K. Genaro:** None. **Y. Gwak:** None. **Z. Luo:** None.

Poster

207. Trigeminal Pain and Headache

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 207.30

Topic: D.02. Somatosensation – Pain

Support: NIH Grant: NIDCR R01DE027929

Title: Nociceptor regulation of apical periodontitis transcriptome

Authors: *K. V. LILLIS[1], O. AUSTAH[1,2], R. GRINCEVICIUTE[1], A. DIOGENES[1]; [1]Endodontics, UT Hlth. San Antonio, San Antonio, TX; [2]Endodontics, King Abdulaziz Univ., Jeddah, Saudi Arabia

Abstract: Every year, over 15 million root canal procedures are performed to treat and prevent apical periodontitis (AP) in the United States. AP is a painful inflammatory disease resulting from tooth infection and is primarily characterized by bone loss. There is growing evidence that the nociceptive fibers densely innervating teeth regulate the pathogenesis of this disease by unique communication with non-neuronal cells within the inflammatory site. Since some of these regulatory mechanisms may involve changes in gene expression, we hypothesized that nociceptors regulate the transcriptomic profile of the periapical lesion in a mouse model of AP. Male and female control (Nav1.8 $^{cre+/-}$) and nociceptor-ablated (Nav1.8$^{cre+/-}$ Diphtheria toxin A$^{lox+/-}$) mice were generated and underwent pulp exposure procedure on all 4 first molars and at 8 weeks of age. At either 0, 7, or 14 days after pulp exposure, periapical tissues, including lesions, were dissected from mice (n=3/strain/time point). Total RNA was extracted from the pooled periapical lesions and submitted for total RNA sequencing and bioinformatic analysis. We found that pulp exposure triggers the differential expression of hundreds of genes within the periapical lesion over the course of infection with marked differences between control and mice with nociceptor ablation. Importantly, at 14 days post-pulp-exposure, 422 genes were differentially expressed between nociceptor-ablated and control mice with greater enrichment of biological processes related to inflammation, specifically immune cell chemotaxis and migration, compared to control mice. Among these inflammatory markers, TNFα, IL-1α, and IL-1β are known to play a crucial role in AP and were significantly upregulated in nociceptor-ablated mice. We also found that nociceptors modulate the infection in a sex-specific manner, as differentially expressed genes in female lesions vastly differ from those observed in male lesions. In conclusion, nociceptor-ablation regulates the transcriptomic profile of periapical lesions in a mouse model of AP, shifting the gene expression profile to a greater enrichment of inflammatory genes, suggesting nociceptors play a role in the kinetics of the immune response in a sex-specific manner. This newly uncovered neuro-immune axis and its mechanisms in AP can potentially be an important therapeutic target for the treatment of this prevalent disease.

Disclosures: K.V. Lillis: None. **O. Austah:** None. **R. Grinceviciute:** None. **A. Diogenes:** None.

Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 208.01

Topic: D.03. Somatosensation – Touch

Support: NIH R01 NS094659
NIH T32 NS064928
NSF GRFP
NIH F99 AG073558
Ludwig Schaefer Scholar Award
Columbia University Class of 1939 Summer Research Fellowship

Title: Learning deficits of various transgenic mouse lines in a cortex-dependent texture discrimination task

Authors: *N. HARANO[1], J. B. DAHAN[2], J. M. PARK[1], R. M. BRUNO[3];
[1]Columbia Univ., New York, NY; [2]Max Planck Inst. of Psychiatry, Munich, Germany;
[3]Physiology, Anat. & Genet., Univ. of Oxford, Oxford, United Kingdom

Abstract: The cerebral cortex is a heterogeneous structure with numerous regions, layers, and cell types. Advances in optogenetics and transgenic Cre driver mouse lines enable us to probe the computational and behavioral function of genetically and spatially defined neuronal populations. However, transgene expression can adversely affect cell health through toxic or leaky expression and cause neural and behavioral abnormalities. Here, we compared the learning rates of various transgenic mice during a whisker-mediated texture discrimination task that requires the primary somatosensory cortex. Specifically, we crossed transgenic Cre lines with conditional enhanced halorhodopsin reporter mice to express inhibitory opsins in excitatory neurons of various cortical layers. Emx1-Halo animals, which express halorhodopsin in all excitatory cortical neurons, were severely impaired when learning the texture discrimination task. They also showed lingering residual effects of photoinhibition on laser-off trials. Such deficits were not observed in a cortex-independent object detection task or in other tested transgenic lines (Rbp4-Halo, Fezf2-Halo, Nr5a1-Halo, Cux2-Halo, and Nex-Halo). These results highlight the importance of characterizing and selecting appropriate transgenic lines for in vivo optogenetic experiments.

Disclosures: **N. Harano:** None. **J.B. Dahan:** None. **J.M. Park:** None. **R.M. Bruno:** None.

Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

Location: SDCC Halls B-H

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Program #/Poster #: 208.02

Topic: D.03. Somatosensation – Touch

Support: NRF-2019M3E5D2A01058328
NRF-2021M3E5D2A01019544

Title: Layer-specific neural coding of textures in the mouse somatosensory cortex

Authors: D. OH, *J. KWAG;
Korea Univ., Seoul, Korea, Republic of

Abstract: Perceiving and discerning different textures from the environment is critical for rodent behavior. It has been established that rough textures evoke higher spike firing rate than smooth textures in cortical neurons of the rodent primary somatosensory cortex (S1). However, whether different texture stimuli can be represented and encoded in different types of neural codes such as spike firing rate and spike timing in cortical neurons across the layers of S1 is poorly understood. To address this issue, we recorded single-unit activities from S1 *in vivo* across the layers using a 32-channel silicon probe during a 1 s-long presentations of two different textures, P80 (rough) and P1200 (smooth), to the mouse whiskers. In response to the texture stimuli, majority of neurons across the cortical layers displayed two distinct periods at which spike firing rates peaked, at the onset and termination of texture stimuli. However, the two peaks had distinctively different peak spike firing rates and latency to peak firing rates, each representing rate and temporal coding of textures, respectively. As for the 1st peak firing rate, rough texture was represented as higher spike firing rate than smooth textures in layer (L) 5/6 of S1, while, smooth texture had a faster peak firing rate onset latency than rough texture. These results indicate rough and smooth textures are differentially represented in L5/6 in spike firing rate and spike timing, respectively. As for the 2nd peak firing rate, while the latency of the 2nd peak firing rates were not significantly different between two textures across the layers, smooth texture evoked higher spike firing than rough textures in L4/5. Together, our preliminary results demonstrate there may be layer-specific texture encoding using different neural codes in S1.

Disclosures: D. Oh: None. **J. Kwag:** None.

Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 208.03

Topic: D.03. Somatosensation – Touch

Title: Interhemispheric integration of tactile inputs during passive and active vibrissa sensation

Authors: *Y. ORAN, Y. KATZ, I. LAMPL;
Neurobio., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Corpus callosum (CC), a major fiber bundle in the mammalian brain, arises from almost all regions of the neocortex and is known to connect predominantly homotopic areas. Despite considerable research devoted to this pathway, the neuronal mechanisms of interhemispheric communication and the role of the CC in mediating them are still largely unknown. In a previous study (Oran, Katz, et. al., Nature Communications, 2021), we found state-dependent interhemispheric correlations both at the supratheshold (action potentials) and subthreshold (membrane potential) levels in the barrel cortex. We then showed that these state-dependent changes causally depend on callosal fibers activity. Here we studied the role of the CC in coordinating interhemispheric sensory integration in this system. First, we explored the specificity of homotopic maps. Secondly, we investigated the role of CC in interhemispheric bilateral integration in behaving mice. The strong innervation of callosal axon at the border between S1/S2, suggests a possibly stronger ipsilateral response for barrels near this border. To test this, we preformed widefield imaging (n=2 mice) during passive unilateral and bilateral whisker stimulation of whiskers in rows A to D. In agreement with our prediction, our data reveal that unilateral whisker stimulation in rows A and B was highly more likely to elicit an ipsilateral response compared to rows C and D. Interestingly, we found that, unlike unilateral stimulation, bilateral stimulation was effective in activation of cortical areas outside the barrel cortex, in particular the retrosplenial cortex. Next, to examine bilateral tactile integration during active sensation,we built a virtual tunnel environment for mice, simulating traversing a tunnel. Mice were head fixed, standing on a treadmill and surrounded by two movable walls (two big wheels). We controlled the distance between these walls to simulate a tunnel of changing width, introducing a semi-natural unilateral and bilateral stimulus. Overall, the responses to unilateral and bilateral stimuli of 48 neurons (n=2 mice) recorded with silicon probes in the barrel cortex were examined. Nineteen cells (40%) responded to ipsilateral stimuli and 13 cells (27%) were responsive to bilateral stimulation. Most cells were tuned to the wall distance from the snout, both to contralateral and ipsilateral stimulation. Importantly, in some cells this tuning either disappeared or significantly changed after blocking corpus callosum activity using chemogentics. In conclusion, our results suggest that the corpus callsoum has a pivotal role in shaping interhemispheric integration during natural sensation.

Disclosures: **Y. Oran:** None. **Y. Katz:** None. **I. Lampl:** None.

Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

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Program #/Poster #: 208.04

Topic: D.03. Somatosensation – Touch

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NIH/NINDS R21NS112783
SNSF Postdoctoral Fellowship P2ELP3_168506
SNSF Postdoctoral Fellowship P300PA_177861

Title: Cell-type specific signatures of bilateral tactile signal integration in awake mouse somatosensory cortices

Authors: *A. PALA, G. B. STANLEY;
Georgia Inst. of Technol. and Emory Univ., Atlanta, GA

Abstract: To produce a coherent sensation of the external world, the brain must integrate tactile information obtained through both sides of the body. Evidence of bilateral tactile signal integration has been found in primary (S1) and secondary (S2) somatosensory cortices in rodents, monkeys and humans. Yet, the cellular organization and the detailed effect of bilateral tactile signal integration on cortical sensory activity are unknown. Further, it is unclear how properties of the bilateral tactile signals, such as their strength, relative timing, and whether they are produced during movements, modulate integration. Finally, to what extent bilateral integration contributes to sensory information coding is unknown. To measure the spiking activity of populations of individual neurons, we targeted laminar silicon probe recordings to the whisker region of S1 and S2 in awake head-fixed mice while delivering unilateral and bilateral sensory stimuli to a single whisker. High-speed videography was used to monitor spontaneous whisker movements. Across S1 and S2, we found that the amplitude of sensory responses evoked in regular-spiking (RS) neurons by bilateral stimuli was most commonly reduced without change in variability or latency when compared to responses evoked by contralateral stimuli. To more precisely understand the nature of the suppressive interactions between ipsilateral and contralateral sensory signals, we compared population velocity response curves measured in response to contralateral stimuli with or without associated ipsilateral stimulation. We observed both subtractive and divisive interactions, as a function of the relative timing between contralateral and ipsilateral stimuli and of the neuron responsiveness to ipsilateral stimuli. Such interactions were predominant and, in some cases, specific to S2. In summary, our results reveal the role of bilateral tactile signal integration in shaping the representation of tactile information in the two main somatosensory cortical areas with cellular resolution. Ongoing work is focusing on investigating the relevance of bilateral tactile signal integration for sensory coding.

Disclosures: **A. Pala:** None. **G.B. Stanley:** None.

Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 208.05

Topic: D.03. Somatosensation – Touch

Title: Single-whisker dependent active ethological navigation paradigm for studying sensory-processing in head-fixed mice in virtual reality.

Authors: *A. G. ARMSTRONG[1], K. HU[2], Y. VLASOV[3];
[1]Univ. of Illinois Urbana-Champaign, Champaign, IL; [2]Univ. of Illinois, Urbana-Champaign, Urbana, IL; [3]Neurosci., Univ. of Illinois, Urbana-Champaign Neurosci. Program, Urbana, IL

Abstract: To gain insight into the functional and dynamic organization of cortical microcircuits that drive behavioral decision making, we developed a tactile virtual-reality navigation paradigm to study the activity of neuronal populations in the mouse barrel cortex during active naturalistic behavior. Mice are head-fixed on a suspended ball that forms a 360° treadmill. A pair of motorized textured walls are placed either side of the snout at a distance reachable only by its vibrissae. The speed and direction of mouse movements are tracked in a 2D virtual plane and are coupled to the wall movement in a real-time closed-loop system creating the illusion of navigating a virtual corridor [1, 2]. In darkness and the presence of white noise from air suspension, the VR leaves whisking against textured walls as the only sensory cue present to guide the navigation. The trial structure first establishes a baseline of straight running guided by active tracking of both walls. The baseline task is followed by a single wall approaching the snout from the left or right causing the animal to change direction of its run to avoid the incoming virtual obstacle. Unlike traditional head-fixed behavioral paradigms, mice can perform this task without weeks of prior training. We found 2-5 training sessions are enough for animals to fully adapt and run for 90% of the 2h session at mean speeds above 15cm/s. In total, 95% of mice (n=20) tested were able to reach 90% success rate of actively tracking walls. Remarkably, there was no significant statistical difference between mice tracking turns with only C2 whiskers compared with all the whiskers intact. Mean run angle as a function of distance to the wall during the turns shows highly repeatable classical psychometric curves (n=5). Acute recordings of neural activity during active navigation are carried out using Neuropixel probes in the C2 barrel column in *Scnn1a-TG3-Cre x Ai32* mice. Interaction of C2 whiskers with the textured walls drives vast spiking activity across all cortical layers. Using optical tagging, lesions, and Cre expression of GFP in layer 4 as a guide, the coordinates of each recorded unit is identified and aligned with the Allen CCF atlas. Synchronous firing of neuron subpopulations across layers is observed that changes rapidly during different stages of the behavioral task. Analysis of firing correlations reveals dynamic organization of neuronal populations across layers. Our results shed light on the dynamic functional organization of cortical circuits and identify specific stages of information flow during perceptual decision making. [1] N.Sofroniew, *et al.* eLife;4:e12559 (2015). [2] A.G.Armstrong, *et al.* SFN 2021:P413.03.

Disclosures: **A.G. Armstrong:** None. **K. Hu:** None. **Y. Vlasov:** None.

Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 208.06

Topic: D.03. Somatosensation – Touch

Title: Temporal coding of sensory cues pre-processed via dispersive high-frequency vibrational modes in rodent's vibrissae

Authors: *Y. DING[1], Y. VLASOV[2];
[1]Univ. of Illinois, Univ. of Illinois, Urbana, IL; [2]Neurosci., Univ. of Illinois, Urbana-Champaign Neurosci. Program, Urbana, IL

Abstract: High-precision spike timing as small as 10μs has been observed during whisker sweeping across textured surfaces in primary afferents [1] temporally aligned with fast acceleration events of whisker tip stick-slip motion. These characteristic times correspond to frequencies of whisker micromotions extending beyond 10-500Hz that are typically studied with high-speed video recordings. What aspects of such fast and complex spatiotemporal micromotions of a whisker are contributing to generation of these precisely timed spike trains? Here, using highly sensitive acoustical methods we performed a systematic study of vibrissae micromotions and identified a regular series of high order vibrational modes (up to 30) spanning frequencies up to 10KHz [2]. Interaction of both mice and rats' vibrissae with a pole excites mostly second order mode (80% of total energy below 500Hz). In striking difference, when vibrissa sweeps over a textured surface the vast majority of the shockwave energy (up to 80%) is redirected to higher order modes at frequencies above 500Hz. Moreover, these higher order modes exhibit up to 100X smaller damping ratio and are propagating 10X faster than lower order modes. It is demonstrated that this strong frequency dispersion results in a single shockwave generated at the tip of vibrissa segregating into individual high order modes that arrive at the whisker base at different times. Higher frequency modes being fast and less damped arrive first within 0.1ms, while slow and heavily damped low frequency modes are significantly delayed by 3-8ms. A single collision event, therefore, is transformed into a time series of individual events at the whisker base as the eigenmode wave packets arrive. To evaluate an upper limit of information capacity available at the whisker follicle for the neural uptake after a single collision event, information theoretic methods were employed. For 1ms bin time the mutual information available for spike encoding is estimated to be 720bits/sec. However, when shorter time intervals are considered (time bin 200μs), the mutual information is increasing dramatically up to 3000bits/sec. This estimate is indicative of an ultrahigh information capacity that is available for encoding in primary afferents that is at least 10-fold higher than what is usually perceived. [1] M.R. Bale et. al., J. Neurosc, 35, 5935 (2015). [2] Y. Ding, Y. Vlasov, bioRxiv, DOI 10.1101/2022.06.15. 496141 (2022)

Disclosures: **Y. Ding:** None. **Y. Vlasov:** None.

Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

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Program #/Poster #: 208.07

Topic: D.03. Somatosensation – Touch

Support: CRCNS R01NS115327
NIBIB R01EB029857
NINDS R21NS112783
NIBIB T32 T32EB025816

Title: Machine learning approaches for estimating cortical states in the awake mouse

Authors: *A. M. F. BORSA[1], D. A. WEISS[2], A. PALA[2], A. J. SEDERBERG[3], G. B. STANLEY[2];
[1]Sch. of Electrical and Computer Engin., [2]Coulter Dept. of Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA; [3]Dept. of Neurosci., Univ. of Minnesota Twin Cities, Minneapolis, MN

Abstract: In awake mammals, cortical activity patterns vary with behavioral context such as locomotion, attention, arousal, or quiescence. These activity patterns, termed cortical states, profoundly modulate cortical function. For example, spontaneous cortical activity as reflected in the local field potential (LFP) has been shown to shape sensory processing in somatosensory, visual, and auditory areas. Despite their important role in contextually modulating cortical processing across sensory modalities, cortical states are defined in an *ad hoc* manner that does not leverage the structure of variability in cortical activity. To address this gap, we applied data driven techniques to extract cortical states from spontaneous LFP activity recorded from the primary somatosensory cortex (S1) in awake, head-fixed mice. We applied unsupervised learning techniques including k-means clustering and principal component analysis (PCA) to Fourier spectrums of short epochs of the recorded LFP. In agreement with the literature, these unsupervised algorithms revealed that the magnitude of low-frequency (LF: ~1-10 Hz) components and broadband high frequency (HF: ~30-90 Hz) components are major sources of variability across LFP epochs, and therefore represent a natural way to cluster periods of similar neural activity into cortical states. For subsequent analyses, we collapsed the Fourier spectrums associated with each LFP segment into a unidimensional LF/HF ratio. The distribution of LF/HF ratios was well fit by Gaussian mixture models (GMMs) when two to three components were used, indicating that a few discrete underlying states may account for the structured variation in spontaneous cortical activity. Building on this finding, we fit a two-state Hidden Semi-Markov Model (HSMM) to cortical activity to capture the evolution of cortical states over time. By accounting for the time spent in a state, this modeling approach provides stability against fast transient changes generated by noisy measurement of the LF/HF ratio. This final approach allows cortical states to be decoded both temporally and probabilistically. In ongoing work, we are comparing our cortical state estimates with behavioral markers such as whisker motion to evaluate the efficacy of our approaches. Moreover, we are analyzing multichannel LFP data to make more robust cortical state estimates. In future work, we will port these approaches onto real-time hardware so that we can estimate cortical states in real-time.

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Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

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Topic: D.03. Somatosensation – Touch

Support: NIH/NNDS R01 Grant NS107599
Whitehall Foundation Grant 2017-05-71

Title: Shift from Suboptimal Sampling to an Optimal Temporal Strategy for a Selective Detection Task in Mice

Authors: *K. MARRERO[1], A. LAM[2], B. ZAREIAN[3], K. ARULJOTHI[3], Z. ZHANG[1], E. ZAGHA[3];
[1]Neurosci., [2]Biomed. Sci., [3]Psychology, Univ. of California, Riverside, CA

Abstract: Ongoing analyses of behavioral performance genuinely seek to disentangle internal cognition from external decisions, but often such analyses neglect the impacts of learning and learning-related components when investigating goal direction. Indeed, the learning process from naïve behavior is often investigated separately from what is considered expert performance. Additionally, amongst a vast variety of detection paradigms (feature or spatial tasks), temporal aspects are often neglected. Yet, depending on the task design, anticipation, expectation, timing, waiting, and withholding are key temporal components. Here, we longitudinally analyze naïve mice who implement suboptimal sampling behavior in a robust selective detection paradigm. We find that mice trained to selectively detect and ignore stimuli also learn to optimize temporal strategies as they reach expert status in a whisker-based discrimination task. Mice improve performance while simultaneously learning to time, wait, and withhold as they anticipate an expected stimulus presentation. Specifically, these mice implement intermediate strategies that enhance stimulus detection, improve temporal methods, and reduce sampling behavior when required to wait for a stimulus. By establishing and implementing standardized measures, we show that impulse control of timing, waiting, and withholding become implicitly required in learning the task design for optimum goal directed behavior. These results provide insight across paradigms where declining performance in mental health disorders, such as ADHD and autism, show temporal deficits with respect to impulsivity, timing, waiting, and withholding.

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Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

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Program #/Poster #: 208.09

Topic: D.03. Somatosensation – Touch

Support: NIH 1R37 NS092367-1

Title: Diversity of neural coding of vibrotactile noise stimuli in whisker somatosensory cortex

Authors: *L. RODRIGUEZ[1,2], D. E. FELDMAN[1,2];
[1]Helen Wills Neurosci. Inst., UC Berkeley, CA; [2]Mol. & Cell Biol. Dept., UC Berkeley, CA

Abstract: Rodents can robustly distinguish fine differences in texture (roughness) using their whiskers, a capacity that depends on neuronal activity in primary somatosensory cortex (S1). The relevant physical cue for roughness discrimination is thought to be the mean speed of whisker vibration, which is classically encoded in S1 by mean population firing rate. However, individual single units in S1 have been reported to encode roughness of sandpaper and grating surfaces in a variety of ways, from a simple linear relationship between roughness and firing rate, to units that are tuned for specific textures, particularly in L2/3. How these different types of single-unit coding, and the different cortical layers, contribute to the overall population code is not understood. To address this question, we are studying neural coding of vibrotactile noise (VTN) stimuli, which vary in mean speed and model the irregular whisker vibrations that occur during texture palpation. We performed Neuropixels recording in S1 of head-fixed anesthetized mice while delivering VTN stimuli at different mean speeds. At the population level, mean speed was encoded linearly. However, at the level of single units we observed substantial coding heterogeneity within and across layers, including units with roughly linear encoding over a broad range of mean speed, units with sigmoid response functions with varying threshold, and units tuned for specific mean speeds. Stimulus identity could be robustly decoded based on single-trial firing rates across populations of simultaneously recorded units. Using this approach, and extending it to awake mice performing VTN discrimination, we will assess the contribution of each neural coding type, and each cortical layer, to population encoding and behavioral discrimination of vibrotactile mean speed.

Disclosures: L. Rodriguez: None. **D.E. Feldman:** None.

Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

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Topic: D.03. Somatosensation – Touch

Support: NIH Intramural Research Program

Title: Brain-wide networks for functionally-distinct principal neurons in the primary somatosensory cortex

Authors: *A. R. INACIO, K. LAM, Y. ZHAO, F. PEREIRA, C. R. GERFEN, S. LEE;
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Abstract: Neuronal connections within and across brain areas provide the scaffolding for neuronal computations. Revealing brain-wide wiring diagrams at the single neuron level constitutes a fundamental step in understanding how distinct patterns of neuronal activity patterns emerge and how these may support behavior. In primary sensory cortices, principal neurons exhibit heterogeneous patterns of activity not only in the presence but also, absence of external sensory stimulation, namely during spontaneous movements. Whether these behavioral-state dependent patterns of activity are supported by random or structured connectivity remains unknown. We show that movement-sensitive neurons receive characteristic excitatory long-range inputs. Using functional imaging, we monitored the activity of principal neurons in layers II/III of the mouse primary somatosensory cortex (S1). Activity during spontaneous movements was dominated by a subset of neurons, spatially intermingled with other functionally-distinct neurons. This subset was stable over time, suggestive of structured connectivity. To investigate brain-wide afferent connectivity onto functionally-defined, individual neurons, we combined functional imaging with single neuron-initiated monosynaptic retrograde tracing. Analysis of brain-wide, presynaptic anatomical ensembles revealed several connectivity features. First, each neuron, independently of activity profile, receives inputs from virtually every brain area known to project to S1. Second, local and long-range presynaptic ensembles of movement-sensitive and movement-insensitive neurons are not spatially distinct. Third, movement-sensitive neurons receive a smaller fraction of inputs from primary and secondary motor cortices, while receiving a larger fraction of inputs from thalamus than movement-insensitive neurons. Yet, the activity of movement-sensitive neurons cannot be explained by sensory feedback from moving whiskers, since it was preserved after unilateral whisker pad paralysis, nor by neuromodulatory inputs. Movement-triggered optogenetic suppression of thalamic inputs altered activity patterns, demonstrating a direct functional role of thalamic inputs in S1 during spontaneous movements. Our study provides an anatomical and functional connectivity rule that supports the representation of spontaneous movements in S1.

Disclosures: **A.R. Inacio:** None. **K. Lam:** None. **Y. Zhao:** None. **F. Pereira:** None. **C.R. Gerfen:** None. **S. Lee:** None.

Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 208.11

Topic: D.03. Somatosensation – Touch

Support: F32 FNS119282A
R01 NS10533

Title: Whisker deprivation alters intrinsic excitability of somatostatin (SST) interneurons in mouse somatosensory cortex

Authors: *B. M. BOHANNON[1], D. E. FELDMAN[2];
[1]Helen Wills Neurosci. Inst., [2]Mol. & Cell Biol. Dept., UC Berkeley, Berkeley, CA

Abstract: Sensory cortex exhibits robust plasticity in response to patterns of sensory experience. The circuit mechanisms of plasticity are best characterized in excitatory circuits but remain poorly understood in inhibitory circuits. Here we focus on somatostatin (SST) interneurons, which provide dendritic inhibition to pyramidal (PYR) cells and are proposed to gate PYR plasticity through disinhibitory circuit motifs. Whether SST cells and circuits themselves are plastic is unknown. We studied this in L2/3 SST cells in whisker somatosensory cortex (S1), where whisker deprivation drives well-characterized plasticity of the L2/3 whisker map. We asked whether whisker deprivation alters the intrinsic excitability of SST cells, as it has been shown to do for parvalbumin (PV) interneurons as part of a rapid homeostatic response to sensory deprivation.We made whole-cell current-clamp recordings from L2/3 SST cells in S1 slices from SST-Cre;tdTomato mice (P18-25). Mice had the D-row of whiskers plucked, and recordings were made in identified D whisker columns. We compared passive (Vrest, Rinput) and active cell properties (rheobase, frequency-current (F-I) curves, spike threshold) in D-row deprived mice vs. sham (anesthesia only) littermates. 1 day of deprivation did not alter SST intrinsic properties, unlike L2/3 PV cells whose excitability is decreased. However, 3 days of D-row deprivation decreased SST intrinsic excitability, depressing F-I curves by ~40% and depolarizing spike threshold by 2.7 ± 0.77 mV ($p = 0.005$). Passive properties were unaffected. 52.9% of SST cells produced action potential bursts in mice with normal whisker experience, but this was reduced to 29.4% after whisker deprivation ($p = 0.0009$).Thus, 3 days of deprivation reduces SST intrinsic excitability, similar to the rapid effects of deprivation on PV cells. This suggests that SST cells, like PV cells, may be involved in network homeostasis in response to reduced sensory drive, with disinhibition shifting from PYR cell bodies to PYR dendrites with longer periods of deprivation. The functional effect of reduced SST bursting is unclear but could permit heightened integration or enhanced plasticity of dendritic inputs to pyramidal cells, thus promoting the onset of whisker map reorganization within excitatory networks after several days of deprivation.

Disclosures: B.M. Bohannon: None. **D.E. Feldman:** None.

Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 208.12

Topic: D.03. Somatosensation – Touch

Support: GR5270671

Title: Diverse behavioral representation in PV interneuron dynamics is conserved across neocortical areas but may diverge between layers 2/3 in mice

Authors: *A. I. MORE, C. A. DEISTER, D. N. SCOTT, C. I. MOORE;
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Abstract: Parvalbumin-positive inhibitory interneurons (PV) sculpt local circuit activity in the Neocortex, and are widely believed crucial to optimal processing. Several studies have tied PV, and their dynamics on single trials, to perceptual success (Siegle et al. 2014, Shin and Moore 2019). Using two-photon imaging of genetically identified PV in primary sensory Neocortex during well-controlled tactile and visual detection tasks, the present study is testing the possibility of laminar differentiation in PV dynamics during motivated behavior. Mice are imaged each day of training to analyze emerging dynamics that may occur with sensory learning. Neocortical depths are sampled each day spanning the range of Layer 2 (L2) and Layer 3 (L3) in the mouse, from 100 to 350 microns deep from the Neocortical surface.
We have confirmed that, as shown previously, PV show greater sensory responsiveness compared to nearby non-PV neurons. Additionally, we find a bimodal distribution of task-predictive PV in primary somatosensory cortex (SI) and primary visual cortex (V1) in our tactile and visual detection tasks. These two ensembles consist of a subgroup that predicts successful performance on hit trials with increased firing rates, and an additional subgroup that predicts failed detection (miss trials) with their rates. In contrast, putative excitatory neurons show a single, detection-predictive sub-group.
Initial analyses indicate that the relative number and type of task-predictive PV may differ across L2 and L3 of the sensory Neocortical column. These preliminary findings were made in V1 during a visual contrast detection task. To address whether this cortical depth result is specific to V1 or generalizes to other sensory cortices, we are now testing if these findings hold in SI during tactile detection. These parallel detection studies in V1 and SI will help to address whether sublaminar functional representations of L2 and L3 differ in primary sensory neocortex.

Disclosures: A.I. More: None. **C.A. Deister:** None. **D.N. Scott:** None. **C.I. Moore:** None.

Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 208.13

Topic: D.03. Somatosensation – Touch

Support: NIH Grant R01-NS117636
NIH Grant K99/R00-NS096108
NIH Grant F31-NS124244
Michigan State Startup

Title: Identification of an inhibitory circuit that mediates motor integration in the somatosensory cortex

Authors: H.-H. KIM, C. JONES, L. E. MARTINETTI, S. DASH, D. M. AUTIO, T. KELLER, A. RACHOR, J. ACKERMANN, K. E. BONEKAMP, ***S. R. CRANDALL**;
Dept. of Physiol., Michigan State Univ., East Lansing, MI

Abstract: According to the corollary discharge theory, motor centers send signals informing sensory areas of ongoing motor actions, suppressing the expected sensory input. However, the mechanism underlying such suppression within the cortex is unclear. Using optogenetics, we find that input from the whisker primary motor cortex (wM1) activates layer 6(L6) parvalbumin(PV)-expressing inhibitory cells whose axons arborize locally within deep layers more strongly than any other interneuron population in whisker somatosensory cortex. The greater responsiveness of these PV interneurons was not due to unique intrinsic properties or local circuit interactions but was produced by synaptic mechanisms. Notably, vM1 axons made stronger excitatory connections onto these PV cells than other neurons. Recordings in behaving mice also reveal that some excitatory neurons are suppressed before whisking, whereas some PV interneurons increase their activity. Our results provide a synaptic circuit mechanism for motor-related corollary discharge in cortex that may help tactile sensation and whisker-dependent behaviors.

Disclosures: H. Kim: None. **C. Jones:** None. **L.E. Martinetti:** None. **S. Dash:** None. **D.M. Autio:** None. **T. Keller:** None. **A. Rachor:** None. **J. Ackermann:** None. **K.E. Bonekamp:** None. **S.R. Crandall:** None.

Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 208.14

Topic: D.03. Somatosensation – Touch

Support: German Research Foundation (grants SFB 1089 and SPP 2041)

Title: Cell type-specific organization of GABAergic interneurons in a cortical column

Authors: *F. YÁÑEZ[1], D. UDVARY[1], L. MESSORE[1], G. QI[2], B. SAKMANN[3], D. FELDMEYER[2], M. OBERLAENDER[1];
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Abstract: Cortical GABAergic interneurons (INs) have been extensively reported as a rich and diverse class of neurons. At the single-cell level, attributes such as morphology and intrinsic physiology exhibit complex patterns of variation, making them difficult to characterize. Here, we systematically assess the degree and character of the variability of these properties across the entire cortical depth of the rat barrel cortex. First, we calculate the laminar distribution of a sample of 306 INs and quantitatively test its representativeness against the absolute number of

INs of the rat barrel cortex. For each neuron, we compute a comprehensive list of descriptive features based on (i) its spiking patterns in response to somatic current injections, and (ii) reconstructions of their axon and dendrite morphology, as well as somatic depth location. Then, we assign neurons into morphological and/or intrinsic physiological types using standardized clustering methods and evaluating the robustness of cluster assignments. This methodology allows us to (i) develop empirical models that reveal the degree to which particular features (such as somatic depth location) are predictive of an IN's type, and (ii) perform cross-species analyzes that provide quantitative insight into the relationships between types in the mouse visual and rat barrel cortices.

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Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 208.15

Topic: D.03. Somatosensation – Touch

Support: GR1021883

Title: Representation of Object Location in Two L5 Cell-types in Barrel Cortex

Authors: *S. KING[1], S. A. HIRES[2];
[2]Biol. Sci., [1]USC, Los Angeles, CA

Abstract: Primary somatosensory cortex (S1) is well-known as being a locus for touch and object location information within cortex. However, which cell-types are the largest constituent contributors to object location representation is still unknown. Layer 5 neurons have been implicated by previous work as being large contributors to object location; however, cell-type-specific identification within layer 5 has been lacking. Here, we investigate the role that two L5 cell subtypes, pyramidal tract neurons (PT) and intratelencephalic neurons (IT), serve in object location encoding.

Disclosures: S. King: None. **S.A. Hires:** None.

Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 208.16

Topic: D.03. Somatosensation – Touch

Support: BMBF/FKZ 01GQ1002
ERC No 633428
NIH Grants R01 NS24328
NIH Grants P40 OD010996

Title: New Definition of the Whisker Motor Areas in the Rat Cerebral Cortex

Authors: *A. MAHARJAN[1], J. M. GUEST[2], J.-A. RATHELOT[3], P. L. STRICK[4], M. OBERLAENDER[1];
[1]Max Planck Inst. for Neurobio. of Behavior – caesar, Bonn, Germany; [2]Max Planck Florida Inst. for Neurosci., Florida, FL; [3]SNI & CNBC, Univ. of Pittsburgh, Pittsburgh, PA; [4]Systems Neurosci. Inst., Univ. Pittsburgh Sch. Med., Pittsburgh, PA

Abstract: Which areas of the rodent cerebral cortex control movements of the facial whiskers (i.e., vibrissae)? Here, we address this question by using retrograde transneuronal transport of rabies virus to identify the cortical areas that have disynaptic access to the motoneurons that innervate a single whisker muscle. For comparison, we also examine which cortical areas have disynaptic access to the motoneurons that innervate a single forepaw muscle, extensor digitorum communis (EDC). We find that five major cortical areas in both the contra- and ipsilateral hemispheres contribute to the control of whisker motoneurons: primary motor (M1) and sensory cortex (S1), secondary motor (M2) and sensory cortex (S2), and anterior insular cortex (AI). The proportions of the neurons that have disynaptic access to the whisker motoneurons are similar in both hemispheres - 50% originate from M1, 35% from S1, and 5% from M2, S2, and AI, respectively. However, the distributions of the neurons within these major cortical areas differ between hemispheres. The vibrissa-related part of S1, the barrel cortex, and a medial-caudal part of M1 are unilateral - i.e., they have access exclusively to whisker motoneurons on the contralateral side. The remaining parts of M1 and S1, as well as M2, S2, and AI are bilateral - i.e., they have access to whisker motoneurons on both the contra- and ipsilateral sides. Notably, the bilateral whisker area of M1 is subdivided further into two parts, where either the contra- or ipsilateral side dominates. Cortical areas that have disynaptic access to EDC motoneurons are restricted to the contralateral hemisphere. The cortical areas that respectively represent the forepaw and whisker muscle are disjoint in S1, partially overlap in M1, and fully overlap in M2 and S2. Insular cortex has no disynaptic access to forepaw motoneurons. Thus, descending influences from multiple, functionally distinct cortical areas contribute to the control of whisker motoneurons. In addition, we discovered new regions for differential control of contra- and/or ipsilateral whiskers, and for coordination in the control of whiskers and forelimb.

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Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

Location: SDCC Halls B-H

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Program #/Poster #: 208.17

Topic: D.03. Somatosensation – Touch

Support: NIH Grant R01-NS117636
NIH Grant K99/R00-NS096108
NIH Grant F31-NS124244
Michigan State Startup

Title: Motor control of layer 6 corticothalamic circuits in the somatosensory cortex

Authors: *L. E. MARTINETTI[1], D. M. AUTIO[2], S. R. CRANDALL[2];
[1]Neurosci. Program, [2]Dept. of Physiol., Michigan State Univ., East Lansing, MI

Abstract: In the rodent vibrissal somatomotor system, projections from the primary motor cortex (M1) to the somatosensory cortex (S1) are thought to facilitate active sensation and sensorimotor integration by conveying motor-related signals that modulate the sensory responsiveness of neurons in the context of vibrissa movement. Although previous work has shown that M1 axons concentrate in layer 1 (L1) of S1, they also branch off in deep layers where they have excitatory effects on excitatory projection neurons, including L6 corticothalamic (CT) neurons. Since L6 CT cells provide a massive input to the thalamus and have local connectivity within the cortex, they can uniquely influence the sensory throughput of the thalamus and cortical responsiveness. Previous work has identified three general classes of L6 CT cells in rodent vibrissal S1 based on their thalamic axonal projections, including (1) those that project only to the ventral posterior medial nucleus (VPm), (2) those that target both the VPm and the posterior medial nucleus (POm), and (3) those that arborize exclusively in POm. However, the afferent connectivity of M1 and the mechanisms by which it controls L6 CT circuits are largely unknown, primarily due to the technical difficulties in identifying the various subclasses and isolating their synaptic responses. To overcome these challenges, we combined retrograde tracing strategies with *in vitro* recordings and optogenetic control strategies to study the synaptic effects of M1 input on different classes of L6 CT cells for the first time. Here, we find differences in intrinsic membrane properties of different CT cells that could influence their propensity to respond to afferent input. We also find that CT classes segregate into sublayers and express distinct neurite intracortical patterns, consistent with previous anatomical work. Optical stimulation of M1 axons/terminals evoked the strongest synaptic currents in VPM/POm projecting CT cells located at a specific sublayer than all other classes. These results start to reveal mechanistically how inputs from M1 can influence the excitability of distinct L6 CT cells and perhaps the activity in specific thalamocortical circuits.

Disclosures: L.E. Martinetti: None. **D.M. Autio:** None. **S.R. Crandall:** None.

Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

Location: SDCC Halls B-H

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Program #/Poster #: 208.18

Topic: D.03. Somatosensation – Touch

Support: Wellcome Trust
BBSRC
ERC
EMBO
Marie Curie Fellowship

Title: All-optical interrogation of behaviorally relevant choice signals in layer 2/3 neurons of the primary somatosensory cortex

Authors: *Z. ZHANG[1,2], C. BUETFERING[1,3], R. RATTO[1], P. DZIALECKA[1], M. HAUSSER[1]; [1]Wolfson Inst. for Biomed. Res., Univ. Col. London, London, United Kingdom; [2]Dept. of Psychiatry & Behavioral Sci., Stanford Univ. Sch. of Med., Stanford, CA; [3]Inst. of Pathophysiology, Univ. Med. Ctr. of the Johannes Gutenberg-University of Mainz, Mainz, Germany

Abstract: Understanding the neural circuit mechanisms that transform sensory information into meaningful behavior is a fundamental goal of neuroscience. Where and how decision signals are generated along the pathway to make an informed choice is key to understanding this transformation. The conventional perspective is that primary sensory cortices extract stimulus information from the sensory inputs, while decision signals important to drive behavior are computed downstream in the processing hierarchy. This has been challenged by a series of recent studies reporting that in the rodent brain, neural activity can correlate with behavioral choice as early as in the primary sensory cortices (Sachidhanandam et al., 2013, Chen et al., 2013, Poort et al., 2015, Kwon et al., 2016, Yang et al., 2016, Francis et al., 2018). However, it is unclear whether this signal reflects behaviorally-relevant decision coding, or a modulation of neural activity by action-related variables, such as motivation and movement.
Here, we used all-optical interrogation, combining two-photon calcium imaging and two-photon optogenetic stimulation, to examine stimulus and choice signals in layer 2/3 (L2/3) of mouse barrel cortex. When mice perform a two-choice texture discrimination task, neurons in L2/3 of barrel cortex exhibit stimulus and choice selectivity before the choice action. Interestingly, distinct populations of neurons carry information about the stimulus irrespective of the behavioral outcome (‘stimulus neurons’) or about the choice irrespective of the presented stimulus (‘decision neurons’). Using targeted photostimulation, we found the stimulus and decision neurons exhibit different activity patterns and functional connectivity in the local circuit: there is stronger like-to-like connectivity in neuronal ensembles encoding the same stimulus identity, and a preferential activation of decision neurons. Crucially, direct activation of the correct choice-related activity in the L2/3 circuit improves behavioral performance and therefore causally links the decision signal to behavior. The finding that neurons encoding either the stimulus or the decision are intermingled within the same circuit challenges the idea of a strict processing hierarchy in the cortex, and the causal manipulation results suggest a direct involvement of the choice signal in S1 in the decision-making process.

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Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

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Program #/Poster #: 208.19

Topic: D.03. Somatosensation – Touch

Support: SNF CRSII5_177237

Title: Quantifications of axonal projections from neurons located in layers 2/3, 5 and 6 of mouse barrel cortex

Authors: *Y. LIU, L. DÉLEZ, S. CROCHET, C. C. PETERSEN;
École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Abstract: Mice are nocturnal animals that rely heavily on their whiskers to sense the outside world. Whisker sensory information arriving from the ventral posterior medial nucleus of the thalamus is processed in the primary whisker somatosensory cortex (also known as the barrel cortex, S1-bfd). Projection neurons in S1-bfd in turn signal to various downstream brain areas including motor cortex, secondary sensory cortex, striatum, superior colliculus, thalamus, pons and brain stem. Different classes of S1-bfd neurons innervate different targets, but the precise organisation and quantification of axonal innervation remains to be determined. Here, we use adenoassociated viral (AAV) vectors to express fluorescent proteins in genetically-defined neuronal classes in S1-bfd. We injected Cre-dependent AAVs into various Cre-driver mice in order to express GFP and/or tdTomato in layer 2/3 (Rasgrf2-dCre), layer 5 (Sim1-Cre, Tlx3-Cre and Rbp4-Cre) and layer 6 (Ntsr1-Cre). After several weeks of expression, the fixed brains were immunostained and cleared through a variant of iDISCO (Renier et al., Cell 2014). Volumetric brain images were acquired by a MesoSPIM light sheet microscope (Voigt et al., Nat. Methods 2019). Voxels containing axons were segmented using TrailMap, a trained 3D convolutional network (Friedmann et al., PNAS 2020). Finally, images were aligned to the Allen mouse brain atlas (Wang et al., Cell 2020), and axonal length was quantified according to the annotated parcellations. Consistent with previous work, we find different projection patterns from each of the Cre-driver lines such as inter-telencephalic projections and pyramidal tract projections. Our results provide anatomical bases for functional connectivity contributing to the brain-wide processing of whisker-related sensorimotor information. In the near future, we also plan to perform similar preparation and analysis aimed to address projections originating from the supplemental somatosensory area.

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Poster

208. Somatosensory Responses in the Barrel Cortex of Rodents

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Program #/Poster #: 208.20

Topic: D.03. Somatosensation – Touch

Support: ERC 633428
DFG SFB 1089
DFG SPP 2041
BMBF Grant 01IS18052

Title: Top-down modulation of cortical output is driven by thalamus

Authors: *A. BAST[1], J. M. GUEST[1], C. P. DE KOCK[2], R. T. NARAYANAN[1], R. FRUENGEL[1], M. ROYO[1], M. OBERLAENDER[1];
[1]Max Planck Inst. for Neurobio. of Behavior, Bonn, Germany; [2]VU Amsterdam, Amsterdam, Netherlands

Abstract: Perception is causally linked to a calcium-dependent spiking mechanism that is built into the distal dendrites of layer 5 pyramidal tract neurons - the major output cell type of the cerebral cortex. It is yet unclear which circuits activate this cellular mechanism upon sensory stimulation. Here we found that the same thalamocortical axons that relay sensory signals to layer 4 also densely target the dendritic domain by which pyramidal tract neurons initiate calcium spikes. Distal dendritic inputs, which normally appear greatly attenuated at the cell body, thereby generate bursts of action potentials in cortical output during sensory processing. Our findings indicate that thalamus gates an active dendritic mechanism to facilitate the combination of sensory signals with top-down information streams into cortical output. Thus, in addition to being the central hub for sensory signals, thalamus is also likely to ensure that the signals it relays to cortex are perceived by the animal.

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Poster

209. Olfactory Central Mechanisms: Invertebrates

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 209.01

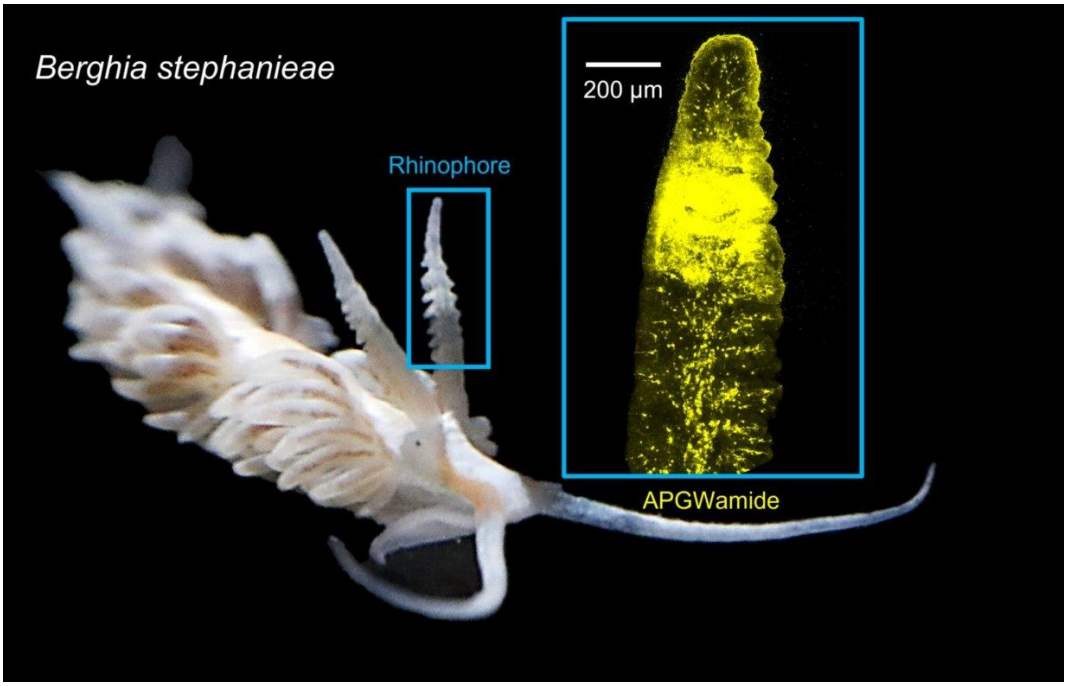
Topic: D.04. The Chemical Senses

Support: NIH U01-NS123972
NIH U01-NS108637

Title: Structure and organization of the olfactory system in the mollusc Berghia stephanieae

Authors: *C. C. TAIT, M. D. RAMIREZ, P. S. KATZ;
Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Olfactory systems in arthropods and vertebrates show remarkable neuroanatomical similarities, with primary sensory neurons projecting to specialized regions in the brain. It is not known if this similarity extends to molluscs. We used neurobiotin tracing, immunohistochemistry, and *in situ* hybridization chain reaction to determine the neuroanatomical organization of the olfactory system in the nudibranch mollusc, *Berghia stephanieae*. Like most gastropod molluscs, *Berghia* has posterior tentacles (termed rhinophores in nudibranchs, see Figure) that are specialized for distance chemoreception. Several populations of primary sensory neurons line the epithelium of the rhinophore, including histaminergic and peptidergic neurons (Figure inset). Some histaminergic primary sensory neurons in the rhinophore project centrally through two rhinophore nerves and terminate in the rhinophore ganglion (RhG), which sits at the base of the rhinophore. A population of primary sensory axons bypass the RhG and project through the rhinophore connective directly into the central ring ganglia (CRG), with many continuing through the commissure to the contralateral brain hemisphere and RhG. Other axons from the rhinophore nerves terminate in many neuropil regions throughout the CRG. In addition to sensory neurons, there are morphologically distinct peptidergic neurons found deeper within the rhinophore tissue, which may be interneurons. This suggests key roles for neuropeptides in gastropod olfaction. Finally, we found extensive efferent innervation of the rhinophores including the axons of identified peptidergic and serotonergic neurons in each ganglion of the CRG. Neurobiotin tracing also filled a photoreceptor in each eye. Thus, there is neurochemical complexity within the rhinophore and widespread multimodal connectivity between the rhinophore and the rest of the brain. The results from this project suggest that the organization of this molluscan olfactory system is substantially different from that seen in arthropods and vertebrates.



Disclosures: **C.C. Tait:** None. **M.D. Ramirez:** None. **P.S. Katz:** None.

Poster

209. Olfactory Central Mechanisms: Invertebrates

Location: SDCC Halls B-H

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Program #/Poster #: 209.02

Topic: D.04. The Chemical Senses

Support: NIH U01-NS123972
NIH U01-NS108637

Title: A connectomics approach to an enigmatic ganglion in a gastropod mollusc

Authors: *H. H. SANT[1], B. D. DRESCHER[1], Y. MEIROVITCH[2], R. SCHALEK[2], Y. WU[2], J. LICHTMAN[2], P. S. KATZ[1];
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Abstract: The central nervous system (CNS) of nudibranchs and other gastropod molluscs are well known for their large identifiable neurons. In addition, there are peripheral ganglia that contain much smaller neurons of unknown identity and function. Here, we took a connectomics approach to determine the structural organization of neurons in the rhinophore ganglion (RhG), which sits at the base of the rhinophore, the chemosensory appendage, in the nudibranch, Berghia stephanieae. Based on its position, the RhG should be analogous to the antennal lobe of insects or the olfactory bulb of vertebrates. However, the neuronal architecture and connectivity of the RhG is unknown. To address this, we serially sectioned an entire RhG at 33 nm thickness and completed imaging each of the 2175 sections at 4x4 nm lateral resolution. We determined that the RhG contains around 9000 neuronal somata, which is almost twice as many neurons as the rest of the brain. The somata ranged from 5 to 15 µm in diameter. The RhG had a variety of regions including distinctive clusters of somata, neuropil, and axon tracts. The axon tracts were separated by glial cells, which were often associated with exosomes. Some axons traversed the ganglion without synapsing. Neurons had unique ultrastructural features including large membranous particles, different staining densities, vesicles of varying sizes, and vesicle-free neurites. We found some neuronal somata that projected an axon into the nerve connective to the cerebral ganglion. This connective contained about 30,000 axons, some as small as 90 nm in diameter. We are applying machine learning algorithms to automatically segment all the cells and neuronal processes intrinsic to and coursing through the RhG to provide a first draft of the connectome. A complete connectome of this ganglion will provide insights into the structural organization of this enigmatic ganglion, which is likely to be involved in higher order olfactory processing in this mollusc.

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Poster

209. Olfactory Central Mechanisms: Invertebrates

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 209.03

Topic: D.04. The Chemical Senses

Title: Parallel neural pathways in the Drosophila olfactory system: subtle structural alignment

Authors: *H. GIAFFAR[1], C. E. RULLÁN[2], M. AOI[1];
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Abstract: Despite many differences, there is a preserved logic to odor processing across the tree of life. One of the preserved features shared by the olfactory systems of diverse organisms is the presence of two (parallel) output channels from the primary olfactory center. In the fly, the lateral horn (LH) pathway has been shown to associate the presence of key ethologically relevant odorants with appropriate innate behavioural programs. The mushroom body (MB) pathway is thought to underlie flexible learning in dynamic and novel chemical environments. In the case of the LH, this is thought to be facilitated by genetically encoded synaptic structure, while in the MB via a set of random synapses. To compare the synaptic structure in these two pathways, we extract two connectivity matrices from the FlyEM Hemibrain connectome dataset: L (51 glomeruli * 1428 principle cells) and M (51 glomeruli * 1761 principle cells). We detect structure in both pathways (significantly more in L than M) by comparing summary statistics between each connectivity matrix and a family of null models in which any structure is removed by various shuffling techniques. To assess the relationship between the structural components in L and M, we compute a matrix inner product (similarity) and compare this to null models, finding a significant and consistent alignment between L and M. To locate this common structure between L and M, we analyse the sensitivity of this similarity score to shuffling the connections made from each glomerulus type (row) independently. This simple sieving procedure yields a subset of sensitive glomeruli, which we relate to both glomerular valance properties and the phenomenon of ephaptic coupling of olfactory channels at the periphery. A comparison of glomerular spaces derived from L and M reveals significant (anti)alignment of the first two principle components, suggesting a subspace in which neural activity in the MB and LH is similarly organized. We explore this idea by modeling odorant representations in the L and M subspace via the incorporation of odorant response data K (37 glomeruli * 84 odorants). We also compute shared information between connectivity (L and M) and neural activity data, K. Overall, this work combines connectivity and response datasets to explore synaptic structure and odorant representations in the parallel pathways. We find limited but consistent alignment between the pathways at the level of synapses and describe a shared representational subspace.

Disclosures: H. Giaffar: None. **C.E. Rullán:** None. **M. Aoi:** None.

Poster

209. Olfactory Central Mechanisms: Invertebrates

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 209.04

Topic: D.04. The Chemical Senses

Support: UF1 NS108213
U01NS094296
Simons Collaboration on the Global Brain Research Awards

Title: Brain-wide circuit representations of sensory and behavioral responses to olfactory stimuli in adult Drosophila

Authors: *W. LI, E. OZEN, E. S. SCHAFFER, R. W. YAN, C. PEREZ CAMPOS, E. M. C. HILLMAN;
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Abstract: Unprecedented anatomical and connectomic studies over the past decade have revealed extensive connectivity between cells across the entire brain, from flies to mice. Frequent synchronies and functional dynamics between multiple brain areas and neural networks have been observed during a wide range of behaviors, suggesting the importance of studying brain activity from a holistic perspective. Recent advances in both fluorescent indicators or neural activity such as GCaMP, and high-speed 3D imaging technologies, have facilitated the recording of brain-wide activity in small organisms such as *Drosophila*, however, many questions still remain. One major challenge is the difficulty of resolving densely packed neuropil and associated neural circuits from cytosolic GCaMP data, which provide extra information of functional connectivity and cell identity compared to nuclear-localized GCaMP recordings. In our study, we focus on pan-neuronal cytosolic GCaMP activity across the whole brain of awake behaving *Drosophila* receiving olfactory stimuli, while simultaneously monitoring their real-time behavior including locomotion. Combining high-speed 3D SCAPE microscopy with novel spatiotemporal unmixing methods, we are able to resolve and extract functional neuronal circuits from near whole brain in real time. We have been able to identify a series of olfactory sensory pathways that differ in temporal coding patterns and odor selectivity. We have also found circuits associated with higher-level brain-wide modulation and locomotion. This functional circuit-based representation has allowed us to observe the information flow of olfactory stimuli across the brain, and explore how real-time sensory-motor integration changes on a trial-to-trial basis. This work is contributing to a better understanding of the relationship between structural and functional connectomes, while enabling new studies of real-time multisensory-motor integration on a brain-wide scale.

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Poster

209. Olfactory Central Mechanisms: Invertebrates

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 209.05

Topic: D.04. The Chemical Senses

Support: NIH Grant 5R01NS107970-04

Title: Investigating the functional consequences of biased connectivity to an associative learning brain center

Authors: *A. MACKENZIE[1], A. BUTTS[2], T. HAYASHI[1], Y. ASO[3], S. J. C. CARON[2,1]; [1]Neurosci., [2]SBS, Univ. of Utah, Salt Lake City, UT; [3]Aso Lab., Janelia Res. Campus, Ashburn, VA

Abstract: The mechanisms by which brains process sensory information for associative learning remains poorly understood. The mushroom body of D. melanogaster is necessary for such learning and is composed of Kenyon cells that are innervated primarily by olfactory projection neurons. Each projection neuron receives input from one of the 51 glomeruli of the antennal lobe. We've previously reported that projection neurons connect to their downstream Kenyon cell partners at varied rates depending on the glomerulus they are connected to. It is not known how this "biased connectivity" affects the transformation of olfactory information from the antennal lobe to the mushroom body but understanding how these layers communicate is critical to uncovering the fundamental ways in which associative learning brain centers function. To this end, we've employed a combination of optogenetic techniques alongside in vivo calcium imaging to stimulate individual glomeruli and observe the Kenyon cell activity elicited. Five different glomeruli were stimulated and the percentage of Kenyon cells each glomerulus activated (VL1 = 0.8% ± 0.22, DL3 = 2.6% ± 0.35, VM4 = 4.9% ±2.8, DA1 = 7.2% ± 1.32, DC3 = 7.6% ± 1.31 SD, n=3, females 2 days post-eclosion) correlates strongly with their connectivity rates to the mushroom body ($r = .98$, $p < .0025$). This demonstrates that while glomeruli are unique in their connectivity rates, the method by which they activate their Kenyon cell partners is uniform. We next examined whether this variability in individual glomerular representation in the mushroom body affects the strength of associative learning these glomeruli confer. Using a T-maze assay, ~100 flies/trial were aversively space-trained on geosmin, an odor that activates an underrepresented glomerulus, or farnesol, an odor that activates an overrepresented glomerulus. A performance index (PI) score was calculated for each odor tested, where a value of -1 = complete aversion and a value of 1 = complete attraction. Flies did not form negative associations with either geosmin or farnesol (PI = .002 ± .016, .014 ± .049 SEM respectively, n = 3), suggesting that the stimulation of a single glomerulus is not sufficient to drive associative learning irrespective of its connectivity rate. Thus, the question remains as to the purpose of biased connectivity; it is possible that biases become relevant in more complex odor spaces as a gain mechanism, for example. Further interrogation of this tractable, numerically simple brain center will permit us to explore this possibility, among others, and should reveal fundamental ways in which brains process sensory information for efficient and accurate associative learning.

Disclosures: A. MacKenzie: None. **A. Butts:** None. **T. Hayashi:** None. **Y. Aso:** None. **S.J.C. Caron:** None.

Poster

209. Olfactory Central Mechanisms: Invertebrates

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Program #/Poster #: 209.06

Topic: D.04. The Chemical Senses

Support: NIH Grant DC-016293

Title: Stimulus specific modulation by serotonin in the olfactory system of Drosophila

Authors: J. JONAITIS[1], M. F. MAZRI[1], T. R. SIZEMORE[1,3], K. L. RAMACHANDRA[1], *A. M. DACKS[1,2];
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Abstract: Olfactory processing is vital for animals to locate food, communicate with conspecifics and avoid environmental hazards. To maintain flexibility in the face of a shifting physiological state, the olfactory system relies on neuromodulation, whereby metabotropic receptors expressed by different neuronal classes allow the fine-tuning of individual aspects of sensory processing. However, there are instances in which a single neuron class has heterogeneous expression of modulatory receptors for a single signaling molecule and the functional significance of this is not fully understood. For instance, in the olfactory system of Drosophila, ventral projection neurons (vPNs) that extend from the antennal lobe (AL) to the lateral horn (LH) express all five insect serotonin (5-HT) receptors. Here we explore the functional significance of a single neuronal population expressing a complex pattern of neuromodulatory receptors. We first found that vPNs can express several different 5-HT receptor combinations and that vPNs expressing a given 5-HT receptor innervate different patterns of glomeruli in the AL. We then identified two driver lines expressed by vPNs that express distinct patterns of serotonin receptors; 5-HT1A/1B vs. 5-HT7. These sets of vPNs innervate different glomeruli and LH regions, consistent with a stimulus-specific functional organization of 5-HT receptor expression. Using two-photon imaging of odor-evoked responses, we determined that the two sets of vPNs have different odor-tuning and are differently modulated by serotonin pharmacology. Finally, using connectomics we demonstrate that the two sets of vPNs target separate populations of third-order neurons within the LH. Our work suggests that the heterogeneous expression of 5-HT receptors by vPNs reflects a stimulus-specific form of modulation by 5-HT in the olfactory system of the fly.

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Poster

209. Olfactory Central Mechanisms: Invertebrates

Location: SDCC Halls B-H

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Program #/Poster #: 209.07

Topic: D.04. The Chemical Senses

Support: NIH 1R01DC020892

Title: Learning odor environment by the inhibitory network of the honey bee antennal lobe

Authors: *S. JOSHI[1], S. HANEY[2], F. LOCATELLI[3], B. H. SMITH[4], M. V. BAZHENOV[2]; [1]ECE, [2]Dept. of Med., Univ. of California San Diego, La Jolla, CA; [3]Univ. of Buenos Aires, Buenos Aires, Argentina; [4]Schl Life Sci., Arizona State Univ., Tempe, AZ

Abstract: A honeybee in search of food identifies nectar producing flowers using their aromas composed of many volatile chemicals. However, the nectar producing floral aromas may share chemicals with the non-producing ones. Hence, the honeybee faces a challenging task in determining the map between chemical sensing and reward prediction. In this study, we examine the mechanism of modification of neural representations of odors due to learning in the early olfactory system - antennal lobe (AL) - using a combination of computational modeling and Calcium imaging of the honeybee AL in vivo. Based on previous work (Sinakevitch et al., 2013) that showed octopamine receptors (modulating reward) co-localized with GABA receptors, we modeled plasticity in the inhibitory AL network. Rewarded odors caused GABA facilitation based on presynaptic firing rates, and non-rewarded (habituated) odors caused GABA facilitation based on post-synaptic firing (Chen et al., 2015). We found that this inhibitory plasticity was sufficient to create many of the changes seen in vivo. This includes the shifting of odor mixtures due to reward, the adaptation to habituated odor presentations, and changes in the neural representations of complex blends when moving from one environment to the other. The model predicted that the cells representing chemical compounds common to both rewarded and habituated odors face increased inhibition from both associative and non-associative plasticity. This prediction was verified in vivo by examining Calcium imaging data where the glomeruli that were common to many odor blends were suppressed by training and those that were unique to the rewarded odor blend were enhanced. We also tested the effects of changing the environment on the inhibitory network structure and found that learning in the AL shapes the coding space to maximize the discriminability between the odors present in a particular environment. Our model demonstrates a learning paradigm where the inhibitory network reshapes coding space to suit the current task and environment. These findings suggest an efficient computational strategy for perceptual learning in complex natural odors through modification of the inhibitory network.

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Poster

209. Olfactory Central Mechanisms: Invertebrates

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Program #/Poster #: 209.08

Topic: D.04. The Chemical Senses

Support: MSU Startup Grant to Dr. Saha
MSU DFI Funding to Dr. Saha

Title: Harnessing the honeybee olfactory sensory system for the detection of cancer

Authors: *S. W. SANCHEZ[1], E. COX[2], M. PARNAS[1], N. LEFEVRE[2], S. MILLER[2], A. FARNUM[1], D. SAHA[1];
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Abstract: We explored a novel cancer detection technology using the honeybee olfactory sensory system for the detection of putative lung cancer biomarkers present in exhaled human breath. The human body emits thousands of different volatile organic compounds (VOCs) via the breath, and the identity and concentrations of these VOCs reflect the underlying metabolic processes and, in turn, the health of an individual. As a result, the analysis of cancer-induced changes in breath volatiles offers the potential for a point-of-care breath-based diagnostic tool that is noninvasive and patient friendly. The component-wise breath analysis performed by gas chromatography-mass spectrometry (GC-MS) is very different from the biological approach of gas mixture classification. The performance of GC-MS is hindered by the low concentrations of chemicals present in human breath (ppb to ppt ranges), and due to the presence of other background odorants. Hence, there is a critical need for new technological advancements that effectively harness the power of biological olfaction, which is superior compared to the existing engineered VOC sensors, for developing next generation, one-shot, robust, and real-time VOC sensors for cancer diagnostics. Current clinical methods for the diagnosis and staging of lung cancer include blood tests, chest X-rays, computed tomography, and tissue analysis. However, lung cancer is the leading cause of cancer-related deaths worldwide and prognoses still remain dismal with a 5-year survival rate of 14%. Identifying breath-based biomarkers for lung cancer patients offers a promising direction for diagnosing cancer early and noninvasively, and can be used for faster implementation of targeted therapy. Biological olfactory systems, such as honeybees, can detect and classify complex odorant mixtures with great efficiency. We obtained putative lung cancer VOC-evoked antennal lobe neuronal responses via extracellular neural recordings from an *in vivo* antennae-attached honeybee brain. Our results show that several cancer biomarkers can be distinguished using the antennal lobe spiking activities. Next, to determine the detection limit and clinical application of this approach, we created simulated healthy and lung cancer breath mixtures that mimic the biological concentrations of six VOCs observed in the exhaled breath of healthy and lung cancer patients. The concentrations of the VOCs in the simulated breath mixtures were in the ppb to ppt ranges. Our preliminary results indicate that the spiking activity of honeybee antennal lobe neurons can also differentiate between these simulated breath samples (lung cancer vs. healthy), successfully.

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Poster

209. Olfactory Central Mechanisms: Invertebrates

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Topic: D.04. The Chemical Senses

Support: MSU Startup Grant to Dr. Saha
MSU DFI Funding to Dr. Saha

Title: Harnessing insect olfactory neural circuits for noninvasive detection of human cancer

Authors: *M. PARNAS[1], A. FARNUM[1], E. H. APU[1], E. COX[2], N. LEFEVRE[2], C. H. CONTAG[1], D. SAHA[1];
[1]Biomed. Engin., [2]Michigan State Univ., East Lansing, MI

Abstract: There is overwhelming evidence that metabolic processes are altered in cancer cells and these changes are manifested in the volatile organic compound (VOC) composition of exhaled breath. Numerous studies using gas chromatography - mass spectrometry (GC-MS) have indicated that specific VOCs are up- or down-regulated due to cancer. However, due to the component-wise identification of compounds used by GC-MS, it has difficulty classifying the entire complex mixture of thousands of VOCs in human breath. Biological VOC sensing systems (i.e., olfactory sensory systems) have evolved to classify complex mixtures found throughout various natural environments. Therefore, here, we used an intact insect (locust) brain to harness the olfactory neural circuit as a VOC sensor for cancer detection. The insect antennae, analogous to vertebrate noses, contain sensory neurons that interact with VOCs. Signals from these sensory neurons travel to the antennal lobe in the insect brain, within which a biological combinatorial coding scheme is used to detect upwards of trillions of odor mixtures. To achieve our goals, we combined *in vivo* extracellular recordings from the antennal lobe with an electrophysiology platform and employed biological neural computation schemes of antennal lobe circuitry for data analysis. Our results demonstrate that cell culture VOC mixtures from three different human oral cancer cell lines, Ca9-22, HSC-3, and SAS, can be robustly distinguished from each other and from a non-cancer cell line, HaCaT, by analyzing the neural responses in the insect antennal lobe during odor presentation to the antenna. Individual putative neurons are combined to produce a population neural response that captures the spatio- (each neuron) temporal (firing patterns) information encoded within the brain. By evaluating cancer vs. non-cancer VOC-evoked population neural responses, we show that olfactory neuron response-based classification of oral cancer is sensitive and reliable. We use a simple linear classifier to achieve 100% accurate classification of each cell line. Not only does this allow for discrimination between cancer and non-cancer, but also differentiation of cancer subtypes. Moreover, our approach is very fast (detection time of 250 ms). Our results also demonstrate that this cancer detection technique is effective across changing chemical environments, reflective of variations in sampling environments and variations between individuals. Our insect brain-based cancer detection system uses a novel VOC sensing method that will spur the development of more forward engineering technologies for the noninvasive detection of cancer.

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Poster

209. Olfactory Central Mechanisms: Invertebrates

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Topic: D.04. The Chemical Senses

Support: DBT–Wellcome India Alliance Intermediate fellowship IA/I/11/2500290
KVPY
CSIR

Title: Invariant neural representations of fluctuating odor inputs

Authors: R. MOHANTA, S. ADITHYAN, ***C. ASSISI**;
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Abstract: Steady odor streams are typically encoded as robust spatiotemporal spike trains by olfactory networks. This suggests a one-to-one mapping between the stimulus (an odor mixed in a steady stream of air) and its representation (a spatiotemporal pattern of spikes in a population of neurons) in the brain. Such a one-to-one mapping between an odor and a spatiotemporal pattern is unlikely to be accurate since natural odor stimuli change unpredictably over time. Odors arrive riding upon chaotically pulsed plumes of air and show unpredictable variation in concentration and in the composition of odorant molecules. These temporal changes often vary over time scales that are similar to the time scales of neural events thought to play a role odor recognition. In the absence of such temporal variations, animals are known to inject intermittency while sampling the odor suggesting that intermittent inputs might be a ‘feature’ not a ‘bug’. Here, we attempt to find the neural invariants of stable olfactory percepts using a computational model of the locust antennal lobe, the insect equivalent of the olfactory bulb in mammals. We show that when time-varying odor inputs intermittently perturb subsets of neurons in the antennal lobe network, the activity of the network reverberates in a manner that depends on both the nature of the inputs it receives and the structure of the neuronal sub-network that these inputs stimulate. We demonstrate that it is possible to decipher the structure of the perturbed sub-network by examining transient synchrony in the activity of the neurons. The ability to reconstruct the sub-network structure is vastly improved when odor inputs arrive or are sampled in an intermittent manner. Thus, the structure of the stimulated sub-network itself serves as a unique invariant code that represents the odor. Recent studies have shown that the response of individual projection neurons in the antennal lobe, to a particular odor, can be approximated using an odor-specific response kernel convolved with the temporal profile of the odor input. The parameters defining this kernel remain invariant to temporal changes in the profile of the input. Our simulations show that this invariance is inherited from the network structure.

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Poster

209. Olfactory Central Mechanisms: Invertebrates

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Program #/Poster #: 209.11

Topic: D.04. The Chemical Senses

Support: DFG Grant EXC 2117 – 422037984
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Title: Social flexibility and olfactory processing in the desert locust.

Authors: I. PETELSKI, Y. GÜNZEL, S. SAYIN, ***E. COUZIN-FUCHS**;
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Abstract: Flexibility in social foraging behavior allows animals to maximize foraging success in nutritionally unpredictable environments. The desert locust *Schistocerca gregaria* exhibits one of the most extreme examples of this flexibility. Usually, solitarious locusts populate sparse landscapes at low densities and forage alone. However, under suitable conditions, mediated by an increase in density of surrounding conspecifics, locusts gradually convert into a gregarious phase. The transition to group foraging entails considerable changes in the type, quality, and quantity of sensory information available to individual animals. In addition to personally acquired evidence, gregarious locusts have access to a plethora of social information, allowing them to integrate socially derived cues on the location and quality of a food source. How is this transition mediated in terms of sensory processing? What role do social cues, such as the smell of conspecifics, play in foraging decisions? How is that modulated with change in conspecifics density? We addressed these questions by investigating the early olfactory processing of food odor cues in the presence and absence of the colony smell. We do so by widefield calcium imaging of antennal lobe projection neurons in gregarious and solitarious locusts. We demonstrate that a simulated olfactory group context increases the overall magnitude of projection neuron activity to food odorants in gregarious animals. Yet, this social modulation is phase-dependent and does not occur in solitarious animals, suggesting it to be a potential adaptation of the olfactory system to facilitate or promote foraging in a group.

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Poster

210. Organization of Retinal Circuitry: From Photoreceptors to Amacrine Cells

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 210.01

Topic: D.06. Vision

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Indiana Wesleyan University University Scholar Award

Title: ATP-mediated H^+ efflux from isolated axolotl Müller cells: contributions from sodium-hydrogen exchange and monocarboxylate transport.

Authors: *M. KREITZER[1], M. VREDEVELD[1], K. TINNER[1], B. K. TCHERNOOKOVA[2], R. P. MALCHOW[2];
[1]Indiana Wesleyan Univ., Marion, IN; [2]Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL

Abstract: Glial cells in the central nervous system are very responsive to purinergic signaling. Sources of ATP-initiated signaling cascades have been suggested to be both neuronal and autocrine in nature, and commonly initiate changes in glial cell intracellular calcium levels. We have previously reported that low micromolar levels of extracellular ATP induce a significant increase in H+ efflux from Müller cells isolated from the retinae of tiger salamanders and other vertebrates, including human. Neurotransmitter release across synaptic layers of the retina has a high sensitivity to alterations in extracellular H+ levels and has been shown to alter neuronal activity. In the present study recordings of extracellular H+ fluxes were obtained from isolated Müller glia of axolotls (ambystoma mexicanum) using self-referencing H+-selective microelectrodes. Recordings were performed with 1mM HEPES as the extracellular pH buffer with no bicarbonate added to the Ringer's solution. We find that stimulation of isolated Müller glia from axolotls with extracellular ATP also leads to an extracellular acidification mirroring studies using tiger salamanders (Tchernookova et al., 2018). H+ efflux from axolotl Müller cells is mediated by activation of purinergic receptors and is dependent on calcium release from internal stores. ATP-induced increases in H+ efflux were attenuated by 2 mM amiloride and were also reduced when extracellular sodium was replaced in the Ringer's solution with choline. These observations point towards NHE activity in mediating part of the ATP-induced increase in H+ efflux. We also found that the ATP-initiated efflux of H+ could be reduced by 1 mM 4-cin, an agent known to inhibit monocarboxylate transporters. Addition of 4-cin in a bath lacking extracellular sodium resulted in an additional reduction in the ATP-mediated increase in H+ efflux. These observations suggest roles for both NHE and MCT transport in mediating the H+-efflux from Müller cells induced by extracellular ATP. Given the very high sensitivity of synaptic transmission to small changes in extracellular H+, we hypothesize that ATP-mediated extrusion of H+ from Müller cells (and glia more broadly) could play an important role in regulating signaling at synapses within the retina and likely throughout the nervous system.

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Poster

210. Organization of Retinal Circuitry: From Photoreceptors to Amacrine Cells

Location: SDCC Halls B-H

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Program #/Poster #: 210.02

Title: WITHDRAWN

Poster

210. Organization of Retinal Circuitry: From Photoreceptors to Amacrine Cells

Location: SDCC Halls B-H

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Research to Prevent Blindness, Inc (UCLA Department of Ophthalmology)

Title: Activity of ρ-subunit containing GABA receptors in horizontal cells is regulated by light and dopamine via intracellular Ca^{2+}

Authors: *S. BARNES[1,2,3], C. F. MCHUGH[1], J. DE LOS SANTOS[3], A. A. HIRANO[3], N. C. BRECHA[3,2,4,5], B. J. SMITH[3];
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Abstract: Dopaminergic actions mediated by horizontal cell (HC) dopamine D1 receptors, activated during light exposure, increases the strength of inhibitory feedback to photoreceptors. Additional actions of light that regulate ρ-subunit containing GABA receptors in HCs are investigated here. The effects of these actions, wherein GABA acts autaptically on HC GABARs to modulate photoreceptor Ca channels, mediate changes in feedback signaling during light- and dark-adaptation. Immunolabelling of GABAR ρ2 subunits at HC synapses was confirmed using super-resolution confocal imaging. Light and dopaminergic induction of GABAR subunit expression at HC synapses was tested in dark-adapted mice exposed for 15 or 60 min to light, light levels of 10, 10^3 and 10^4 lux, and dopamine alone or with SCH23390, a D1R antagonist. Brighter light, longer exposure times and D1R activation showed higher levels of ρ2 immunoreactivity. Patch clamp recording in tangential retinal slices showed that TPMPA-sensitive GABAρR mediated currents are larger in light-adapted retinas than dark-adapted retinas. Testing with focal puffs of GABA via micropipet showed that in light-adapted

conditions, SR96631 (non-GABAρR blocker) blocked ~66% of GABA-activated currents while TPMPA blocked ~33%. Under dark-adapted conditions, TPMPA produced no significant reduction. HCs respond to their own tonic release of GABA, so we assessed these currents using TPMPA, finding no effect on tonic GABA currents in dark-adapted HCs while there was significant reduction in light-adapted HCs. In dark-adapted retina, dopamine levels are low, and we found the TPMPA-sensitive currents in HCs were greatly increased by the D1R agonist, SKF38393, with TPMPA blocking ~33% of the GABA-activated current, similar to light-adapted retinas. HC membrane potential was tested for its effect on GABAρRs with CNQX, which hyperpolarizes HCs, and it increased current blocked by TPMPA to ~25%. A link between higher [Ca^{2+}] reducing GABAR activity was tested with cyclosporin A, which blocks calcineurin, itself activated by Ca^{2+} to reduce GABAR expression elsewhere. In dark-adapted retinas superfused with cyclosporin, TPMPA reduced GABAR currents by ~30%, just shy of the reduction seen in light-adapted conditions. These results suggest that feedback inhibition is turned down under dark-adapted conditions due largely to elevated [Ca^{2+}] caused by HC depolarization by high glutamate release from photoreceptors. Depolarization under darkness normally produces higher [Ca^{2+}] via Ca_v channel activation, which has also been shown to be suppressed by D1Rs in HCs, likely contributes to the reduction of [Ca^{2+}].

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Poster

210. Organization of Retinal Circuitry: From Photoreceptors to Amacrine Cells

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 210.04

Topic: D.06. Vision

Support: NIH grant R01-EY029777

Title: Gaba mediated lateral excitatory signaling between the dendrites and spinules of horizontal cells in the retina is modulated by light/dark adaptation

Authors: *M. GOEL, H. J. CHOI, S. C. MANGEL;
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Abstract: Background- In goldfish and other vertebrate retinas, cone photoreceptors use glutamate to signal cone bipolar cells and cone horizontal cells (cHCs) at triad synapses. Evidence suggests that cHCs use GABA to signal cone bipolar cells following maintained (>30 min) bright illumination (light adaptation) (Chaffiol et al., 2017), but whether and how cHCs signal cones and/or other cHCs following light adaptation is unresolved. cHC dendrites in fish retina form "finger-like" evaginations into cone membranes following light adaptation but are absent following maintained darkness (dark adaptation). It has been suggested that these small spine-like structures or spinules are involved in feedback from cHCs to cones, but the evidence

remains unclear. Methods- To understand the physiological role of spinules in cHC signaling, we performed immunolabeling for GABAa receptor (GABAaR) expression in light- and dark-adapted goldfish retinas during the day and obtained high magnification images of cone pedicles with transmission electron microscopy. Because the chloride cotransporter, NKCC, which mediates GABA excitation, has been observed on cHC dendrites, we also studied the effects of gabazine (50 uM), a selective GABAaR antagonist, and bumetanide (10 uM), a selective inhibitor of NKCC, on cones and cHCs following light- and dark-adaptation during the day. Results- cHC spinules were observed significantly more often in light-adapted compared to dark-adapted retinas. Intense GABAaR expression was observed on cHC spinules following light adaptation and significantly more often on cHC dendrites at the cone triad synapses following light adaptation compared to dark adaptation. In contrast, cone plasma membranes at cone triad synapses exhibited minimal GABAaR immunolabeling in the day following light or dark adaptation. Gabazine and bumetanide hyperpolarized cHCs by ~10 mV following light adaptation but had little effect when applied following maintained darkness. Gabazine had minimal effect on cones following light or dark adaptation during the day. Conclusions- These results suggest that GABAaRs on cHC spinules and dendrites following light adaptation in the day mediate excitatory signaling between cHCs. The finding that GABAaRs are not expressed on the plasma membrane of cones at the cone triad synapses suggests that GABA-mediated feedback from cHCs to cones is minimal during the day following both light and dark adaptation. The appearance of cHC spinules following light adaptation therefore may increase the lateral extent of GABA-mediated cHC to cHC excitatory signaling.

Disclosures: **M. Goel:** None. **H.J. Choi:** None. **S.C. Mangel:** None.

Poster

210. Organization of Retinal Circuitry: From Photoreceptors to Amacrine Cells

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Program #/Poster #: 210.05

Topic: D.06. Vision

Support: 5RO1DC012938

Title: Modulation of a retinal AII amacrine cell crossover inhibitory circuit by dopamine

Authors: *P. STRAZZA JUNIOR, M. MEADOWS, H. VON GERSDORFF;
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Abstract: The AII amacrine cells (AII-ACs) play a central role in retinal crossover inhibition where they convert excitatory light responses from Rod bipolar and ON-Cone bipolar cells into glycinergic inhibition of OFF-Cone bipolar cells (OFF-CBCs). Recently our group has shown that glycine release by AII-ACs is potentiated by cAMP through a signaling pathway relying on Ca2+ release from internal Ca2+ stores, but the mechanism initiating this potentiation remains elusive. We've started to address this question by investigating how one candidate

neuromodulator, dopamine, affects the glycinergic neurotransmission between AII-ACs and OFF-CBCs. The cAMP mediated D1-receptor signaling pathway can change gap junction (GJ) coupling, but we hypothesize that the potentiating effects on glycine release are independent of GJ modulation. We tested this by applying 10 µM dopamine and 10 µM of the D1-receptor antagonist SCH23390 to mouse retinal slices. During drug washes we performed whole-cell patch clamp experiments to measure presynaptic AII-AC spiking (current-clamp) and postsynaptic OFF-CBC glycinergic sIPSCs (voltage-clamp). We observed spiking in AII-ACs with a current injection, holding the cell from -65 to -50 mV. Dopamine caused a brief (< 3 min) hyperpolarization of the AII-AC membrane potential, which abolished the spiking in most cells tested. Comparing the long term dopamine effects at the same membrane potential in each condition, we found that dopamine decreased the frequency of single and spikelet bursting. On the other hand, SCH23390 permanently depolarized the AII-ACs, but surprisingly, it also decreased the frequency of spikelets when we compared the spiking at about the same membrane potential in each condition. Furthermore, dopamine and SCH23390 effects were delayed and partialy blocked when we used a high EGTA internal solution, suggesting that the underlying mechanisms may be dependent on changes of internal Ca2+. In our voltage clamp experiments, we found that dopamine decreases the frequency of the glycinergic sIPSCs, while SCH23390 had the opposite effect. To better control for effects of light signals transmitted through GJs between AII-ACs and ON-BCs, all experiments were repeated using AMES containing 10 µM L-AP4 (mGluR6 agonist), which chemically mimics scotopic conditions. The results were qualitatively the same. Taken together the results suggest that dopamine modulates the AII-ACs crossover inhibition circuit by activating voltage and/or Ca++ dependent conductances on AII-ACs. These findings can contribute to a better understanding of how the retina adapts crossover inhibition circuits during changes in environmental luminance.

Disclosures: **P. Strazza Junior:** None. **M. Meadows:** None. **H. von Gersdorff:** None.

Poster

210. Organization of Retinal Circuitry: From Photoreceptors to Amacrine Cells

Location: SDCC Halls B-H

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Program #/Poster #: 210.06

Topic: D.06. Vision

Title: Effect of gap junctions on the firing activities of retinal AII amacrine cells

Authors: *F. TAMALU, N. MIWA;
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Abstract: Retinal AII amacrine cells generate TTX-sensitive repetitive spikes that are dependent on glutamatergic synaptic input and are reciprocally connected via gap junctions. To examine physiological roles of the gap junctions, we performed whole-cell patch clamping of AII amacrine cells in the mouse retinal slice. In current-clamp mode, we observed large fluctuations of membrane potential in AII amacrine cells. Adding gap junction blockers to the external

solution diminished the voltage fluctuations, suggesting that the fluctuations are originated from the activities of neighboring AII amacrine cells. In addition, under these conditions, AII amacrine cells fired spikes at the highest frequency even when a small amount of current injection (about 10 pA) was applied at a membrane potential of about -65 mV. This is because the gap junction blockers made the input resistance higher by closing the shunt via gap junctions. Significant differences were found in the input resistance of recorded cells (740 ± 56 MΩ, control, n = 36 vs 1253 ± 222 MΩ, gap junction blocker, n = 20). Our data suggested that gap junctional connections of AII amacrine cells might have a crucial role to respond wide range of input, probably by controlling shunt current.

Disclosures: F. Tamalu: None. **N. Miwa:** None.

Poster

210. Organization of Retinal Circuitry: From Photoreceptors to Amacrine Cells

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Topic: D.06. Vision

Support: R01 EY032057

Title: Using genetic tools to characterize a single retinal amacrine cell subtype

Authors: *A. ISAKHAROV[1], K. M. WRIGHT[2];
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Abstract: The retina is a structured and accessible extension of the central nervous system that provides an ideal landscape to study neuronal fate specification and circuitry. The diversity within its five major neuronal classes includes at least 120 subtypes, making the retina an ideal model to understand how distinct neuronal subtypes are defined and work toward the larger goal of visual processing. Of these subtypes, around half are Amacrine Cells (ACs), the inhibitory neurons of the inner retina, which tune visual inputs from bipolar cells onto retinal ganglion cells. The function of many neurons in the retina are poorly defined due to a lack of tools to identify and manipulate individual subtypes. Here, we describe a genetic approach that allows us to characterize a novel subtype of amacrine cell in the mouse retina. These cells appear to comprise a single AC subtype based on their morphological properties, including dendritic arbor size, branching patterns, and stratification within the inner plexiform layer. Ongoing work will define the intrinsic physiological properties of these neurons, and test the hypothesis that this AC population provides input to the direction selective circuit in the retina. This project will give us both a broader understanding of neuronal identity and development, and a more thorough model of a critical circuit for visual processing.

Disclosures: A. Isakharov: None. **K.M. Wright:** None.

Poster

210. Organization of Retinal Circuitry: From Photoreceptors to Amacrine Cells

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Support: MEXT KAKENHI 20K09836
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MEXT KAKENHI 19K09939
MEXT KAKENHI 22K09823

Title: ON and OFF starburst amacrine cells are differentially regulated through distinct acetylcholine receptors.

Authors: *M. GANGI[1], T. MARUYAMA[2,1], T. ISHII[1], M. KANEDA[1];
[1]Physiol., Nippon Med. Sch., Tokyo, Japan; [2]Physiol., Tokyo Women's Med. Univ., Tokyo, Japan

Abstract: In the retina, visual information is processed in parallel in ON and OFF pathways. Starburst amacrine cells (SACs) are interneurons that release acetylcholine (ACh) and GABA as transmitters. They comprise two spatially segregated populations that form circuits in the ON or OFF sublamina in the inner plexiform layer. Because the morphological properties of ON and OFF SACs are mirror-symmetrical, ON and OFF SACs have been assumed to function as a mirror-symmetrical manner. However, in a series of experiments, we have shown that SACs exert asymmetric signal processing between the ON and OFF pathways. The P2X2-purinergic receptors selectively excite OFF SACs, while the inhibitory actions of glycine receptors mainly work in ON SACs. In addition, we have shown the possibility that choline, a precursor of ACh, is transported through P2X2-purinergic receptors and used for ACh synthesis in OFF SACs. In the present study, therefore, we examined whether such differences of choline transport between ON and OFF SACs can produce further difference in cholinergic signaling pathways. We used IG-8 line transgenic mice, which express GFP signals in ON and OFF SACs, and monitored total charge transfer (TCT) of postsynaptic currents (PSCs) from SACs using patch-clamp recordings. When we bath-applied ACh, TCT of PSCs increased in both ON and OFF SACs. The increase of PSCs by ACh was completely inhibited by SR95531, an antagonist of $GABA_A$ receptors, indicating that both ON and OFF SACs receive GABAergic inhibitory feedbacks driven by ACh from neighboring neurons. An application of tetrodotoxin (TTX), a voltage-gated Na^+ channel blocker, did not block ACh-induced GABAergic feedback in both ON and OFF SACs, suggesting that presynaptic neurons are non-spiking amacrine cells. Additionally, an application of TTX decreased spontaneous PSCs in OFF SACs but not in ON SACs. Oxotremorine, a muscarinic ACh receptor agonist, increased TCT of PSCs in ON SACs but not in OFF SACs, while nicotine, a nicotinic ACh receptor agonist, increased TCT of PSCs in both SACs at >P28. In early developmental stage (P7, P8), nicotine but not oxotremorine increased TCT of PSCs in

both ON and OFF SACs. These results indicate that ON and OFF SACs have different cholinergic pathways which are regulated through distinct acetylcholine receptors in adult.

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Poster

210. Organization of Retinal Circuitry: From Photoreceptors to Amacrine Cells

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Grant-in-Aid for Scientific Research (C) from JSPS (KAKENHI No. 19K09939 and 22K09823; to T. Ishii)

Title: Starburst amacrine cells form gap junctions in early postnatal stage of the mouse retina.

Authors: *T. ISHII, T. MARUYAMA, M. KANEDA;
Nippon Med. Sch., Nippon Med. Sch., Tokyo, Japan

Abstract: In the central nervous system, neuronal networks via gap junctions are lost during postnatal development. On the other hand, in the retina, several types of neurons have known to use gap junctional coupling for signal processing in adulthood. Currently, starburst amacrine cells (SACs) are known to play an important role in the formation of direction selectivity in the adult retina, and recognized as a neuron which does not form gap junctional coupling in adulthood. However, it remains unclear whether SACs form gap junctional coupling during the developmental stage. In the present study, therefore, we examined whether SACs form gap junctional coupling in the developmental stage in the mouse retina using immunohistochemical, electrophysiological, and RT-PCR technique. When we injected Neurobiotin into SACs, many tracer-coupled cells were detected before eye-opening. The tracer coupled cells were RNA-binding protein with multiple splicing (RBPMS)-positive cells, retinal ganglion cells, but not SACs. Number of tracer coupled cells was significantly reduced after eye-opening. Consistently, the membrane capacitance, an indicator of gap junctional coupling, of SACs was also reduced after eye-opening. At the mRNA level, connexin 43 expression before eye opening was significantly higher than that after eye opening in SAC. An application of meclofenamic acid, a gap junction blocker including connexin 43, to SACs resulted in a significant decrease in membrane capacitance of SACs. Dark rearing did not change the extinction process of gap junctions during development in SAC. These results suggest that SACs form gap junctions in

early postnatal period, and reduce the number of gap junctional coupling to neighboring cells during the development.

Disclosures: T. Ishii: None. **T. Maruyama:** None. **M. Kaneda:** None.

Poster

210. Organization of Retinal Circuitry: From Photoreceptors to Amacrine Cells

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Program #/Poster #: 210.10

Topic: D.06. Vision

Support: NIH R01 NS109990

Title: Voltage-gated mechanisms compartmentalize starburst amacrine cell dendrites for motion detection

Authors: H. ACARON LEDESMA[1], J. DING[1], S. OOSTERBOER[1], X. HUANG[2], Q. CHEN[3], S. WANG[4], M. Z. LIN[5], ***W. WEI**[3];
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Abstract: In the mammalian retina, direction selectivity first arises in the radially symmetric dendrites of starburst amacrine cells (SACs). Dendritic quadrants of the SAC exhibit independent output properties: they exhibit distinct directional preferences and inhibit postsynaptic targets. In contrast, synaptic inputs are not clustered based on dendritic quadrants, but rather distributed concentrically based on the radial distance from the soma. How concentrically organized synaptic inputs are transformed by SAC dendrites into sector-specific outputs is not fully understood. Here, we combine two-photon subcellular voltage and calcium imaging and somatic electrophysiological recording to determine the computational architecture of SAC dendrites. We found that motion-evoked synaptic inputs are processed by two concentric dendritic zones with distinct modes of integration. The distal, excitable zone is capable of local calcium spike initiation, and the proximal, subthreshold zone accrues motion-evoked depolarization over the entire dendritic field. mGluR2 signaling sets the proper calcium spike threshold in the distal zone, while voltage-gated potassium channels (Kv) maintain subthreshold depolarization and prevent spike propagation in the proximal zone. Together, we show that independent directional tuning of SAC dendritic sectors is critically dependent on the synergistic action of these voltage-dependent and metabotropic mechanisms. Although mGluR2 and Kv signaling are well studied for their roles in modulating synaptic transmission and action potential waveforms, our study highlights their importance in dendritic compartmentalization.

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Poster

210. Organization of Retinal Circuitry: From Photoreceptors to Amacrine Cells

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Topic: D.06. Vision

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NIH K99EY030909

Title: A role for retinal waves in development of retinal direction selectivity

Authors: *K. BISTRONG, A. TIRIAC, M. B. FELLER;
Univ. of California Berkeley, Berkeley, CA

Abstract: In the developing visual system, neurons in the retina form precise connections to accurately represent features of the visual scene and transduce them into physiologically-relevant outputs. For some features, like motion detection, these connections are already established before the onset of vision. The factors that set up these connections during this developmental period remain unknown. During development, the retina exhibits spontaneous waves of neural activity that sweep across its surface. We tested the influence of these retinal waves on the development of the direction-selective circuit by characterizing direction-selective responses in a mouse model (β2-nAChR-KO) that exhibits perturbed cholinergic retinal waves. These mice present a severe deficit in direction-selective responses to motion along the horizontal axis. This phenotype is reminiscent of a recent study where genetic deletion in mice of a gene linked to human congenital nystagmus, FRMD7, leads to a severe reduction in horizontal direction selectivity in the mouse retina. We therefore tested whether FRMD7 expression is influenced by cholinergic retinal waves during development. Using fluorescent in situ hybridization and immunohistochemistry, we find that starburst amacrine cells have normal levels of FRMD7 expression in β2-nAChR-KO mice, indicating retinal waves and FRMD7 may play independent roles in the establishment of direction selectivity. We will present our latest findings based on targeted whole-cell voltage clamp recordings to determine whether the synaptic basis of direction selectivity is altered similarly between FRMD7 and β2-nAChR-KO mice and whether excitatory and inhibitory inputs are altered in these mice.

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Poster

210. Organization of Retinal Circuitry: From Photoreceptors to Amacrine Cells

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Program #/Poster #: 210.12

Topic: D.06. Vision

Title: Utilizing cell-type-specific surface proteomics to decode the formation of retinal neural circuits

Authors: *S. SKARLATOU[1], J. LI[3], H. SAPINOSO[1], A. SHUSTER[4], L. LUO[3], Y.-R. PENG[2]; [2]Jules Stein Eye Institute, Dept. of Ophthalmology, [1]UCLA, Los Angeles, CA; [3]Stanford Univ., Stanford, CA; [4]Dept. of Biol., Howard Hughes Med. Inst., Stanford, CA

Abstract: A complex task in the nervous system is to make precise synaptic connections, which are the functional units for sensation, perception, cognition, and behavior. How billions of neurons find their partners and make proper synaptic choices has been a challenging and long-standing question. High-throughput genomic and transcriptomic methods revolutionized the characterization of gene expression in individual cell types, allowing for indirect curation of genes that might be involved in synaptic connections. However, directly measuring surface proteins in individual cell types remains challenging. Here, we used a novel high-throughput cell-surface protein labeling reaction (Li et al., 2020) adapted to the mouse to study the formation of synapses during development. The method relies on an unbiased proximity-based biotinylation of proteins carried out by a cell-membrane-bound horseradish peroxidase (HRP) that is genetically targeted to specific cell populations in a Cre-dependent manner. We used a highly-ordered neural structure, the retina, as a model system to demonstrate the efficiency, specificity, and resolution of this method. By using different Cre-lines, we are profiling the surface proteomes of five cell types with synaptic laminations at select layers of the outer plexiform layer (OPL) and the inner plexiform layer (IPL). We present our preliminary analysis of the corresponding proteomics datasets and identify candidates with potential functions in the establishment of specific synapses in the OPL and the IPL, respectively. Investigating the roles of these candidates further, will shed some light onto the developmental mechanisms that endow the visual circuits with the necessary precision during the assembly of connectivity patterns.

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Poster

211. Visual Motion

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Topic: D.06. Vision

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NSF GRF 2020305476

Title: Laminar and cell class distinctions for pre-saccadic attention in marmoset MT/MTC

Authors: *A. BUCKLAEW[1], S. H. COOP[2], G. H. SARCH[4], J. F. MITCHELL[3];
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Abstract: Attention leads eye movements producing perceptual enhancements at the saccade targets immediately prior to saccades (Deubel and Schneider, 1996; Kowler et al, 1995; Rolfs and Carrasco, 2012). Pre-saccadic attention has been related to enhanced neural responses before saccades made into a neuron's receptive field in macaque visual cortex (Moore and Chang 2009). However, much remains unknown about the underlying circuit mechanisms. Using the marmoset, a small New World monkey with a smooth lissencephalic brain, we examined laminar and cell class distinctions during pre-saccadic attention in motion selective areas MT/MTC. Recordings were made with linear arrays and current source density (CSD) was used to mark the reversal point between input and deep layers in cortex. We also examined the distribution of extracellular waveform shapes and found a bi-modal distribution of narrow and broad spike durations consistent with previous work (Mitchell et al., 2007). Several basic measures validated these circuit level distinctions including a higher baseline firing and broader motion tuning bandwidth among narrow spiking neurons, which support that narrow spiking cells are predominantly fast spiking PV interneurons. We investigated how pre-saccadic attention varied by cell class and layer in a saccade foraging paradigm. In each task trial marmosets made a saccade from a central fixation point to one of three equally eccentric stimuli (full coherence dot fields with motion sampled independently between apertures). We positioned the stimuli such that one foraged location overlapped the receptive fields of neurons under study and examined how tuning functions for motion direction changed. Tuning curves were fit with an adjusted Von Mises curve that estimates baseline, gain, and tuning width. Similar to previous studies of covert attention (McAdams and Maunsell, 1999), we found in two animals that neurons on average exhibited increases in baseline and gain with pre-saccadic attention, but no systematic changes in tuning width. In a single animal we were able to dissect the population by cell class and layer. We found that increases in gain were predominantly among broad spiking neurons in superficial layers whereas additive increases in rate were shared across layers and cell types. The increases in gain correlated with increases in neural sensitivity while baseline increases did not. This suggests that superficial layer broad spiking neurons, the putative projection neurons that would relay information to downstream cortical areas, have a privileged role for encoding enhanced motion sensitivity during pre-saccadic attention.

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Poster

211. Visual Motion

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Title: Efficient mapping of joint tuning for retinal and eye velocities in macaque area MT

Authors: *Z.-X. XU[1,2], A. ANZAI[1,2], G. C. DEANGELIS[1,2];
[1]Brain and Cognitive Sci., [2]Ctr. for Visual Sci., Univ. of Rochester, Rochester, NY

Abstract: Humans and other animals constantly move their eyes to explore the environment and track objects of interest. However, eye movements distort the retinal image projection of the world, making the source of retinal image motion ambiguous. Thus, the visual system must combine signals about eye movements with retinal motion to infer scene structure. This process is traditionally thought to happen in higher-level areas such as parietal cortex. However, recent studies suggest that neurons in macaque area MT incorporate extra-retinal signals about eye movements to process information that goes beyond retinal motion (Kim et al. 2015, 2017; Nadler et al. 2008, 2009; Xu & DeAngelis 2022).

To better characterize the interaction between retinal motion and eye movements in MT, we used two uncorrelated white noise sequences, one for retinal motion and one for eye velocity, to efficiently measure the joint velocity tuning of MT neurons. We trained the animal to track a moving target on the screen using smooth pursuit, and we presented a patch of random dots at the average receptive field location of the neurons under study. The velocity profile of the pursuit target was a pseudorandom Gaussian white noise sequence generated from a binary m-sequence (Kashiwagi & Sakata 1978); the movement of the patch followed the same sequence with a half-cycle shift, such that the two sequences were uncorrelated.

We recorded responses of MT neurons using 32-channel linear electrode arrays and analyzed the data using both encoding and decoding approaches. For the encoding approach, we first computed a modified version of spike-triggered average (STA). Traditional STA assumes a linear response function and cannot capture nonlinear neural tuning. We extended this method to discretized velocity signals, which allows nonparametric estimation of temporal kernels for each velocity value. Preliminary results show a clear diagonal structure in the joint tuning of some MT neurons, extending our recent findings (Xu & DeAngelis 2022). We then quantified the extent of inseparability in the joint tuning using a Fourier domain approach. We also fit a Poisson Generalized Additive Model (P-GAM; Balzani et al. 2020) to the population responses for better characterization of the neural activities.

In addition, single-session decoding analysis showed that a simple linear regression can capture a significant amount of variance in both retinal and eye velocity signals. Our experiment reveals detailed evidence that neurons in macaque area MT encode both retinal motion and pursuit eye movement velocity and demonstrates that our method is efficient for measuring the joint neural tuning for these two variables.

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Poster

211. Visual Motion

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Program #/Poster #: 211.03

Title: WITHDRAWN

Poster

211. Visual Motion

Location: SDCC Halls B-H

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Program #/Poster #: 211.04

Topic: D.06. Vision

Support: R124985

Title: Three-dimensional unsupervised probabilistic pose reconstruction (3D-UPPER) for freely moving animals

Authors: *A. EBRAHIMI[1], P. ORLOWSKA-FEUER[2], Q. HUANG[3], A. ZIPPO[4], R. PETERSEN[3], R. STORCHI[2];
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Abstract: A key step in understanding animal behaviour relies in the ability to quantify pose and movements. Methods to track body landmarks in 2D have made great progress over the last few years but accurate 3D reconstruction of freely moving animals still represents a challenge. To address this challenge here we develop the 3D-UPPER algorithm, which is fully automated, requires no a priori knowledge of the properties of the body and can also be applied to 2D data. We find that 3D-UPPER reduces by >10 fold the error in 3D reconstruction of mouse body during freely moving behaviour compared with the traditional triangulation of 2D data. To achieve that, 3D-UPPER performs an unsupervised estimation of a Statistical Shape Model (SSM) and uses this model to constrain the viable 3D coordinates. We show, by using simulated data, that our SSM estimator is robust even in datasets containing up to 50% of poses with outliers and/or missing data. In simulated and real data SSM estimation converges rapidly, capturing behaviourally relevant changes in body shape. Such changes, observed in 2D and 3D datasets, include head and body pitch typically occurring during rearing and left/right head and body torsions observed during orienting behaviours and spontaneous exploration. Altogether 3D-UPPER represents a simple tool to minimise errors in 3D reconstruction while capturing meaningful behavioural parameters.

Disclosures: A. Ebrahimi: None. **P. Orlowska-Feuer:** None. **Q. Huang:** None. **A. Zippo:** None. **R. Petersen:** None. **R. Storchi:** None.

Poster

211. Visual Motion

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 211.05

Topic: D.06. Vision

Title: A spatially invariant representation of motion arises via feedforward projections in the mouse visual cortex

Authors: *S. M. GANNON[1], N. J. PRIEBE[2], L. L. GLICKFELD[1];
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Abstract: Global motion perception requires representations that are invariant to the specific spatial features of the stimulus. In the primate visual system invariant motion signals emerge from a processing cascade in which spatial invariance first arises in primary visual cortex (V1; e.g., complex cells) and then motion invariance emerges in area MT (e.g., pattern cells). In the rodent visual system, however, pattern selective cells are found in V1, suggesting that spatial and motion invariance may be constructed in parallel. We initially examined the global motion signals in primary visual cortex using drifting gratings and plaids and have found that a subset of V1 neurons do appear pattern selective (16.9%, n=291/1719 cells, 7 mice). To examine the robustness of this global motion signal we altered the spatial features of drifting plaids by systematically changing the stimulus spatial phase. We find that pattern selectivity in mouse V1 is highly sensitive to the spatial features of the plaid, and that the classification of neurons as pattern or component shifts with spatial phase. Only 7.6% (22/291) of neurons maintain global motion selectivity across spatial phase, a fraction that is consistent with a reshuffling of encoding at chance. Thus, in order to achieve a representation that is both spatially and directionally invariant, additional transformations, potentially in the higher visual areas, are needed. To test this hypothesis, we used two-photon calcium imaging to compare the global motion sensitivity of neurons in V1 and the higher visual area (HVA) LM while changing plaid spatial phase. Consistent with an increase in invariance in the HVAs, we found that neurons in LM are less phase-dependent than those in V1 (phase modulation amplitude: V1- 0.18±0.01, n=280 cells; LM- 0.12±0.01, n=205 cells; 4 mice). To determine whether this increase in invariance is transmitted by sub-populations of neurons in V1, or achieved through a transformation in LM, we measured the phase-dependence of V1 axon terminals in LM. We found that V1 projections to LM are only weakly modulated by stimulus phase and are more similar to the population of neurons in LM than V1 (V1- 0.13±0.01, n=14571 boutons, 3 mice). Our data suggest that one way an invariant representation of motion arises in mouse visual cortex is via specific feedforward projections of an invariant subpopulation in V1 to the higher visual area LM.

Disclosures: S.M. Gannon: None. **N.J. Priebe:** None. **L.L. Glickfeld:** None.

Poster

211. Visual Motion

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Program #/Poster #: 211.06

Topic: D.06. Vision

Support: 5R01EY026286-06

Title: Encoding of Pattern and Component Motion in the Mouse Early Visual System

Authors: *V. DEPIERO[1], C. CHEN[2], E. SAVIER[1], H. CHEN[1], W. WEI[4], J. CANG[2,3];
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Abstract: An important function of the visual system is to process and encode motion information, such as the movement direction and speed. Motion processing starts in the retina, where direction-selective ganglion cells (DSGCs) respond selectively to stimuli moving along a preferred direction. In mice, DSGCs project to the superficial layer of the superior colliculus (SC) and give rise to direction selectivity there (Shi et al., Nature Neuroscience, 2017). Additionally, retinal output, including that from DSGCs, reaches the primary visual cortex (V1) via the lateral geniculate nucleus (LGN). Direction selectivity is well studied throughout the visual system but exactly how the SC process motion patterns compared to V1 remains unknown. It is also unclear how much of the motion responses originate in the retina or how they are transformed to downstream brain areas. Here we investigate how neurons in the superficial SC and V1 responds to complex motion patterns known as plaid stimuli. This visual stimulus consists of two sinusoidal gratings superimposed over one another moving in different directions giving the perception of a global motion pattern. Using two-photon calcium imaging in awake mice, we show that most DS neurons in the SC are primarily pattern selective, meaning they respond preferentially to the global motion direction of the plaid stimulus. On the other hand, very few V1 neurons are selective for pattern motion, whereas some neurons show component responses. This population of V1 neurons respond to the motion axes of both moving gratings that make up parts of the plaid stimulus. Finally, we use patch-clamp electrophysiology to measure responses of DSGCs to plaid stimuli in a whole-mount *ex vivo* retinal preparation. Our preliminary data indicate that DSGCs are predominantly selective for pattern motion.Together, our data reveal a drastic difference in how V1 and SC compute motion signals. SC neurons most likely inherit their pattern motion sensitivity from DSGCs in the retina, whereas V1 neurons are more likely to encode component motion.

Disclosures: V. DePiero: None. **C. Chen:** None. **E. Savier:** None. **H. Chen:** None. **W. Wei:** None. **J. Cang:** None.

Poster

211. Visual Motion

Location: SDCC Halls B-H

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Program #/Poster #: 211.07

Topic: D.06. Vision

Title: Dissecting the perceptual and oculomotor representations of moving objects using the double drift illusion

Authors: *N. M. DOTSON[1], Z. W. DAVIS[1], J. M. SALISBURY[2], S. E. PALMER[2], P. CAVANAGH[3], J. H. REYNOLDS[1];
[1]SNL-R, Salk Inst., La Jolla, CA; [2]Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL; [3]Glendon College, York Univ., Toronto, ON, Canada

Abstract: Generating an internal representation of the external world is a fundamental role of the nervous system. However, it is apparent that the internal simulation that we construct does not always match physical reality. This dissociation is made abundantly clear by visual illusions that can generate profound differences between the physical and the perceived stimulus. These differences provide us with a unique tool for dissecting this construction process. Here we use the double drift illusion, where the perceived path of a moving Gabor patch differs from the physical path by ~45°. Results from human subjects indicate that the neural representation of the illusory position must be computed within areas that can represent spatial positions in an allocentric reference frame and that have the capacity to integrate information over long time scales, such as the lateral prefrontal cortex (PFC). This illusion also reveals a major gap in our understanding of the perceptual and oculomotor systems. When humans are asked to report the position of the stimulus with a saccade, they accurately locate the physical position indicating that the oculomotor system is unaffected by the illusion. This apparent paradox suggests that the oculomotor system - including the frontal eye fields (FEF) - may only encode recent retinotopically referenced positional information, not the accumulated allocentric, illusory representation that is perceived. To address these gaps in our knowledge, we trained marmosets, which have a visual system that is specialized for hunting small moving objects, to perform the Double Drift Delayed Choice (DDDC) task in which they report the direction of a moving Gabor patch by choosing the target that the Gabor would have intercepted. We have begun recording from lateral PFC and FEF using laminar probes, including Neuropixels. Importantly, because the marmoset cortex is lissencephalic, we are able to record across layers within a column. Our preliminary behavioral results demonstrate a modulation in behavior that is indicative of the presence of an illusory percept. Preliminary neural results indicate that we can identify laminar compartments in PFC using current source density analysis. We have also verified that neurons in PFC encode the position of the Gabor patch as it moves across the screen. Identifying where in the brain these illusory representations are encoded and how they are computed will be major milestones toward our understanding of the stages of processing leading to spatial perception. Results may also lead to insights into the mechanisms underlying psychiatric illnesses, like schizophrenia, in which distortions of reality are a hallmark.

Disclosures: N.M. Dotson: None. **Z.W. Davis:** None. **J.M. Salisbury:** None. **S.E. Palmer:** None. **P. Cavanagh:** None. **J.H. Reynolds:** None.

Poster

211. Visual Motion

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Title: Hierarchical processing of 3D motion in macaque MT and FST

Authors: *L. W. THOMPSON[1], B. KIM[1], B. ROKERS[2], A. ROSENBERG[1];
[1]Neurosci., Univ. of Wisconsin - Madison, Madison, WI; [2]Psychology, New York Univ. - Abu Dhabi, Abu Dhabi, United Arab Emirates

Abstract: Determining how objects move through three-dimensional (3D) space is an essential function of the visual system. Many neurophysiological studies have examined the cortical processing of motion in two dimensions (2D). However, the extent to which those findings generalize to 3D motion processing is unclear because the cues that signal 2D and 3D motion are distinct. Recent work has shown that 3D motion can be decoded from activity in the macaque middle temporal area (MT) based on combinations of 2D motion tuning and ocular dominance (OD). These properties are ubiquitous across early visual cortex, making it unclear whether the activity reflects genuine 3D motion selectivity or the processing of lower-level features that naturally covary with an object's 3D motion. To distinguish these possibilities, we measured the responses of neurons in MT and the fundus of the superior temporal sulcus (FST) to multiple 3D motion cues during a 'toward'/'away' discrimination task. The stimuli were comprised of a volume of dots (3° diameter; ±1° disparity) that moved toward or away from the monkey's "cyclopean eye" and contained either: (1) combined stereoscopic and perspective cues, (2) stereoscopic cues only, or (3) left- or right-eye perspective cues only. Critically, 3D motion toward or away from the cyclopean eye produces left- and right-eye retinal motion signals with opposite net 2D directions. Lower-level (2D) processing would thus be evidenced by opposite 3D motion direction preferences in the left- and right-eye perspective cue conditions. We found that a similar percentage of MT and FST neurons showed significant combined-cue 3D motion selectivity (MT = 54%; FST = 58%). However, three converging lines of evidence suggest that the MT responses largely reflected lower-level feature selectivity, whereas the FST responses reflected a robust representation of 3D motion. First, 3D direction preferences in MT were typically opposite in the left- and right-eye perspective cue conditions, and the direction preferences across cue conditions were well explained by the neurons' 2D motion tuning and OD. In contrast, in FST, the 3D direction preferences were typically cue-invariant. Second, neurometric sensitivities in FST, but not MT, were well correlated with the behavioral sensitivities across cue conditions. Finally, 3D motion direction could be linearly decoded in a cue-invariant fashion from FST, but not MT because 2D and 3D motion signals were conflated in the non-dominant eye. Together, these results suggest that a crucial 2D-to-3D visual transformation occurs along the MT to FST pathway to create a robust cue-invariant representation of 3D motion.

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Poster

211. Visual Motion

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Topic: D.06. Vision

Support: NIH Grant EY027023

Title: Modeling nonlinear interactions underlying spatial integration of visual inputs in the V1 to MT pathway

Authors: ***Z. YAO**[1], W. BAIR[2];
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Abstract: The primate visual cortical motion area MT receives convergent inputs from V1 neurons and combines such signals across space to achieve receptive fields (RFs) in MT that are up to 10 times the diameter of those in V1. Britten and Heuer (1999, J Neurosci) demonstrated that this spatial summation process is nonlinear by comparing the response of MT neurons to a pair of spatially separated stimuli against the responses to the same stimuli presented alone. Such nonlinearity is often interpreted in the framework of normalization and suppression, and several modeling studies have suggested that the integration of V1 signals in MT should be nonlinear (Simoncelli & Heeger, 1998, Vision Res; Tsui et al., 2010, J Neurophysiol); however, the underlying neural mechanisms have remained unclear. We adapted a previously published MT model developed in our laboratory (Baker & Bair, 2016, J Neurosci) by incorporating spatial integration of V1 inputs, and implementing nonlinear mechanisms at various stages of the model - V1 surround suppression, V1-to-MT input normalization, and MT population normalization. We aimed to identify the potential mechanisms that can account for Britten and Heuer's results that describe how the response to two separate moving stimuli depends in a nonlinear way on both the amplitude of the responses to the individual stimuli and on the relative positions of the two stimuli within the MT RF. At the same time, the model was required to also explain other important and well-established properties of MT neurons, including pattern vs. component direction selectivity, direction bandwidth, and RF size. In addition to testing various normalization schemes, we also tested how the topographic arrangement of the RFs of the V1 inputs used in constructing an MT unit can impact the spatial extent of the nonlinear interaction in MT by varying the level of centralization of V1 locations in the MT RF. In our model, we found that both V1-to-MT input normalization and MT population normalization can explain the normalizing interaction in terms of the apparent power-law behavior observed by Britten and Heuer, but the input normalization was not able to account for the suppressive spatial interactions that they and others have observed. Given that V1-to-MT input normalization may be more difficult to implement biologically, whereas MT population normalization can be easily implemented by lateral inhibition, we speculate that MT population normalization is more likely

the cause of the nonlinear summation in the MT RF, while other mechanisms, such as V1 surround suppression and the centralization of V1 locations, regulate the nonlinear spatial interaction in MT more moderately.

Disclosures: Z. Yao: None. **W. Bair:** None.

Poster

211. Visual Motion

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Topic: D.06. Vision

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Title: Parallel processing of optic flow signals in central self-motion estimation networks

Authors: *M. ERGINKAYA[1], M. BROTAS[1], K. STECK[2], A. NERN[3], D. BOCK[4], M. B. REISER[3], M. CHIAPPE[1];
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Abstract: Sensory experience critically depends on our own movement: the brain must incorporate information about self-movement to guide behavior, or discount self-generated sensory signals to detect external events in the world. How do sensory circuits process self-generated signals in different ways to meet specific demands?
In insects, the pattern of visual motion generated by self-movement, or optic flow, contributes to an internal estimate of self-motion and is thought to actively control the walking course. Lobula Plate Tangential Cells (LPTCs) of the fly optic system respond to specific patterns of optic flow. LPTCs also receive extra-retinal signals related to head/body movements during locomotion, which suppress or enhance their activity depending on the task. Thus, the LPTC network is suitable to investigate task-dependent processing of self-generated sensory signals for behavioral control. Although some pathways that connect LPTCs to head rotations have been identified, the full extent of the central circuitry that enable optic flow signals to control behavior remains largely unidentified.
Using a complete adult *Drosophila* brain electron microscopy data, we identified synaptic partners of two classes of LPTCs, the Horizontal (HS) and Vertical System (VS) cells. We found that HS and VS cells receive synaptic inputs at their axon terminals located at a premotor center. Interestingly, neurons that receive the strongest convergent input from HS and VS cells are three distinctive classes of GABAergic interneurons that, in turn, provide the majority of synaptic inputs back onto HS and VS cells at their axons, likely modulating LPTC activity. By mapping the inputs and outputs of these key interneurons, we found two major subnetworks that contact

distinct populations of central and descending neurons. Functional imaging revealed that these key interneurons are sensitive to specific optic flow patterns that are either induced by forward or turning movements. The same population of cells also respond in darkness, when the fly is either walking forward or turning, respectively.
The combination of the circuit architecture and functional responses suggest that one of the subnetworks can be used to detect voluntary turns and discount the resultant optic flow, whereas the other monitors the angular deviations and elicits turns to maintain a straight course.
Altogether, our results reveal two central networks that process complex patterns of optic flow field into translational and rotational directions, which in combination with extra-retinal signals, modulate the activity of visual neurons according to the ongoing task of an animal moving through space.

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Poster

211. Visual Motion

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Program #/Poster #: 211.11

Topic: D.06. Vision

Support: Howard Hughes Medical Institute

Title: Lobula Plate Tangential Cells and frontal optic flow in Drosophila

Authors: *S. KOSKELA, A. NERN, A. ZHAO, E. M. ROGERS, S. MILLER, R. ARRUDA, C. LAUGHLAND, M. B. REISER;
HHMI / Janelia Reseach Campus, Ashburn, VA

Abstract: When navigating their environment flies rely on visual feedback to regulate their flight behaviors. Wide-field motion sensitive neurons called the Lobula Plate Tangential Cells (LPTCs) have receptive fields (RF) that are well-matched to certain visual patterns (optic flow) induced by self-motion and are presumed to contribute to visually guided stabilization and course control. The LPTCs reside in the Lobula plate (LP), the fourth neuropil of the fly visual system. The LP has four synapse-dense layers that correspond to four directions of motion: front-to-back, back-to-front, upward and downward. Some LPTCs have been studied for decades in larger flies with sharp microelectrodes, but only recently has it been possible to describe their anatomy and explore their function in *Drosophila*. Thus far, no LPTC has been conclusively linked as a critical conduit for visual motion in a specific optomotor reaction, such as yaw stabilization or lift control. Here, we present our work to describe the LPTC types innervating LP layers 1 and 2 and their potential roles in behavioral optic flow responses.
Based on extensive EM reconstructions, we identified 59 LPTC types in *Drosophila melanogaster*. We quantified their morphology and layers of innervation, from which we

computationally predicted their RFs. Based on these predictions, we designed wide-field visual stimuli and quantified the behavioral responses of tethered, flying flies using an updated high speed, high spatial resolution LED display. Steering reactions were tracked by an optical detector that measures the amplitude of individual wing strokes. We found that the optomotor response of flying wildtype flies (CantonS x w^{1118}, females) to a drifting vertical grating was strongest for motion patterns presented in front of the fly (centered at 0° from the fly's heading; 37.5° wide window) while stimuli presented at the sides (±90° to ±120° from the fly's heading) evoked no response. To identify the LPTC types underlying this behavior, we examined LPTCs with frontal innervation in layers 1 and 2 of the LP. We generated candidate driver lines for these cell types, that morphologically match EM reconstructions. We used these split-GAL4 driver lines to silence specific LPTCs by expressing Kir1.2 and compared the behavioral responses to different wide-field motion patterns (yaw, sideslip, roll, lift) with genetic control flies. We present the results for the well-known LPTC cell types, the HS and H2, and new candidate cell types.
In our ongoing work, we characterize the phenotypes of all the LPTCs, including cell types innervating LP layers 3 and 4 and test contributions of individual cell types for diverse optomotor behaviors.

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Poster

211. Visual Motion

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Program #/Poster #: 211.12

Topic: D.06. Vision

Support: ZIA-MH-002909

Title: Object position estimation through periods of occlusion

Authors: *A. K. BEHEL, L. TEICHMANN, G. EDWARDS, C. I. BAKER;
NIMH, Bethesda, MD

Abstract: We have strong predictions about what happens to an object when it becomes occluded. In the current pre-registered study, we examined the underlying mechanisms supporting single object tracking through periods of occlusion. In particular, we tested how we represent motion trajectories during occlusion and how motion information pre-occlusion is integrated with information post-occlusion. Participants (n=20) completed two separate tasks. In the first task, we tested the accuracy of position representation as an object moved on a straight trajectory and became occluded by a rectangle. The object moved at a constant velocity across the screen and was associated with a sound. While the object was behind the occluder, the sound stopped indicating that the object stopped moving. The participant was then asked to estimate the object's stopping position behind the occluder. Within the first 100 ms of occlusion, participants

accurately report stopping position. However, perception of object position soon lagged behind the true object position with decreasing performance as occlusion time increased. Eye movement recordings demonstrated initial accurate tracking of the object into the occluder. Gaze then increasingly lagged behind the object the longer it remained occluded, consistent with the behavioral data. In the second task, we tested whether visual information post-occlusion is useful in the reconstruction of the motion trajectory during occlusion. While the object was visible, it moved at a constant rate. However, during occlusion speed varied and participants were then asked to determine whether the object reappeared too early or too late. We found that participants were accurate at determining whether an object reappeared too early or too late given its initial velocity. This shows that visual information post-occlusion is important to represent motion trajectories through periods of occlusion. In the eye movement recordings, gaze position focused on the points of disappearance and reemergence. The focus on the reappearance position grew stronger with increased delay of object reemergence. Across the two tasks, we find evidence for initial extrapolation into the occlusion period. Post-occlusion information was critical to integrate the whole motion trajectory. The eye-tracking data further highlight the task-dependent nature of gaze patterns as well as the importance of context before and after occlusion for understanding motion trajectories. Overall, our results shed light on our abilities to bridge perceptual gaps to perceive the dynamic visual world as continuous.

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Poster

211. Visual Motion

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Topic: D.06. Vision

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NIH 1U01NS111695-01

Title: Neural mechanisms of eye-head coordination during active head-unrestrained gaze shifts in mice

Authors: *B. M. VERDONE, H. V. CHANG, D. ROBERTS, K. E. CULLEN;
Johns Hopkins Univ., Baltimore, MD

Abstract: To reorient the axis of visual gaze relative to space, animals can generate both head and eye movements. A prevailing view is that animals lacking the high-acuity visual center known as the fovea (afoveates) predominately perform head-directed gaze, while foveate animals use their eyes to redirect gaze, followed by compensatory head movement. Though mice are now a prevailing afoveate model in the study of vision, little is known regarding the neural mechanisms that underlie their natural gaze reorientation strategy. One hypothesis is that voluntary (active) gaze shifts are generated by voluntary head movements with eye movements

produced by involuntary reflexes. Alternatively, mice may generate both voluntary eye and head movements to redirect their gaze. In this study, we assess the dynamics of mouse gaze during active and passive movements to define their natural gaze behaviors and distinguish between these two possibilities.
First to investigate gaze during active head movements, male mice (n=6) were trained to perform small (~ ±10°) and large (~ ±40°) horizontal, goal-directed head rotations to orient between two waterspouts for reward. Horizontal and vertical eye position relative to the head was tracked using a miniature camera system, head position relative to space was measured using a high resolution potentiometer, and gaze position was calculated as the sum (gaze-in-space = eye-in-head + head-in space). We found that mice generated rapid saccade-like eye movements in the direction of the head motion within 10-20 ms of head motion onset. Second, for comparison, we recorded the eye movements evoked in response to comparable passive movements (ie., ±10°, ±40°) generated by applying an external torque to the mouse's head. Passive head motion-generated eye movements demonstrated a robust and sustained vestibulo-ocular reflex (VOR) response as evidenced by equal and opposite eye and head position persisting for ~100ms, only after which a corrective quick phase was generated. Further, during larger movements, subsequent compensatory and quick-phase VOR were generated until the head stabilized. Taken together, our results contrast with the prevailing view and suggest that mice generate both voluntary eye and head movements to redirect gaze. The onset of the saccade-like eye movements generated during active orienting head movements occurred an order of magnitude faster than VOR quick-phase eye movements generated during comparable passive head movements. We propose that although mice lack a fovea, they can reorient gaze using a combined eye-head strategy.

Disclosures: B.M. Verdone: None. **H.V. Chang:** None. **D. Roberts:** None. **K.E. Cullen:** None.

Poster

211. Visual Motion

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Topic: D.06. Vision

Support: NIH Grant EY027373-02

Title: Degraded motion reliability reduces eye speed during the initiation of smooth pursuit eye movements through changing the signal-to-noise ratio of the population response in area MT.

Authors: *S. BEHLING, S. G. LISBERGER;
Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: Primates use smooth pursuit eye movements to track moving objects. Smooth pursuit is initiated by sensory estimates of stimulus speed represented in the middle temporal area of

extrastriate visual cortex (MT). We showed previously that degraded motion reliability caused by reduced coherence in a pursuit target created from a moving patch of dots reduces the eye speed during the initiation of pursuit. To understand the representation of image motion during smooth pursuit and to ask why degraded motion reduces eye speeds, we recorded from isolated single neurons in extrastriate visual area MT. We sought to determine whether eye speed lags behind target speed for low dot coherence because (i) the speed representation in MT is compromised or (ii) the representation of image speed remains accurate in MT and eye speed is eroded in downstream circuits. We presented moving patches of dots of varying speeds and coherences while recording neurons in area MT. During pursuit initiation, the amplitude, but not the tuning, of MT responses depends on dot coherence. The population response gets noisier as coherence reduces the amplitude of neural (and eye movement) responses, but image speed still can be decoded by applying traditional vector averaging strategies to the MT population response. To account for the effects of degraded motion reliability on the initiation of pursuit, we propose a downstream gain signal based on the population amplitude of MT. The smooth eye movement region of the frontal eye fields (FEF_{SEM}) receives input from area MT and has outputs that control the gain of visual-motor transmission for pursuit. We conclude that MT consistently represents image speed as motion reliability is degraded, while the decrease in signal-to-noise ratio in MT reduces the gain of visual-motor transmission (and presumably the output of FEF_{SEM}) and eye speed in the initiation of pursuit.

Disclosures: S. Behling: None. **S.G. Lisberger:** None.

Poster

211. Visual Motion

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Topic: D.06. Vision

Title: Exploring the role of active conductances in the Drosophila motion vision circuit.

Authors: *F. G. RICHTER, A. BORST;
MPI For Neurobio., MPI For Neurobio., Munich, Germany

Abstract: Detecting the direction of visual motion is crucial for the survival of all sighted animals. In the fruit fly the first direction-selective cells in the motion vision circuit are T4 cells for ON motion and T5 cells for OFF motion, respectively. Current biophysical models of motion detection in these cells rely exclusively on passive conductances.
However, according to mRNA levels the voltage-gated sodium channel *Paralytic* (*Para*) is highly expressed in T4 and T5 cells. Conditional labeling of the endogenous *Para* protein with the recently described 'FlpTag' tool showed that *para* expression in T4 and T5 cells is limited to the fiber connecting the dendrite to the axon terminal. The functional role of these channels in T4 and T5 cells has not been explored so far.
Using *in vivo* 2-photon calcium imaging, we find that blocking voltage-gated sodium channels

pharmacologically with tetrodotoxin (TTX) abolishes all calcium responses in T4 and T5 cells elicited by visual stimuli. Surprisingly, the visual responses of the main inputs to T4 and T5 cells are unaffected. Knocking down *Para* in T4 and T5 cells with RNAi leads to similar results as TTX application. These findings indicate an important role of voltage-gated channels in motion sensing neurons in *Drosophila*.

Disclosures: F.G. Richter: None. **A. Borst:** None.

Poster

211. Visual Motion

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Topic: D.06. Vision

Support: SFB 870

Title: A single cell type implements multi-level visual motion opponency in Drosophila

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Abstract: The interaction between opponent sensory channels is a widespread circuit motif across diverse brain areas and species. Here, we investigate the structure and function of a multi-level opponent neural network in the motion vision system of the fruit fly *Drosophila*.
Small-field T4/T5 cells are the first direction-selective neurons in the *Drosophila* visual system. They come in four subtypes, each of which preferentially responds to one of the four cardinal directions of motion and sends axonal projections to a specific layer in the lobula plate. There, large-field lobula plate tangential cells (LPTCs) pool excitatory input from multiple T4/T5 cells, thereby inheriting their directional preference. T4/T5 cells also target inhibitory LPi cells, which in turn project onto LPTCs in the neighboring, oppositely tuned layer. Thus, LPTCs receive excitatory input in their preferred direction and inhibitory input in their null direction, rendering them fully motion opponent.
To study additional inhibitory interactions in this circuit, we performed 2-photon voltage imaging of all circuit elements. Interestingly, LPis already showed fully motion-opponent voltage responses, similar to postsynaptic LPTCs. Furthermore, we detected signs of opponent inhibition in the axon terminals of T4/T5 cells. Genetic silencing experiments indicate that motion-opponent signals arise from cross-inhibitory input originating in the oppositely tuned lobula plate layer. Analysis of connectomic EM reconstructions revealed that LPis, in addition to LPTC dendrites, target axon terminals of T4/T5 cells and LPis of opposite directional preference. Thus, LPi cells implement motion-opponent inhibition at three consecutive levels in the lobula plate circuitry. In agreement with these findings and the glutamatergic nature of LPi cells, we confirmed the presence of the inhibitory glutamate receptor GluClα on LPi dendrites and on T4/T5 axon terminals.

Lastly, we investigated the functional consequences of multi-level opponent inhibition using electrophysiological recordings and computational modelling. These experiments suggest that the different opponent levels serve distinct functional roles. While the opponent subtraction stage at the level of LPTCs is essential for generating flow-field selectivity, the motion-opponent computation at the level of T4/T5 and LPis suppresses correlated noise while allowing LPTCs to operate in a high gain regime. The computational structure we uncovered thus provides a twofold benefit for visual processing: it ensures that the visual system remains sensitive to noisy input while staying selective for relevant sensory signals.

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Poster

212. Visual Cognition and Decision Making in Mammals

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Title: Rhythmic Attentional Sampling during Multialternative Decision Making

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Abstract: The oscillatory nature of the large-scale neuronal signal has been implicated in a wide range of cognitive tasks. Recent work indicates that rhythmic neuronal processing directly shapes behavior, in the detection of near-threshold stimuli and the allocation of spatial attention between different stimuli. It has remained unclear if and how rhythmic neuronal processing (i) affects the sampling of sensory evidence in multi-target perceptual decisions; and (ii) is integrated along the processing hierarchy from perceptual encoding to motor preparation. Here, we recorded magnetoencephalography (MEG) from 20 participants (9 female) during a novel 3-alternative visual decision making task. Participants were simultaneously shown 3 Gabor patches with varying contrast levels. Randomized across trials the participants had to choose either the highest or lowest contrast. The target contrast was only revealed 1 second into the stimulus presentation (sensory phase) with a framing cue (high/low). This effectively orthogonalizes each options value from the physical contrast. After the subsequent decision phase, a Go signal cued the motor response (button press with left hand, right hand or foot). We applied Inverted-Encoding-Models to reconstruct the sample-by-sample sensory and motor-related activity for each of the three choice options. We found that the distribution of MEG-reconstructed attention shifts between the three options were rhythmic: the allocation was nested within an underlying

10-15 Hz oscillation. This attentional rhythmicity was prolonged during the complete sensory and decision phases (3.5 s in total). By contrast, motor planning did not show any rhythmicity. Sensory encoding and motor preparation further dissociated in their temporal relationship to the trial structure: The sensory signal shows peak discriminability between the options with highest and lowest decision value early in the decision phase. The relationship between decision value and motor activity builds up towards the end of the trial starting after peak sensory discriminability. We conclude that stimulus encoding during decision-making is generally rhythmic. Further, the targets of attention-allocation appear to follow a top-level strategy - i.e. attend to lowest value first ("rejection") - early in the decision phase. As such the "when" vs. "where" to attend appear to be independently shaped by the trial structure. Additionally, the ramping of motor preparatory activity follows after the stimulus sampling and without rhythmicity. Overall, our results hint at separate mechanisms underlying rhythmic sampling of choices and the build-up of an action plan.

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Poster

212. Visual Cognition and Decision Making in Mammals

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Topic: D.06. Vision

Support: EU Horizon 2020 Research and Innovation Program (ERC starting grant no. 802905)

Title: Flexible information sampling in multialternative choice

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Abstract: Choice among multiple alternatives requires sampling and accumulating decision-relevant information. An influential view, based on eye-tracking, holds that humans and other primates tend to choose the option they have looked at more. However, eye-tracking does not measure covert shifts of attention and hence cannot fully characterize the interplay between attention and choice. Here we combined a multialternative perceptual choice task with magnetoencephalography (MEG) to simultaneously track the locus of attention across choice alternatives (**sampling**) and the propensity to choose one alternative over the others (**decision variable**).
Human participants (N = 20) viewed arrays made up of three Gabor patches that had distinct contrasts and orientations. On different trials, they were instructed to choose the stimulus with either the *highest* or *lowest* contrast. The same physical stimuli thus mapped onto distinct,

orthogonal decision values dictated by the two task *frames*. Each trial began with a *sensory* phase where the stimulus array occurred without task information. This was followed by a *frame-cue* phase instructing the task (high/low), and then a *decision* phase, which was intercepted by a *Go* cue that probed the choice (button/foot-pedal press).
We used an inverted-encoding model (trained on independent localizer data) to assay the time course of attention allocation. The MEG amplitude measured at each sensor was modelled with spatial channels (rectified sinusoids), each tuned selectively for a different angular position. The encoding model was then inverted to estimate the channel responses from the pattern of MEG signals across the scalp in the main choice task. We showed that MEG activity patterns encoded sensory (*contrast*) or *value* information in the *sensory* or *decision* phases, respectively. Although the decoded attention aligned with the decision value for most of the *decision* phase (peaked ~ 270 ms post frame-cue offset), attention was briefly deflected to the low-value option soon after the presentation of the frame cue. Only in this early period, the propensity to choose the attended alternative (depicted in the decision variable) was reduced and not enhanced, suggesting a process of first 'rejecting the worst'. Importantly, this early deflection to the worst was covert and not evident in the eye position.
Our data shed light on how attentional states are structured as a multialternative decision unfolds in time: choosing the highest-value target among many is preceded by an elimination of the worst. This finding may reflect a more general sequential strategy that reduces a complex N-alternative choice into more tractable 2-alternative choices.

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Poster

212. Visual Cognition and Decision Making in Mammals

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Whitehall Research Foundation

Title: A cortical information bottleneck during decision-making

Authors: *M. KLEINMAN[1], T. WANG[2], E. K. LEE[2], N. CARR[2], E. FEGHHI[1], D. XIAO[1], C. CHANDRASEKARAN[2], J. C. KAO[1];
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Abstract: Decision-making signals are observed in multiple brain areas, but an explanation for why multiple areas are involved in decision-making tasks is still missing. The success of deep

artificial neural networks with multiple areas (or layers) has been explained by the implicit optimization of an information bottleneck, where unnecessary information is filtered out so that only the task-relevant information remains. These networks form optimal representations of task inputs that are sufficient to perform the task well, but minimal so that they are invariant and robust to nuisance variables. We hypothesized that the brain also implements an information bottleneck, using multiple areas to form minimal and sufficient representations. We combined electrophysiological recordings in behaving monkeys and multi-area recurrent neural networks (RNNs) to investigate this hypothesis.

We recorded single neurons and multiunits in dorsolateral prefrontal cortex (DLPFC) and dorsal premotor cortex (PMd) in monkeys performing a decision-making task. In this task, monkeys discriminated the dominant color of a checkerboard composed of red and green squares and touched the target that matched the dominant color of the checkerboard. The target configuration was randomized so that color choice (red vs. green) was separated from action choice (left vs. right). We found that while DLPFC represents target configuration, color choice, and action choice, the downstream PMd contains a minimal sufficient representation of only the action choice, reflecting the formation of an optimal representation in cortex in later areas.

To obtain a mechanistic understanding of how the cortex forms these optimal representations, we trained a multi-area RNN to perform the decision-making task. Remarkably, DLPFC and PMd resembling representations emerged in the early and late areas of the multi-area RNN, respectively. We quantified similarity between model and data by quantifying information and comparing low-D projections of the neural and artificial activity. We found that like DLPFC, the RNN orthogonalized axes for action choice information and target configuration. Action choice information was preferentially propagated to downstream areas through alignment of the choice axis with the inter-area connections, whereas target configuration and color choice were not. This led to a downstream representation that only encoded the action choice (and hence both sufficient and minimal).

Our results suggest that cortex implements an information bottleneck through coordinated intra-area computation and inter-area communication, resulting in minimal sufficient representations.

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Poster

212. Visual Cognition and Decision Making in Mammals

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Whitehall Research Foundation

Title: Distinct roles of prefrontal and premotor cortex in decision-making

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Abstract: Dorsolateral prefrontal cortex (DLPFC) and dorsal premotor cortex (PMd) are two clinically relevant brain regions implicated in decision making. However, whether these brain areas have similar or distinct decision-related dynamics and representations remains an open question. Here, we addressed this question by comparing the neural population dynamics within these two brain areas of monkeys performing a red-green reaction time checkerboard discrimination task.

In the task, two targets (red and green), were shown to the monkey with two target configurations (Red left and Green right, or vice versa) to unmix signals related to the color of target chosen to the direction of reach. After a target viewing period, a checkerboard composed of red and green squares appeared. Monkeys discriminated the dominant color in the checkerboard and reached to and touched the target corresponding to the dominant color.

We examined the activity of 2810 units in DLPFC of monkey T, and 996 units in PMd from monkeys T and O. Both single cell and population dynamics (using demixed principal component analysis and decoding) showed neurons in DLPFC are modulated during both the target and checkerboard epochs, where PMd neurons only demonstrated activity after the checkerboard onset.In DLPFC, target configuration related activity emerged shortly after target onset and persisted into the checkerboard period. Here, robust action choice and modest color choice signals were present after checkerboard onset. In contrast, neural activity in PMd was predominantly action choice related, with minimal color and target configuration signals present across the whole trial.

Prior work using tasks that do not unmix signals related to action choice and sensory signals report that decision-related signals are largely similar in multiple brain areas (Cisek, 2012). In contrast, our novel task and electrophysiological recordings provide one of the first clear demonstrations of richer task representations in DLPFC compared to PMd. These results also suggest that DLPFC is upstream of PMd and a likely source of choice signals observed in PMd, an important question that may be addressed by simultaneous recordings in these brain areas. Finally, these results provide rigorous data constraints for developing the next generation of multi-area recurrent neural network models of decision-making (Kleinman et al. 2021).

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Poster

212. Visual Cognition and Decision Making in Mammals

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Title: Initial conditions in dorsal premotor cortex covary with RT, are altered by trial outcome, and combine with sensory evidence to induce choice-related dynamics

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Abstract: How the time-varying and heterogeneous responses of neural populations drive decision-making behavior is a fundamentally unresolved problem. Recent motor planning and timing studies have leveraged a "dynamical systems" perspective to succinctly describe neural computations. Here, we investigated if such a perspective could bridge decision-related dynamics and behavior.

The dynamical systems perspective posits that neural computations are described by a state equation that evolves in time by combining recurrent activity and inputs. Distinct neural state space trajectories (i.e., dynamics) are thereby induced by varying the initial conditions and inputs. For decisions, our two key predictions from this approach are that 1) initial conditions substantially predict the speed and location of subsequent dynamics and behavior and 2) sensory evidence (i.e., inputs) combines with initial conditions to affect the speed of choice-related dynamics.

We tested these predictions by investigating the spiking activity of 996 heterogenous units (single neurons and multi-units, 141 sessions) recorded from the dorsal premotor cortex (PMd) of 2 monkeys performing a red-green reaction time (RT) checkerboard discrimination task where we varied the sensory evidence.

Dimensionality reduction, trajectory analysis, and decoding revealed that the initial condition (as indexed by the prestimulus neural state) predicted the evolution of poststimulus neural trajectories (speed and location) and behavior, specifically RT but not eventual choice. Furthermore, faster RTs were associated with faster pre- and poststimulus dynamics as compared to slower RTs, with these effects observed within a stimulus difficulty. Poststimulus dynamics depended on both the sensory evidence and initial condition, with easier stimuli and "fast'' initial conditions leading to the fastest choice-related dynamics, whereas harder stimuli and "slow" initial conditions led to the slowest dynamics. Finally, the initial condition was altered by the outcome of the previous trial, with slower pre- and poststimulus dynamics and RTs on trials following an error as compared to trials following a correct response.

Together our results suggest that neural activity in PMd is well described by a dynamical system where inputs combine with initial conditions to induce decision-related dynamics. At a broader level, these results naturally bridge previously disparate findings from speed-accuracy tradeoff, post-error adjustment, motor planning and timing research, providing a common framework for deriving models of neural computations underlying decision-making.

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Poster

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Title: Stimulus encoding, working memory, and action selection in dorsolateral prefrontal cortex during perceptual decision making

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Abstract: Perceptual decision-making across time necessitates three processes: encoding and deliberation on relevant sensory stimuli, maintenance of information in working memory, and action selection. Recent studies demonstrate that premotor (PMd; Wang and Montanede et al. 2019) and parietal (LIP; Shushruth et al. 2022) cortices are involved in action selection but not sensory evidence or working memory leaving open the question of where and how these are represented during decision-making. One area potentially involved in these processes is hypothesized to be the dorsolateral prefrontal cortex (DLPFC). We tested this hypothesis by examining neural activity in DLPFC of a macaque monkey performing a red-green checkerboard decision-making task that separates stimulus deliberation and encoding processes from action selection by a working memory delay.
In this task, the monkey viewed a central static checkerboard cue composed of red/green squares. The checkerboard cue then disappears for a variable delay and two targets, red and green, appear to the left and right. The target configuration (left red/right green or vice versa) and sensory

evidence (ratio of red and green squares) are randomized across trials. The task of the monkeys was to touch the target whose color matches the dominant color in the checkerboard. While the monkey performed the task, we used a 32-channel V probe to record single neurons and multi-units from DLPFC (36 sessions, 1,187 units).
We found that DLPFC neurons were responsive in multiple epochs of the task. To identify the variables encoded in these epochs, we performed a regression analysis with sensory evidence, color choice, target configuration, and action choice as predictors of firing rates.
We found that during the checkerboard viewing period, ~25% of units responded to sensory evidence and ~10% of units encoded color choice. Importantly, during the working memory period, the percentage of units modulated by sensory evidence decreased to 10% while the percentage of units encoding color choice remained the same (~10%). When targets appeared, the proportion of units encoding sensory evidence further decreased to zero while 10% of units continued to encode the color choice. In addition, in this epoch, ~15% of units encoded target configuration and ~74% of units later encoded the action choice.
Together, these results indicate that when perceptual decisions span time, areas like PMd, LIP, and DLPFC all participate in action selection. However, only DLPFC carries representations of sensory evidence, working memory, and the target configuration. These results implicate DLPFC as upstream of PMd and LIP during perceptual decision-making.

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Poster

212. Visual Cognition and Decision Making in Mammals

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Topic: D.06. Vision

Title: Anticipatory causal interactions initiates the brain processes subserving visual object identification: An EEG connectivity study

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Abstract: Visual object recognition is an essential behavior of most organisms due to its survival importance. Detection, categorization, and identification are considered to be the component processes of object recognition. Existing literature on visual object processing consider that the visual information processing involves both bottom up and feed forward pathways during visualization of objects. But the anticipatory neural interactions just before seeing a visual objects is not largely known. Since the neural processing involved in visual object recognition occurs in the range of milliseconds, high-density electroencephalogram (HD-EEG) due to their high time resolution and improved spatial resolution, can be considered the best technique for

studying effective connectivity of visual object recognition. We aimed to elucidate the causal interactions underlying the 'Prestimulus' period (100 ms) of visual object identification using effective EEG brain connectivity. In the current study, visual object recognition task (VORT) designed using one hundred images (each for male and female participants) with neutral valence belonging to four categories (Human, animal, plant and inanimate) from the International Affective Picture System (IAPS) and Nencki Affective Picture System (NAPS) was used. Low-level perceptual features of the images were equalized using SHINE (spectrum, histogram, and intensity normalization and equalization) toolbox in MATLAB. EEG was acquired from healthy participants (n = 55) during baseline (eyes open) and 'identification' of visual objects. Standardized low-resolution brain electromagnetic tomography (sLORETA), phase slope index (PSI) and BrainNet Viewer were used for the source analysis, effective brain connectivity and plotting the effective connectivity results respectively. In the "Pre-stimulus" period (100 ms), there were significantly different ($p < 0.05$) causal interactions during the visual object identification when compared to the baseline condition i.e. eyes open: Within as well as between the cortical hemispheres. This study is novel in using EEG derived effective brain connectivity during visual object identification. Current study findings indicate the anticipatory widespread interaction between higher order cortical areas in initating the brain processes subserving visual object identifcation in the prestimulus period. Future research using complex neural network analysis and neural network modelling could help to better understand the strategic anticipatory neural processes underlying visual object recognition in healthy and diseased conditions.

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Poster

212. Visual Cognition and Decision Making in Mammals

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Topic: D.06. Vision

Support: Whitehall Foundation
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Title: Confidence gates the effects of reinforcement on subsequent decision bias and reaction time

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Abstract: To survive in a changing and uncertain world, we must learn from experience to improve our future choices. Decision confidence, the subjective belief that a choice is correct, is thought to play a key role in adjusting decision strategy after feedback, but direct quantification

of this effect using explicit confidence reports remains elusive. Moreover, it is unclear whether and how confidence regulates post-feedback adjustments of decision speed (e.g., post-error slowing). We examined the role of decision confidence in mediating history-dependent choice biases and adjustments of reaction time (RT) in a visual motion discrimination task. We hypothesized that variability in confidence, even for a fixed level of stimulus strength, affects both the speed and bias of subsequent decisions. We trained two rhesus monkeys to perform a choice-RT-wagering version of a random dot motion task ('peri-decision wagering'). The monkeys viewed a dynamic random-dot stimulus and, when ready, reported both the perceived direction of motion (left or right) and a wager on the decision outcome with a single saccadic eye movement to one of four targets: left-high, left-low, right-high, or right-low. Multiple lines of evidence and a control task suggest that the 'high' vs. 'low' wager serves as a reliable index of confidence. We found that the monkeys were biased to repeat a previously rewarded choice, and this bias was inversely proportional to stimulus strength, as shown previously. Here we extend this finding by showing that (1) the history-dependent choice bias is modulated by decision confidence per se, and that (2) confidence also modulates post-feedback adjustments of RT (post-error slowing and post-correct speeding up). Conditioned on motion strength, the bias was greater in magnitude following a low-confidence vs. a high-confidence correct choice, and both monkeys showed larger changes in RT on the subsequent trial following a high-confidence error or a low-confidence correct trial. The results can be explained by a reinforcement learning model incorporating confidence into the reward prediction error (RPE) upon feedback. A reward should lead to a larger RPE for a low-confidence trial due to its smaller expected value. On the other hand, an error should cause a more negative RPE for a high-confidence trial due to its higher expected value. In both cases, a larger magnitude of RPE will result in a larger update to the subsequent decision strategy. This work contributes to understanding confidence-guided learning in perceptual decision making and furnishes an opportunity to explore the neural basis of trial-by-trial adjustments of decision strategy.

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Poster

212. Visual Cognition and Decision Making in Mammals

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Title: Assessing perceptual confidence with and without social context in a continuous perceptual report paradigm

Authors: *F. SCHNEIDER[1], A. CALAPAI[1], A. GAIL[2], I. KAGAN[3], S. TREUE[1];
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Abstract: Perceptual decision making typically combines sensory evidence with the subject's perceptual confidence, yet many perceptual paradigms do not assess confidence and are often designed to minimize effects of confidence variations. The influence of social interactions on perceptual confidence is even less well understood. We aim to assess (i) sensory evidence and perceptual confidence in a subject's real-time perceptual decision-making and (ii) the influence of a (real or simulated) partner's real-time report of these two parameters on the subject's decision-making. We tested individuals and pairs of humans using a newly developed continuous perceptual report (CPR) paradigm, where one's own sensory evidence and perceptual confidence in a visual motion task could be combined with that of a partner to improve task performance. The CPR task presents a long-duration random dot motion pattern (RDP) that is frequently changing in its direction and can have various levels of coherence (signal-to-noise ratios). The RDP's motion direction is predictive of the location of randomly appearing reward targets. Subjects control a response arc with a joystick to indicate the perceived motion direction. The angular width of the response arc is coupled to the joystick's displacement from its center position, with higher displacements causing a smaller arc. Reward is collected if the response arc overlaps with the location of briefly presented reward targets. Both, response accuracy and joystick displacement, determine reward magnitude, implementing a "peri-decision wagering": accurate and confident responses yield high reward, inaccurate confident responses are not rewarded, less accurate low confidence responses yield low reward. In solo settings, subjects see only their own response arc, whereas the behavioral response of a partner is visible in dyadic settings, where two differently colored response arcs are shown. Our findings validate the CPR task: the subjects' behavior contains proxy measures of perceptual confidence. Joystick displacement and correlation measures between the motion pattern and the joystick response change distinctly with the noisiness (coherence) of the stimulus. High RDP coherence causes higher perceptual confidence. In dyadic human interactions subjects' responses change, compared to solo CPR performance. Furthermore, assuming they are interacting real-time with a human, subjects integrate the stimulus evidence provided by the response arc of a highly reliable simulated computer player to maximize their reward outcome.

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Poster

212. Visual Cognition and Decision Making in Mammals

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Title: Change of head direction as a potential indicator of decision making in freely moving ferrets

Authors: *S. ZHU, M. MANCARELLA, F. BRIGGS;
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Abstract: Freely-moving paradigms of visual behavior have many advantages including ease of training, no head fixation, and closer approximations to natural behaviors. However, the inability to control head movement is regarded as a disadvantage. Here we propose that head movement may be useful for inferring the temporal dynamics of decision-making in freely-moving ferrets. We designed a visually-guided behavioral box (after Dunn-Weiss et al, 2019), in which ferrets discriminate the contrast of visual stimuli presented on a monitor while passing through a narrow corridor to encourage forward head position. Ferrets reported their decision by going to reward ports on either the left or the right side of the monitor, corresponding to the higher contrast stimulus. While ferrets passed through the corridor, their nose and neck were automatically tracked using DeepLabCut to calculate their head direction. We trained ferrets to perform the contrast discrimination task in which the two stimuli were briefly presented for 200-250 msec. We quantified the predictability of animals' decisions based on their head direction by computing the area under the receiver operating characteristic curve (AUROC) at various time points during the visual stimulus display. Preliminary analysis indicates that head direction is a moderate predictor (AUROC >= 0.75) of ferrets' decision at time points half way through the visual stimulus display duration. Interestingly, head direction is a strong predictor (AUROC >=0.85) of the upcoming decision by 171 to 229 msec of the visual stimulus display. Predictability of decision by head direction increased over time and AUROC exceeded 0.9 by the end of the visual stimulus display for all ferrets, suggesting that change of head direction may be a good indicator of decision-making processes during freely moving behaviors.

Disclosures: S. Zhu: None. **M. Mancarella:** None. **F. Briggs:** None.

Poster

212. Visual Cognition and Decision Making in Mammals

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 212.11

Topic: D.06. Vision

Support: Whitehall Foundation
France-Merrick Foundation

Title: Population dynamics in areas MT and LIP during concurrent deliberation toward a choice and confidence report

Authors: *M. VIVAR-LAZO, C. R. FETSCH;
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Abstract: The process of forming a perceptual decision encompasses not only the choice itself, but also the time of decision termination (measured by reaction time, RT) and the establishment of a degree of confidence. For visual motion discrimination, previous work has established that cortical area MT represents the momentary evidence on which both choice and confidence are based. Downstream of MT, a network of association cortices (including parietal area LIP) and subcortical structures appears to implement the accumulation of this evidence up to a threshold, which governs RT. Although the relationship between activity in MT and LIP has been studied previously for a fixed-duration choice task, exactly when and how population activity in the two areas contributes to decision termination and confidence remains unclear. We trained two monkeys to discriminate the net direction of motion (left or right) in a dynamic random-dot display, and whenever they were ready, to indicate both their choice and confidence with a single saccade to one of four targets: left-high, left-low, right-high, or right-low, where high and low refer to a wager on the correctness of the direction choice. Juice rewards and time penalties were given according to a 2x2 contingency table that reinforced metacognitive sensitivity, and behavioral analyses validated the assay as a reliable indicator of confidence. During the task, we recorded simultaneously from neural ensembles in MT (N=301 neurons) and LIP (N=64), positioning the motion stimulus and saccade targets to maximize overlap with the receptive fields of neurons in the two areas, respectively. We quantified the probabilistic relationship between spike trains and external variables (behavior, spike history, other recorded neurons, etc.) using generalized linear models. The encoding model for MT shows temporal differences in readout weights for choice and confidence, suggesting possible influence of feedback from decision-related areas. The model also reveals that neurons whose preferred direction is aligned with the choice are the ones most informative of the confidence report, consistent with a 'positive evidence bias' shown in previous studies. Lastly, preliminary results from an encoding model for LIP suggest that encoding of choice and confidence is not identical for a given LIP neuron, but instead can vary in strength and time course for the two decision outcomes. This multiplexed encoding suggests that LIP is not merely reflecting the planned saccade, but contains a dynamical representation of both the perceptual choice and a prediction of its accuracy, i.e. confidence.

Disclosures: **M. Vivar-Lazo:** None. **C.R. Fetsch:** None.

Poster

212. Visual Cognition and Decision Making in Mammals

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 212.12

Topic: D.06. Vision

Support: NIH Grant R01EY019041
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NIH Grant F31MH124395

Title: Abstract category encoding in primate oculomotor circuits: A novel role of the superior colliculus in higher-order cognition

Authors: *B. PEYSAKHOVICH[1], S. TETRICK[2], A. A. SILVA[6], S. LI[3], O. ZHU[1], W. J. JOHNSTON[4], G. IBOS[7], D. J. FREEDMAN[5];
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Abstract: Many animals have a remarkable ability to categorize complex stimuli into behaviorally meaningful groups. Investigations of visual categorization in primates have focused on a hierarchy of cortical areas that transform sensory information into abstract categorical representations. However, categorical behaviors are evident throughout the animal kingdom, including in species without a neocortex, raising a question about the contributions of subcortical regions to primate cognition. One candidate structure is the superior colliculus (SC), a brainstem region that is evolutionarily conserved across vertebrates. Although traditionally thought to mediate reflexive orienting behaviors such as eye movements, the SC is also involved in cognitive tasks that require spatial orienting. However, the role of the SC in non-spatial cognitive functions is unknown. Here, we show that the primate SC robustly encodes learned categories during a non-spatial visual categorization task, and that reversible inactivation of the SC markedly impairs monkeys' performance on the task. Our results suggest that the SC plays an unexpected key role in higher-order visual cognition.
We trained two monkeys to perform a visual categorization task and compared neuronal population activity in the SC and the lateral intraparietal area (LIP), a cortical region previously shown to causally contribute to categorical decisions. Monkeys learned to group dot motion stimuli into two categories defined by an arbitrary rule. The task required monkeys to maintain fixation on a central cue and to report their decisions with a hand movement. We show that single neurons and neural populations in the SC strongly encode monkeys' categorical decisions. This category encoding in the SC arises with a similar latency to the LIP, is evident during both stimulus viewing and a memory delay period, and is independent of the actions monkeys used to report their decisions. Additionally, reversible inactivation of the SC with a GABA agonist (muscimol) strongly impaired monkeys' performance on the categorization task. These results extend the SC's well-established role in spatial orienting functions to abstract, non-spatial cognitive processing, and provide a novel perspective on subcortical contributions to primate cognition.

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Poster

212. Visual Cognition and Decision Making in Mammals

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 212.13

Topic: D.06. Vision

Support: NIH Grant U01 NS098981

Title: Intracranial neural dynamics of cognitive control in rapid visual recognition

Authors: *M. MCCARTY[1], O. WOOLNOUGH[2], X. SCHERSCHLIGT[3], P. ROLLO[4], N. TANDON[5];
[1]MD Anderson UT Hlth. Grad. Sch., Houston, TX; [2]Vivian L Smith Dept. of Neurosurg., UTHealth, Houston, TX; [3]Texas Inst. For Restorative Neurotechnologies, UTHealth Houston Neurosci. Grad. Program, Houston, TX; [4]UTHSC at Houston, Houston, TX; [5]Neurolog. Surgery, Univ. of Texas Med. Sch. at Houston, Houston, TX

Abstract: The ability to select important information from the environment and flexibly adapt this process based on behavioral goals is an essential feature of human cognition. Within human visual processing, flexible cognitive control enables rapid direction of attention to salient stimuli. In a visually crowded environment, in a matter of milliseconds, humans are able to recognize a familiar face in a crowd of people or the words on a street sign. This process is hypothesized to involve a distributed top-down cognitive control network that modulates earlier bottom-up visual processing within the ventral visual stream. fMRI work has revealed that task-based attentional conditions can modulate category selective regions of ventral occipitotemporal cortex (vOTC), such as the visual word form area.
In this study, we utilize the robust spatial and temporal resolution of intracranial EEG recordings to characterize the modulation of category selective regions in vOTC by changing task demands. Data was collected from 21 patients undergoing electrode implantation for seizure localization of intractable epilepsy. We used a rapid visual recognition task, with visual stimuli of different categories (Words, Faces, Scenes, Animals). For the same stimulus set, patients performed multiple tasks with varying cognitive demands: for example, tracking color changes of a fixation point, performing a one back task, or making semantic decisions (e.g., finding names of fruits).We used broadband gamma activity (BGA; 70-150 Hz) as an index of local neural activity.
Within vOTC, we isolated regions selective for words relative to other stimulus categories. Consistent with previous intracranial findings, this region extended more anteriorly than the fMRI-derived visual word form area. Category selective regions of vOTC showed distinct scaling of BGA responses with selectivity increasing as attentional task demand increased, suggesting a task-driven recruitment of distinct cortical substrates. We quantified inter-areal dynamics within patients with concurrent vOTC and inferior frontal gyrus (IFG) coverage. The onset of peak BGA was earliest for vOTC relative to IFG across task conditions. Within IFG, we found a greater and more sustained increase in BGA for trials in which specific stimuli were selectively attended to. This suggests distinct changes in local neural activity within category-selective regions of the vOTC are scaled by attentional task conditions. These findings point to a complex interplay beyond a feedforward model of visual recognition, illustrating that attentional task demands and behavioral state play a critical modulatory top-down role in this process.

Disclosures: M. McCarty: None. **O. Woolnough:** None. **X. Scherschligt:** None. **P. Rollo:** None. **N. Tandon:** None.

Poster

212. Visual Cognition and Decision Making in Mammals

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Program #/Poster #: 212.14

Topic: D.06. Vision

Support: NIH Grant 5U19NS118246

Title: Online estimation and tracking of perceptual biases in animal behavior

Authors: *Y. DONG, G. LENGYEL, S. SHIVKUMAR, A. ANZAI, G. F. DIRISIO, R. M. HAEFNER, G. C. DEANGELIS;
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Abstract: Studying the subjective percepts of animals poses a fundamental challenge in neuroscience. Unlike human participants, animals cannot be verbally instructed to report what they see, hear, or feel. Instead, they must be trained to perform a task using some form of reward. Researchers, then, have to infer from their responses what the animals likely perceived. However, animals' responses are shaped by the reward feedback they receive. If their subjective percepts result in responses that are biased away from the rewarded responses, the animals will learn to compensate for their biased subjective percepts to receive more rewards. In this case, the researcher may incorrectly infer from the animals' responses that they had unbiased percepts. Furthermore, animals' subjective percepts may actually change over time through reinforcement-based learning. This is a concern for most animal studies of the neural basis of perceptual biases since rewards must be provided continuously during the experiment to keep the animals motivated. Addressing these problems for binary forced-choice tasks, we developed a method that estimates the animal's perceptual bias while they are performing the task and then computes the trial's reward based on the animal's estimated subjective percept. Our method relies on data from multiple perceptual contexts that allow us to infer the perceptual bias separately from other decision-related biases. Since individual binary choices provide little information about any bias, we developed a Bayesian method for updating a posterior over the perceptual bias after each trial. This approach allows researchers to specify prior distributions over perceptual and decision biases based on past sessions, thus reducing the variance of the online estimation and allowing it to converge to a stable estimate using only a small number of trials. After validating our method on synthetic data for which the perceptual and decision biases were known, we applied it to estimate perceptual biases of monkeys in a motion direction discrimination task with varying background optic flow conditions that are known to influence motion perception in a subject-specific way. The method could estimate the animal's perceptual biases caused by the different optic flow conditions with relatively high certainty (+/- 2deg) and deliver rewards based on the estimated percept of the animal. This method can be broadly applied by neuroscientists who wish to measure biases in animal perception.

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Poster

212. Visual Cognition and Decision Making in Mammals

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 212.15

Topic: D.06. Vision

Support: NIH Grant EY022979

Title: Sensory responses of cortical output neurons in primary and secondary visual cortices

Authors: *J. COUTO[1], A. K. CHURCHLAND[2];
[1]UCLA, [2]Neurobio., UCLA, Los Angeles, CA

Abstract: Cortical areas influence animal behavior by projecting to not only other cortical regions, but also to subcortical structures, many of which are involved in movement planning. Because cortical neurons often reflect both sensory and movement signals, a natural assumption is that descending outputs convey primarily information about movements. We recently tested this by measuring and manipulating pyramidal tract (PT) neurons in secondary visual areas A/AM. Surprisingly, PT neurons in A/AM were modulated by and causal for auditory discrimination. This observation left many open questions. First, in previous work, activity was measured with widefield imaging which reflects the pooled activity of many neurons. Second, auditory clicks were used exclusively, limiting interpretation of responses in retinotopically organized areas. Here, we set out to surmount these limitations. As in previous studies, we targeted a developmentally defined class of PT neurons: FezF2 neurons, and restricted GCaMP6s expression using the Ai162 reporter line. We used 2-photon imaging to simultaneously measure activity from thousands of neurons and retinotopically targeted primary and secondary visual cortices (V1 and AM). We measured responses in a novel task where visual stimuli (gratings, 25° patches) are presented stochastically at different spatial locations. Lastly, we used encoding and decoding models to determine which task features were represented in neural activity. FezF2 neurons in V1 had robust and reliable responses to the visual patches. These were in the minority but were encountered more frequently than in CaMKII recordings. In addition, most FezF2 neurons responded during reward delivery. This contrasted with measurements from CaMKII recordings where a smaller fraction of neurons were modulated by reward. Similar observations were made in area AM. Linear decoding analysis revealed that the stimulus location can be decoded from AM responses. Interestingly, and in contrast with CaMKII recordings, the decoding accuracy remained elevated during the reward period.
Taken together, these results provide new insights into the sensory responses of AM PT neurons. Our single neuron measurements during visual stimulation confirm what was suggested by widefield imaging, that PT neurons are driven by sensory stimuli. FezF2 neurons were sharply modulated during reward delivery, an effect that was reduced for CaMKII neurons recorded in superficial layers. Unraveling the sensory responses of FezF2 neurons is an initial step to specifying their role, and the role of other PyN types, in cognitive behaviors that rely on visual inputs, such as perceptual decision-making.

Disclosures: **J. Couto:** None. **A.K. Churchland:** None.

Poster

212. Visual Cognition and Decision Making in Mammals

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 212.16

Topic: D.06. Vision

Support: R01 MH118487

Title: High amplitude local field potentials in area V4 impair rapid shape detection

Authors: ***R. S. WAHLBERG**[1,2], T. I. NETOFF[3], G. M. GHOSE[2];
[1]Anesthesiol., Univ. of Michigan, Ann Arbor, MI; [2]Neurosci., [3]Dept Biomed Eng, Univ. of Minnesota, Minneapolis, MN

Abstract: Previous work from our lab has established that individual neurons in area V4 are reliable over a fine timescale in signaling shape presentations and animals' choices on a shape detection task (Weiner and Ghose, 2014). While LFPs in V4 have been shown to exhibit choice-related signals in discrimination tasks (Krishna et al, 2021), their behavioral significance and relationship to single unit firing during rapid visual decision making has yet to be established. In the present study, we simultaneously recorded in two male rhesus macaques single unit and LFP activity across 96-electrode 4x4 mm Utah arrays while animals were engaged in a rapid shape detection task. In this task, saccades to a briefly and peripherally presented shape (~100 ms) embedded in noise were rewarded. Because the frequency band alpha (10 - 30 Hz) has been described as a potential source of interference in the brain and gamma (50 - 70 Hz) a carrier of information, we separately analyzed signals within those two ranges. We found that alpha band interelectrode coherency was higher and had a broader spatial distribution with a space constant greater than 10 mm. To examine the behavioral relevance of the LFPs, for each electrode (local) and across all electrodes (global), we computed the Hilbert transform of band-pass filtered LFP to resolve phase and amplitude over time. We then analyzed the transformed LFP around shape onset to see whether LFPs were predictive on a trial by trial basis of the animals' subsequent performance (correct detection, false alarm, or miss). We found that LFP quiescence in both frequency bands was strongly predictive of correct detections. To quantify this, we computed the F1 performance of a cross-validated linear discriminator using both bandwidths and both local and global LFP signals. Between 44 total sessions, we found considerable variations in F1 (ranging from 0.1 to 0.4), but within sessions we found consistency between how well global and local LFPs predicted behavior. To assess the impact of LFPs on V4 neurons, we then computed trial by trial spike field coherence by averaging spike-locked Hilbert transforms of LFP activity in the epoch preceding shape onset. We found that, on average, spike locking was stronger for alpha, and the absence of alpha spike locking was more consistently associated with correct decisions, as defined by F1 performance, than the absence of gamma spike locking. Given the extended spatial coherence of alpha and gamma band signals, their associations with errors, and

the correspondence with LFP-spike coupling and performance, our results suggest that these LFPs reflect correlated noise that strongly constrains detection performance.

Disclosures: R.S. Wahlberg: None. **T.I. Netoff:** None. **G.M. Ghose:** None.

Poster

212. Visual Cognition and Decision Making in Mammals

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 212.17

Topic: D.06. Vision

Support: Whitehall Foundation
E. Matilda Ziegler Foundation for the Blind
France-Merrick Foundation

Title: A primate model to investigate neural correlates of decision confidence during self-motion

Authors: *S. J. JERJIAN, C. R. FETSCH;
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Abstract: Accurate perception of self-motion is thought to be achieved through integration of multiple sensory cues, particularly those arising from optic flow and inertial motion. The reliability, or information content, of these inputs can vary rapidly over time, and the neural mechanisms underlying optimal integration across time and modalities in this context, particularly when decision speed is determined by the subject, have not been fully uncovered. Furthermore, the correspondence between integration processes informing decisions about self-motion, and the emergence of confidence in those decisions, has not been investigated.
Two adult rhesus macaque monkeys (1 male, 1 female) have been trained to report their perceived heading direction in the horizontal plane (leftward or rightward of straight ahead), based on inertial motion ('vestibular'), full-field optic flow of varying reliability ('visual'), or synchronous presentation of both cues together ('combined'). Subjects first report their left/right choice via saccade to one of two lateral targets as soon as they are ready, yielding a measure of reaction time (RT). They then make a subsequent saccade up or down to indicate a 'high' or 'low' post-decision wager (PDW) on their initial choice. High bets yield larger reward than low bets for correct choices, but longer timeouts for incorrect choices. Behavioral analyses strongly indicate that PDW serves as a proxy for subjective confidence, as stimulus conditioned PDW is predictive of choice accuracy, and inversely related to RT.
Extracellular recording of neural activity is being made during task performance using deep linear probes (Diagnostic Biochips, Glen Burnie, MD) inserted via transdural guide tubes. Recordings are targeted to areas of the parietal cortex with tuning to vestibular and/or visual task stimuli, with locations guided by anatomical MRI images, gray/white matter transitions, receptive field properties, and observed physiological responses to translations in the horizontal plane and/or optic flow stimuli. Analysis of neural activity is expected to provide insight into

population encoding of momentary evidence regarding self-motion bearing on choice and wager outcomes and inform the biological plausibility of accumulation models accounting for choice, RT, and decision confidence in dynamic multisensory environments.

Disclosures: S.J. Jerjian: None. **C.R. Fetsch:** None.

Poster

212. Visual Cognition and Decision Making in Mammals

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 212.18

Topic: D.06. Vision

Title: The trajectories of the early eye movements go through special visual attractors which shed light on the parallel mechanism of human visual recognition

Authors: *O. LEVASHOV;
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Abstract: The theory of Scanpaths (Norton and Stark, 1971) predicts that the trajectories of eye scans are repeated for a familiar sketchy line picture. To test how this theory will work for complex color visual scenes we recorded the eye fixations at complicated pictures of up to 25o size with the help of short exposures (300--900 ms) of five different pictures in the random series and the presentation immediately at 300 ms of a post-exposition matrix of color digits from 1 to 9. According to the subject's (S) report, (a total of 52 Ss and more than 2000 expositions) we can determine the 3 consistent eyes in the pictures. The adequacy of this technique was confirmed using an eye tracker. No repetitive trajectories were obtained. We found that all the Ss have a hierarchy of VISUAL ATTRACTORS, where they direct their eye saccades from the most to the less important. The most important attractors were the reflections in the water and the distorted shapes of familiar objects (as in the paintings of S. Dali). The less important attractors were faces, figures of people and animals, written words, and familiar textures. We also identified two groups of Ss with different scanning patterns. The first group ("inspectors") can see the whole scene, and the second group ("analysts") can see only the central part of the image and do not grasp the whole plot of the scene. Paradoxically, during the exposure of 300 ms, all subjects were able to choose one of their important attractors and make a saccade to transfer this fragment to the fovea. Since the latent period of the saccade is 200-250 ms. this means that the preliminary analysis of a complex picture and the choice of its important fragment takes no more than 50-100 ms. The data obtained indicates against the traditional theory of visual recognition based on the sequential collection of features and comparing them with memory samples (Treisman A., Gelade G. 1980, Wolfe J.M. 1994). We can assume that recognition occurs according to the BOTTOM-UP parallel scheme. Our model assumes interaction in the recognition of M- and P systems (dorsal and ventral canals). We propose that the first work is the M system, which recognizes the low-frequency "silhouettes" of scene fragments and thereby reduces the search in

memory. The proposed technique for early eye recording makes it possible to find the early predictors of abnormal visual perception in dementia, AD, and PD.

Disclosures: O. Levashov: None.

Poster

213. Cerebellum: Climbing Fibers and Learning

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 213.01

Topic: E.02. Cerebellum

Support: NIH NS116854

Title: A contribution of voltage-gated K currents to the spike afterdepolarization in mouse inferior olivary neurons

Authors: *Z. W. SULTAN, M. NAJAC, I. M. RAMAN;
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Abstract: Neurons of the inferior olive (IO) fire action potentials with a large, long-lasting afterdepolarization (ADP), which is shaped by hyperpolarization-activated cation current (Ih), T-type Ca current, SK current, and Cl(Ca) current, and modulated by subthreshold oscillations. Longer ADPs support more spikes in climbing fiber (CF) axons and evoke longer bursts of complex spikes in Purkinje cells, which affect the magnitude and sign of cerebellar synaptic plasticity. Here we tested how IO action potential waveform and the ADP change with different stimulus conditions by making whole-cell recordings in brainstem slices from C56BL6/J mice (N=7 male, 1 female; P25-31). IO cells (N=8) fired at a mean rate of 0.45 ± 0.19 spikes/s. Action potentials were of high amplitude, reaching a peak of 42.4 ± 2.3 mV, with ADPs of 34.3 ± 1.9 ms. The max rate of rise, proportional to Na current, occurred near 0 mV and was 369.6 ± 39.0 V/s (comparable to Purkinje simple spikes). The max rate of fall, however, was nearly an order of magnitude lower, only -49.6 ± 7.9 V/s, indicative of unusually small K currents. It occurred just negative to the action potential peak and decayed to nearly zero near 0 mV, at of the onset of the ADP. These results suggest that the ADP results not only from actively depolarizing currents but also from a lack of repolarizing K current. F-I curves indicated a maximum firing rate of 12.2 ± 2.2 spikes/s (N=7), and refractory period analysis suggested a minimum interspike interval of ~50 ms. In both cases, ADPs became briefer with depolarization, consistent with deactivation of Ih and inactivation of T-type current. After 500-ms hyperpolarizations, however, which should maximally activate/recruit Ih and T-type current, anode break action potentials repolarized rapidly to ~ -50 mV and lacked ADPs altogether, consistent with recovery of voltage-gated K currents at negative voltages. Pilot voltage-clamp studies confirm the presence of voltage-gated, rapidly inactivating K current in IO cells. Thus, inactivating K currents may regulate spike shape in IO cells, shortening the ADP after oscillation troughs or inhibition. The number and duration

of propagating CF action potentials may therefore be maximized in a narrow range of voltages, which may influence cerebellar plasticity.

Disclosures: Z.W. Sultan: None. **M. Najac:** None. **I.M. Raman:** None.

Poster

213. Cerebellum: Climbing Fibers and Learning

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 213.02

Topic: E.02. Cerebellum

Support: DFG (SCHO 820/6-1, SFB 1089)
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Title: Rim4 deficiency in purkinje cells disrupts neuronal signals in cerebellar circuit and therefore causes a motor dysfunction in mice

Authors: *H. KIM, N. MELLITI, E. SCHÖNHENSE, K. MICHEL, S. SCHOCH, D. DIETRICH;
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Abstract: Signal transmission from a neuron to its target cell is tightly controlled by synaptic proteins. Rab3-interacting molecules (RIMs) are key proteins in regulating neurotransmitter release at the presynaptic active zone. Among four members of the RIM family, the function of the short RIM isoforms, RIM3 and RIM4, has not been fully resolved yet. In this study, we investigated the functional role of RIM4, using RIM4 lacking mice. We generated constitutive RIM4 KO mice (RIM4 KO_{const}) that develop an episodic motor phenotype that strongly impairs their hindlimbs. Unlike the large RIM isoforms, we find that RIM4 has no critical role in the regulation of presynaptic neurotransmitter release. In accordance with its previously described role in the regulation of neurite outgrowth dendritic arborization of Purkinje cells is decreased in RIM4 KO_{const} mice. *In vitro* recordings of spontaneous spikes of Purkinje cells show that RIM4 KO_{const} mice display a disturbed pace-making activity and strongly altered spikes upon caffeine application. The functional alteration in spontaneous activity induced by RIM4 deficiency but not the effect on dendritic growth is reversible in neonatal and adult cerebellar Purkinje cells at the cellular level by re-expression of RIM4 after via rAAV transduction. We further examined the functional role of RIM4 in mediating calcium signaling in cerebellar network by performing calcium imaging in slices and *in vivo*. Climbing fiber-mediated calcium transients in Purkinje cells are strongly reduced in RIM4 KO_{const} mice. *In vivo* calcium imaging also showed a reduced

synchronicity of the spontaneous calcium events in neighboring Purkinje cells in RIM4 KO_{const} mice. Our slice recordings suggest that Purkinje cells without RIM4 show a deficit in the integration of synaptic input from the inferior olive.Taken together, our data shows that RIM4 deficiency unexpectedly shows a much more severe phenotype than the RIM1/2 KO even though synaptic transmission is not altered.

Disclosures: H. Kim: None. **N. Melliti:** None. **E. Schönhense:** None. **K. Michel:** None. **S. Schoch:** None. **D. Dietrich:** None.

Poster

213. Cerebellum: Climbing Fibers and Learning

Location: SDCC Halls B-H

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Topic: E.02. Cerebellum

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Italian Ministry of Health (Ricerca Corrente)

Title: Activity-dependent structural plasticity reshapes cerebellar climbing fiber morphology and connectivity

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Abstract: Cerebellar climbing fibers (CFs) convey a teaching signal to Purkinje cells (PCs) that is crucial for learning. It has been shown that CFs can undergo activity-dependent synaptic plasticity and that they are able of major lesion-induced structural plasticity. However, it is still not known whether they are able of activity-dependent structural modifications during adulthood and how this affects the architecture and function of the olivocerebellar circuit. Here we investigate whether CF activity controls the morphology of the fiber itself and of its target PC, as well as the underlying molecular mechanism. To this aim, by using shRNA-expressing lentiviral vectors, we chronically reduced CF intrinsic excitability *in vivo* in C57BL/6J juvenile mice by knocking-down voltage-gated sodium channels (NaV) or the growth-associated protein GAP-43 (which we have previously shown to mediate both lesion-induced and lesion-independent CF structural modifications) and we assessed the morphological effects on CF and PC morphology by immunofluorescent staining, confocal microscopy, and 3D reconstructions. We confirmed that knocking-down GAP-43 causes a CF atrophy (affecting its length and branching) also in the

animal model chosen for this study. We then observed that knocking-down NaV causes a similar CF atrophy, mimicking GAP-43 knock-down, and additionally induces a compensatory increase of the density of synaptic terminals as well as of PC dendritic spines. Our data, therefore, show that CFs can undergo activity-dependent structural plasticity, affecting PC morphology as well, and suggest that this plastic modification may be mediated by GAP-43.

Disclosures: **M. Musto:** None. **M. Bergamini:** None. **A. La Terra:** None. **A. Marte:** None. **F. Benfenati:** None. **G. Grasselli:** None.

Poster

213. Cerebellum: Climbing Fibers and Learning

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 213.04

Topic: E.02. Cerebellum

Support: NIH Grant NS062771
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Title: Climbing fiber multi-innervation and signal independence across branches in adult cerebellar Purkinje cells with multiple primary dendrites

Authors: *S. E. BUSCH, C. HANSEL;
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Abstract: Our burgeoning ability to probe, perturb, and model ever larger neuronal populations promises to shed light on incredibly complex and deep-seated questions in the field of neuroscience. Before this can happen, however, it is imperative that we understand the operations performed by individual neurons. Even within defined cell types, neurons have diverse morphology, connectivity, and intrinsic properties that can influence their computational capacity, yet many such relationships between cellular properties and function are poorly understood or unknown. In our work, we use electrophysiology and calcium imaging techniques in vitro and in live mice to describe new morphology-dependent properties of cerebellar Purkinje cells (PC), which integrate input from granule cell parallel fibers and inferior olivary climbing fibers to produce the sole cortical output that coordinates motor, cognitive, and emotional performance. First, we show that 10-20% of adult (P21-65) PCs have multiple climbing fiber inputs (CF), contradicting a widespread understanding that every adult PC receives input from one CF. All multi-CF PCs have a proximal bifurcation of their primary dendrite or multiple primary dendrites and the CFs appear to isolate to individual branches in preliminary results. Second, using 2-photon imaging of CF-dependent GCaMP6f signal in PC dendrites of awake mice (>P80), we identify ‘heterogeneous’ events that are either branch-specific or globally expressed but with asymmetric amplitude across branches. The subset of PCs with heterogeneous events also have segregated dendritic compartments and their proportion mirrors multi-CF PCs observed in vitro. Third, we find that PCs with signal heterogeneity also exhibit

branch-specific responses to single whisker stimulation. This confirms that some PCs with segregated compartments sample multiple CF receptive fields representing independent signals from inferior olive. As a result, these PCs exhibit more complex integration of synaptic inputs than previously appreciated. Collectively, these results reject the principle of universal CF→PC mono-innervation and the idea that CFs exclusively provide a homogenous, global dendritic signal. Instead, we suggest that PCs perform a range of input-output operations and that the subset of PCs described in this work - whose output represents multiple CF→PC circuits - could enhance salience and contrast operations relayed from the cortex to cerebellar nuclei, thus optimizing cerebellar output during motor, cognitive, and emotional tasks.

Disclosures: S.E. Busch: None. **C. Hansel:** None.

Poster

213. Cerebellum: Climbing Fibers and Learning

Location: SDCC Halls B-H

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Topic: E.02. Cerebellum

Support: NIH R21EY031639-01A1
NIH R01DC004154-18
NIH R01NS072406-09S1

Title: Plasticity mechanisms for bidirectional oculomotor learning

Authors: *H. SHIM, A. FANNING, J. RAYMOND;
Neurobio., Stanford Univ., Stanford, CA

Abstract: Activity-dependent neural plasticity has long been thought to be a cellular substrate for experience-driven behavioral changes. A fundamental unanswered question is the direction of plasticity (long-term potentiation, LTP vs. long-term depression, LTD) at each synaptic site within a given circuit during learning. In the cerebellum, LTD at the parallel fiber-to-Purkinje cell synapses (PF-PC LTD) is well established as a cellular mechanism supporting learning. Yet accumulating evidence suggests that PF-PC LTD contributes selectively to certain cerebellum-dependent learning tasks, and not others, raising the question of how other plasticity mechanisms in the cerebellum contribute to learning. Here, we identify a role of LTP at the PF-PC synapses in bidirectional vestibulo-ocular reflex (VOR) learning. Published results reported PF-PC LTD in slices of the cerebellar flocculus prepared from mice that had learned to increase the amplitude of their VOR (VOR-increase learning; Jang et al., 2020). Here, we performed slice electrophysiology following learning to decrease the amplitude of the VOR, induced by vestibular-visual pairing (VOR-decrease learning) and following habituation of the VOR, induced by vestibular stimulation alone. We observed PF-PC LTP following both VOR-decrease learning and VOR habituation. In addition, 100 Hz optogenetic stimulation of granule cells, whose axons form the PFs, to induce PF-PC LTP occluded subsequent VOR-decrease or

habituation learning. To gain insight about which subset of PF-PC synapses may undergo LTP, we measured the generalization of VOR learning to the optokinetic reflex (OKR), the reflexive eye movement driven by motion of a visual field. During VOR-decrease learning, the population of PF-PC synapses in the flocculus carries visual as well as vestibular signals, whereas during VOR habituation only the PF-PC synapses carrying vestibular signals would be activated. The OKR was increased after VOR-decrease learning, consistent with previous work, but not after habituation. Hence, during VOR-decrease learning, LTP may occur selectively in PF synapses carrying vestibular signals, while PF synapses carrying visual signals may undergo LTD, which has been implicated in increasing the OKR. Together, the results indicate that bidirectional oculomotor learning is supported by bidirectional changes at the PF-PC synapses, and highlight the necessity for additional work to integrate analyses of coding of sensory and motor signals as well as plasticity at these synapses.

Disclosures: H. Shim: None. **A. Fanning:** None. **J. Raymond:** None.

Poster

213. Cerebellum: Climbing Fibers and Learning

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Topic: E.02. Cerebellum

Support: NINDS R01 NS072406
Simons Collaboration on the Global Brain 543031

Title: Sensitivity of oculomotor learning to the timing of climbing fiber activation during training

Authors: *A. SHAKHAWAT, J. L. RAYMOND;
Stanford Univ., Stanford, CA

Abstract: The timing between neural events is a key determinant of what is learned. In the cerebellum, error signals carried by its climbing fiber input from inferior olive play a critical role in inducing plasticity, presumably by weakening synapses that were active around the time than an error was generated. During oculomotor learning, results from recording and stimulation experiments have indicated that activation of the climbing fiber input to the cerebellar flocculus during an ipsiversive vestibular stimulus induces a learned increase in the amplitude of the vestibulo-ocular reflex (VOR), whereas activation of the climbing fibers during a contraversive vestibular stimulus induces either a learned decrease in the amplitude of the VOR or no learning. Here we used optogenetic stimulation of the climbing fibers to analyze in more detail how the timing of climbing fiber activation relative to a sinusoidal vestibular stimulus influences VOR learning. The results reveal greater sensitivity to the timing of climbing fiber activation than expected. When VOR-increase learning is induced by pairing a sinusoidal vestibular stimulus with oppositely directed image motion, climbing fiber spiking occurs throughout much or all of

the ipsiversive vestibular half-cycle. However, when optogenetic stimulation of the climbing fibers was substituted for image motion, VOR-increase learning was induced only if climbing fiber activation was paired with peak ipsiversive head velocity, and not if the climbing fibers were activated 125 ms before or after peak ipsiversive head velocity during the 1 Hz vestibular stimulus. These findings constrain the rules governing learning in the oculomotor cerebellum.

Disclosures: A. Shakhawat: None. **J.L. Raymond:** None.

Poster

213. Cerebellum: Climbing Fibers and Learning

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Program #/Poster #: 213.07

Topic: E.02. Cerebellum

Support: ANR-19-CE37-0011

Title: In vivo recordings in the cerebellar cortex reveal sagittal bands of activity that adapt to sensorimotor mismatch

Authors: V. NGUYEN, *B. STELL;
SPPIN, CNRS, Paris, France

Abstract: The cerebellum plays an important role in motor adaptation and this is highlighted in individuals with cerebellar degeneration; in addition to ataxia, they are unable to adapt to sensorimotor disturbances while performing simple motor tasks. This is evidenced in a simple reaching task where subjects wear glass prisms that laterally shift their visual field; the prisms initially cause healthy subjects to miss to the side but they adapt their reach after several trials. Subjects with cerebellar damage fail to adapt.The cerebellum likely achieves motor adaptation via implementation of forward models to generate predictions of expected sensory feedback (expected reafference) based on a copy of the motor command. This expected reafference is hypothesized to be compared to the actual, perceived reafference to compute differences that can be used to modify future behavior. In the aforementioned reaching task, the prisms cause differences between perceived and expected reafference that drive changes in behavior. To unravel the cellular and synaptic changes that underlie this adaptation requires a behavioral task that recapitulates the essential components of a task like prism adaptation while allowing access to the brain cells involved. Using a reaching task in head-restrained mice we impose differences between expected and perceived reafference and use imaging and electrophysiology to observe activity in the cells of the cerebellar cortex.We find sagittal bands of synchronized activity timed to movement onset that disappear when the same afferent signal is consistently accompanied by a motor command. This finding suggests that the activity in these bands of cells signal unexpected sensory input. To confirm this, we altered the reafference associated with the motor command and observed activity returning to the same sagittal bands. Our findings demonstrate a new reaching paradigm in mice that provides access to components of the cerebellar forward

model that signal sensorimotor mismatch while mice adapt to differences in expected and perceived reafference.

Disclosures: V. Nguyen: None. **B. Stell:** None.

Poster

213. Cerebellum: Climbing Fibers and Learning

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Topic: E.02. Cerebellum

Support: Wellcome Trust 224668/Z/21/Z to MH
Wellcome Trust 221674/Z/20/Z to LFR

Title: Fast and slow learning signals in cerebellar climbing fibers shaped by differential brain-wide inputs to olivary neurons

Authors: S. CLOTHIER, L. F. ROSSI, M. HAUSSER, *D. KOSTADINOV;
Wolfson Inst. for Biomed. Res., Univ. Col. London, London, United Kingdom

Abstract: Climbing fiber (CF) inputs to cerebellar Purkinje cells (PCs) encode sensorimotor and reward-related instructive signals that are utilized to learn and refine goal-directed behaviors. However, it remains unclear how these instructive signals evolve during the acquisition and refinement phases of learning, whether they target the same PCs, and whether different brain-wide pathways drive functionally distinct populations of CFs.
Here, we used 2-photon calcium imaging to record CF inputs to PCs chronically over weeks in two cerebellar forelimb regions - lobules V (LV) and simplex (LS) - while head-fixed mice learned to rotate a wheel to move a visual stimulus to a target location and obtain a reward. Once mice were experts, we probed fast, adaptive learning by altering the gain between object translation and wheel movement. Mice adapted to these gain changes within 100 trials of a single training session. To quantify which sensorimotor and reward-related factors best explained CF activity and how these inputs change with learning, we fit regression models and compared how models trained on recordings from expert mice generalized to naive mice, and vice versa. Finally, to elucidate the circuits driving functionally distinct CFs, we are utilizing monosynaptic rabies tracing (TRIO method) to identify the brain-wide inputs to olivary neurons projecting to LV or LS.
CFs inputs to LV preferentially signaled movements predictively, and these signals became more reliable as mice became experts in the task. In contrast, CFs inputs to LS preferentially encoded reward-related signals. These reward signals were dynamic on both slow and fast time-scales: they were progressively suppressed as mice became experts but re-emerged when rewards became scarce during gain adaptation. Consistent with these functional differences, anatomical tracing showed that forebrain motor structures preferentially innervate LV-projecting olivary neurons. Surprisingly, LS-projecting olivary neurons only received sparse input from midbrain

reward circuitry, indicating that their reward signals may not be inherited directly from the brain's canonical reward pathway.
Our results suggest that instructive CF signals define functionally distinct cerebellar regions: LV primarily receives sensorimotor instruction and mediates the slow acquisition of goal-directed behaviors; LS preferentially receives reward-related instruction that can guide fast adaptation to dynamic environmental contexts. We are exploring whether this regional functional specialization may result from the differential brain-wide circuits driving distinct populations of presynaptic olivary neurons.

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Poster

213. Cerebellum: Climbing Fibers and Learning

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Program #/Poster #: 213.09

Topic: E.02. Cerebellum

Support: R01NS096289

Title: Cerebellar Climbing Fiber Signal Context-Dependent Reward Predictions

Authors: *C. VIGNALI, M. MUTERSBAUGH, C. HULL;
Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: The cerebellum plays a key role in generating appropriate motor output, and can operate according to the principles of supervised learning instructed by error-based climbing fiber (CF) signals in many behaviors. However, we have also shown that CFs can signal reward-related predictions, suggesting the possibility that the cerebellum can also operate according to reinforcement learning principles. It is necessary to reveal whether CFs can deliver true reward prediction error (rPE) signals, the instructional signals required for reinforcement learning, to the cerebellar cortex. To test this hypothesis, we have used 2-photon calcium imaging to measure CF input to Purkinje cells of the lateral cerebellum during a modified classical conditioning task. Specifically, we have measured CF activity in response to two visual stimuli, one that predicts reward and one that predicts no reward. Surprisingly, we find that CFs generate equivalent responses to cues that predict reward and no-reward, but only if these cues are both highly similar and presented pseudorandomly in time. In this condition, CFs respond to both cues, even though the mice can accurately distinguish them behaviorally. To test whether this result reflects stimulus generalization, we altered the context of the cues by separating them in time, and by changing their sensory modality. These experiments revealed that CF responses are not generalized, but instead vary according to the context in which the cues are presented, with responses becoming more divergent as the cues are made more distinct. To distinguish whether CF responses indeed reflect the context of stimulus presentation or are instead related to training history, we reversed the learning rule in trained animals. These experiments revealed that CF

responses can flexibly re-learning reward associations, and are not fixed by initial learning or training history. We find that reward-predictive CF responses depend on behavioral context in the same manner as dopamine neurons of ventral tegmental area (VTA). Together, these results suggest that CFs can generate learned, reward predictive responses that depend on stimulus context, and can rapidly update the cerebellar cortex in response to changing sensory information and current reward-related learning rules.

Disclosures: **C. Vignali:** None. **M. Mutersbaugh:** None. **C. Hull:** None.

Poster

213. Cerebellum: Climbing Fibers and Learning

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Topic: E.02. Cerebellum

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Title: Signaling agency of an erroneous outcome via synchronization of complex spikes

Authors: *J. PI, E. SEDAGHAT-NEJAD, M. FAKHARIAN, P. HAGE, R. SHADMEHR; Johns Hopkins Univ. Dept. of Biomed. Engin., Baltimore, MD

Abstract: We learn how to move because the inferior olive provides a teaching signal to the Purkinje cells (P-cells) of the cerebellum, modulating the rates of complex spikes (CS) to indicate that the sensory consequences of a movement were in error. However, these rate changes are not specific to erroneous movements. Rather, the changes in CS rates follow a variety of sensory events and precede a variety of movements. How does the brain claim agency for an erroneous outcome, and thus learn from it, if a multitude of sensorimotor events modulate the CS rates? To answer this question, we trained two marmoset monkeys (Callithrix jacchus) on a saccade adaptation task in which a primary visual target was jumped at saccade onset to a random location (2nd target), inducing a motor error. This task included conditions in which visual events sometimes but not always instructed a movement, and movements that were sometimes but not always preceded by a visual event. Some of the visual events were unpredictable, producing sensory prediction errors (SPEs), while others were predictable. Critically, only one of the SPEs signaled the erroneous outcome of a movement. While the marmosets were performing these tasks, we recorded from vermis lobules VI and VII of the cerebellum and isolated 268 individual P-cells and 99 simultaneously-recorded, pairs of P-cells. We found that the CS rates were modulated both in response to the visual events that produced movements and the movements that followed. Moreover, the CS rates were modulated similarly following predictable and unpredictable visual events. Critically, the CS rates could not distinguish the visual event that indicated the motor error.Whereas the CS rates were strongly

modulated following the onset of the primary and center targets, and before the onset of primary and center saccades, CS synchrony was not affected. Instead, CS synchrony increased only after the sensory event that signaled the motor error.We next devalued the erroneous sensory consequences: in some sessions the 2nd target was presented briefly and reward no longer required a corrective movement. Remarkably, even though devaluation of the 2nd target led to reduced CS rates, the CSs synchronized as the transient 2nd target still caused motor error at the end of primary saccade.In sum, in the cerebellum the erroneous consequences of a movement were not signaled via modulation of firing rates. Indeed, regardless of predictability, the CS rates were modulated by the visual events that led to movements, and could not distinguish motor errors from other events. Rather, to identify the erroneous consequences of movements, the CSs became synchronized.

Disclosures: J. Pi: None. **E. Sedaghat-Nejad:** None. **M. Fakharian:** None. **P. Hage:** None. **R. Shadmehr:** None.

Poster

213. Cerebellum: Climbing Fibers and Learning

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Topic: E.02. Cerebellum

Support: NIH Grant R01NS096289

Title: Cerebellar climbing fiber activity driven by unexpected reward influences both neural and behavioral learning

Authors: *S. JIN, C. HULL;
Duke University, Dept. of Neurobio., Durham, NC

Abstract: The cerebellum plays a key role in motor coordination and learning. Classical models posit that cerebellar learning is instructed by teaching signals from climbing fibers (CFs) that act according to supervised learning principles by reporting motor errors. Recently, we have revealed that CFs can also signal reward-predictive information in some behaviors, suggesting the possibility that CFs can operate according to reinforcement learning principles. To test this hypothesis, and determine whether CFs can signal reward prediction errors (rPEs) in a manner similar to dopamine neurons of the ventral tegmental area (VTA), we have measured CF responses in Purkinje cells of the lateral cerebellum during a modified classical conditioning task using 2-photon calcium imaging. Specifically, we have performed stimulus blocking experiments to determine whether CF activity meets the requirements of an rPE signal for transfer from an unexpected reward to a reward-predictive cue. Consistent with the principles of reinforcement learning, we find that once CF activity is transferred to a conditioned stimulus, and there is no longer a response to reward, CFs cannot generate learned responses to another conditioned stimulus that carries the same reward prediction. In addition, by expressing the inhibitory opsin

GtACR2 in neurons of the inferior olive, and optically inhibiting these neurons across behavioral training at the time of unexpected reward, we find that the transfer of CF signals to the conditioned stimulus is impaired. Moreover, this optogenetic inhibition also impairs learning, and results in licking that is not appropriately timed. Together, these results indicate that CF signals exhibit several characteristics consistent with rPEs and reinforcement learning, and that the cerebellum can harness this form of learning to generate accurate motor behaviors.

Disclosures: S. Jin: None. **C. Hull:** None.

Poster

213. Cerebellum: Climbing Fibers and Learning

Location: SDCC Halls B-H

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Program #/Poster #: 213.12

Topic: E.02. Cerebellum

Title: Complex spikes perturb movements, revealing the direction of action of cerebellar Purkinje cells

Authors: *S. MULLER[1], J. PI[2], E. SEDAGHAT-NEJAD[3], R. SHADMEHR[4];
[1]Columbia Univ., Columbia Univ., New York, NY; [2]Biomed. Engin., [3]Johns Hopkins Univ., Baltimore, MD; [4]Dept Biomed. Eng, Johns Hopkins Univ. Dept. of Biomed. Engin., Baltimore, MD

Abstract: Cerebellar Purkinje cells (P-cells) generate two types of spikes: complex spikes (CS) that are generated in the dendrites and have a baseline firing of ~1 Hz, and simple spikes (SS) that are generated in the axon and have a baseline firing of ~50 Hz. SS dominate the output of the P-cells, influencing nucleus neurons and their downstream targets, while CS have been shown to drive learning at the parallel fiber P-cell synapses and thus modulate SS firing. However, theories of cerebellar function have long postulated that CS may directly affect behavior via the potent effect of one CS on the pattern of SS firing. We examine this question in the context of saccadic eye movements in primates. We measured activities of n=270 P-cells in the marmoset cerebellum during visually guided saccades. In response to the presentation of a visual target, and before the onset of the saccade, the CS firing rates were modulated, increasing above baseline when the target was in the preferred direction (labeled CS-on), and suppressed below baseline when it was in the opposite direction (CS+180). Because the occurrence of a CS was a probabilistic event, on some occasions a saccade was made to a given target but immediately preceded by a CS, while in other times the same saccade took place without a CS. We compared the trajectories of these pairs of saccades and found that when a CS took place around saccade onset, the trajectory was perturbed during the saccade. Remarkably, this perturbation was not random, but consistently pushed the eyes in the CS-on direction, with little or no component of the perturbation occurring in direction CS+90. Because occurrence of a CS is followed by a brief cessation of SSs, these results suggest that CSs produce a disruption of movements by disinhibiting the nucleus. The result of this disinhibition is a perturbation in

direction CS-on, thus suggesting a congruence between the sensory inputs that a P-cell receives from the inferior olive, and the direction of motor output that the nucleus cell receiving P-cell output produces. Our results also provide an explanation for earlier work which had reported that CS firing rates during reaching appear to foretell future errors (Streng et al. 2015).

Disclosures: S. Muller: None. **J. Pi:** None. **E. Sedaghat-Nejad:** None. **R. Shadmehr:** None.

Poster

213. Cerebellum: Climbing Fibers and Learning

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Topic: E.02. Cerebellum

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Title: Temporal dynamics of Purkinje cells output drives the early phase of cerebellum-dependent systems consolidation

Authors: *J. SEO[1,2], Y. KIM[1,2], S. KIM[1,2];
[1]Dept. of Biomed. Sci., [2]Dept. of Physiol., Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Memory transfer, a physiological process adopted by the cerebellum to strength the fragile memory, is a fundamental feature of animals to survive and reproduce. Initially encoded motor memory in cortex stays feeble and only lasts shortly, hence, the cerebellum leads the memory to be transferred to the nuclei, which eventually accomplishes the systems consolidation for long-term storage. To properly facilitate these processes, Purkinje cells (PCs), a sole output of cortical circuit, and its associated intrinsic excitability (IE) is known to play pivotal roles. However, a specific spatiotemporal profile of the excitability remains largely unknown. We developed novel surgical procedure to approach flocculus, a lobe-like cerebellar region that is responsible for oculomotor learning, and adopted optogenetics. Thus, we were able to selectively enhance floccular PC excitability within the temporal window that we defined to be essential for early phase of consolidation. Furthermore, the newly found time window was accompanied by a timely dynamics of IE after oculomotor learning. To better understand the process of memory transfer, the synaptic events in vestibular nuclei following the manipulation was further investigated. Overall, our findings elucidated the precise timing and characteristic contributions of IE for systems consolidation.

Disclosures: J. Seo: None. **Y. Kim:** None. **S. Kim:** None.

Poster

213. Cerebellum: Climbing Fibers and Learning

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Topic: E.02. Cerebellum

Support: NRF Grant 2018R1A5A2025964

Title: Revisiting the cerebellar memory consolidation mechanism from ML perspective: The cerebellum as a dual learning machine

Authors: *H. BAE[1], J. SEO[2], C.-E. KIM[1], S. KIM[2];
[1]Gachon Univ. Col. of Korean Med., Seongnam, Korea, Republic of; [2]Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: The cerebellum is known for the critical site for motor learning, and many studies have been conducted to explore the neural circuits and mechanisms responsible for the memory formation and consolidation. Although they have provided detailed observations in several cerebellum-dependent motor learning paradigms, our knowledge of the underlying processes remains fragmentary. In this study, we employ statistical learning theory in machine learning to propose a novel framework that can explain why and how the cerebellum learns and consolidates memories by transfer mechanism.
We model the cerebellar system as dual learning machine which composed of two systems with different dimensions-cerebellar cortex and vestibular nuclei. The cerebellar cortex represents a “complex” system, which can be characterized as complex representation, fast adaptation, but relatively large overheads. The vestibular nuclei represent a “simple” system, which can be characterized as simple representation, slow adaption, but low overheads. Based on modified empirical risk minimization theory, we predicted that adaptive learning occurs first in the cerebellar cortex and simple components are then transferred to the vestibular nuclei, and the extent and timing of the transfer can vary depending on the task difficulty. Predictions were validated with both computer simulation and optomotor behavioral experiments.
In this study, we tried to model and interpret the cerebellar system from the machine learning perspective. This framework can provide a comprehensive understanding on the cerebellar learning and contribute to further elucidating the essence of cerebellar computations.

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Poster

213. Cerebellum: Climbing Fibers and Learning

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Title: Heatstroke-induced late-onset neurological deficits in mice caused by white matter demyelination, Purkinje cell degeneration, and synaptic impairment in the cerebellum

Authors: K. MIYAMOTO[1], H. OHTAKI[2], *A. YOSHIKAWA[3], M. NAKAMURA[4], K. SUZUKI[4], H. YAMAGA[7], K. YANAGISAWA[5], T. SHIMADA[4], M. HAYASHI[8], K. HONDA[6], K. DOHI[4];
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Abstract: Background: Global warming will increase the incidence of heatstroke in the near future. After heatstroke, patients are bedridden and exhibit some neurological symptoms , suggesting cerebellar damage. However, the potential long-term adverse outcomes are poorly understood. Therefore, our study focused on the effects of heatstroke on the cerebellum.
Methods: Male C57/BL6J mice (10 weeks) were divided into heatstroke (HS) and control (Con) groups. The HS group mice were exposed to high ambient temperature (41 °C) and relative humidity for 1 h. Rotarod tests were performed at 1, 3, 5, 7, and 9 weeks post heatstroke. We prepared another group (Con, HS), and the brains from the Con and HS groups were dissected at 1, 3, and 9 weeks post HE. Kluver-Barrera staining was performed to evaluate the medulla demyelination at the cerebellum and corpus callosum in the Con and HS groups. We also performed calbindin immunostaining to clarify the Purkinje cell numbers in the Con and HS groups. Moreover, synaptophysin (pre-synaptic markers) and postsynaptic density-95 (PSD95, post-synaptic markers) immunostaining were performed to evaluate the Purkinje cell's synapse.
Results: Motor coordination disorder significantly appeared 3 weeks after heatstroke and gradually improved to some extent. Although white matter demyelination was detected at 1 and 3 weeks after heatstroke in the cerebellum, it was not found in the corpus callosum. The Purkinje cell numbers significantly decreased at 1, 3, and 9 weeks after heatstroke. The intensity of synaptophysin and postsynaptic density-95 temporarily appeared to attenuate at 3 weeks after heatstroke; however, both appeared to intensify at 9 weeks after heatstroke. Motor coordination loss occurred a few weeks after heatstroke and recovered to some extent. Late-onset motor impairment was suggested to be caused by cerebellar dysfunctions morphologically assessed by myelin staining of cerebellar white matter and immunostaining of Purkinje cells with pre- and postsynaptic markers. Purkinje cell number did not recover for 9 weeks; other factors, including motor coordination, partially recovered, probably by synaptic reconstruction, residual Purkinje cells, and other cerebellar white matter remyelination. **Conclusions**: Motor coordination loss occurred a few weeks after HE and recovered to some extent. Purkinje cell number did not recover for 9 weeks, the others, including motor coordination, were partially recovered, probably by synaptic reconstruction, residual Purkinje cell, and other cerebellar neuron remyelination. These phenomena were associated with late-onset neurological deficits and recovery after heatstroke.

Disclosures: **K. Miyamoto:** None. **H. Ohtaki:** None. **A. Yoshikawa:** None. **M. Nakamura:** None. **K. Suzuki:** None. **H. Yamaga:** None. **K. Yanagisawa:** None. **T. Shimada:** None. **M. Hayashi:** None. **K. Honda:** None. **K. Dohi:** None.

Poster

213. Cerebellum: Climbing Fibers and Learning

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 213.16

Topic: E.02. Cerebellum

Support: OIST intramural support

Title: Nucleo-olivary pathway bidirectionally modulates inferior olive activity in living mice

Authors: *D. GUO, M. Y. UUSISAARI;
Neuronal Rhythms in Movement Unit, OIST, Okinawa, Japan

Abstract: Inferior olive (IO), cerebellar cortex and nuclei (CN) form the interconnected olivo-cerebellar system (OCS) that is crucial for motor control and learning. The IO receives excitatory ascending and descending afferent axons from most parts of the central nervous system. Its axons, called the climbing fibers, target Purkinje neurons (PN) in the cerebellar cortex. Climbing fiber activity evokes distinctive depolarization bursts in PN known as complex spikes (CS), central to most theoretical models of OCS function. In addition to the afferents from the extracerebellar regions, the IO is also innervated by a subset of CN neurons that form a nucleo-olivary (NO) pathway. While the cerebellum could regulate IO activity and thereby its own CS via the NO axons, it has not been directly demonstrated in living animals. To examine how the NO pathway modulates the way IO relays signals to the cerebellum, changes in IO spike probability and waveform were analyzed in experiments with local optogenetic (OG) activation of NO axons and periorbital airpuff. GCaMP6s and ChrimsonR were expressed via viral transfection in IO and CN neurons, respectively, for calcium imaging and optogenetics. A miniscope with gradient-index lens was used for imaging the IO neurons and stimulating the NO axons residing in the recorded area. To record IO activity without damaging its afferent connections or neighboring brainstem regions vital for survival, we approached the superficial ventral areas of IO through the neck viscera in anesthetized animals. Spontaneous spiking of IO neurons (0.1±0.04 Hz, comparable to activity recorded in vitro) was significantly reduced (to 0.06±0.07 Hz, $p < 0.005$; n = 84 cells in 8 animals) during OG activation of NO axons (5s, 20 Hz). Furthermore, there was a slight but significant ($p < 0.005$) decrease in the GCaMP6s fluorescence during OG stimulation, suggesting that NO pathway may modulate the tonic calcium levels in IO cells. In 72 out of 84 cells, rebound spikes were observed (in 40±19% of stimulation trials) after the OG stimulations. The rebound spikes were broader than those evoked by airpuff (rise time 0.70±0.24 s vs 0.61+-0.13 s, $p < 0.05$; rebound spikes: n = 169 in 8 animals; control spikes: n = 37 in 3 animals). Thus, in addition to inhibiting IO spiking, activity in NO axons can evoke post-inhibitory rebound spikes and provide a means for the cerebellum to bi-

directionally modulate its own CS firing via NO pathway. Furthermore, as the width of CS has been proposed to influence direction of plasticity events in PN, our results suggest that the broader rebound spikes evoked by cerebellar feedback could result in distinctive modifications of cerebellar cortical circuits.

Disclosures: D. Guo: None. **M.Y. Uusisaari:** None.

Poster

213. Cerebellum: Climbing Fibers and Learning

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 213.17

Topic: E.02. Cerebellum

Support: JPSP KAKENHI Grant Number JP22J23214
MEXT KAKENHI Grant Number 17H06310
RIKEN CBS Collaborative Technical Development in Data-Driven Brain Science

Title: Developing a spiking network model of the cerebellum as a reinforcement learning machine

Authors: *R. KURIYAMA[1], H. YOSHIMURA[2], R. HOASHI[3], T. YAMAZAKI[1];
[1]The Univ. of Electro-Communications, Tokyo, Japan; [2]Neural Computation Unit, Okinawa Inst. of Sci. and Technol. Grad. Univ., Okinawa, Japan; [3]KOEI TECMO GAMES CO., LTD., Kanagawa, Japan

Abstract: The cerebellum has been considered a supervised learning machine, based on various anatomical and physiological research about long-term depression at parallel fiber-Purkinje cell synapses. Recent studies have revealed existence of other synaptic plasticity mechanisms in the cerebellum, particularly those at parallel fiber-molecular layer interneuron synapses. Functional roles of such synaptic plasticity in the cerebellum are gradually being investigated, and based on these investigations, several studies proposed that the cerebellum might be performing reinforcement learning, in addition to conventional supervised learning. In particular, Yamazaki and Lennon (2019) proposed a concept of the cerebellar actor-critic model. However, the model with a spiking network model has not been implemented and validated. Therefore, in this study, we implemented a reinforcement learning machine based on known anatomical properties of the cerebellum. In our implementation, Purkinje cells and molecular layer interneurons act as an actor and a critic, respectively, in the form of an actor-critic model of reinforcement learning. Parallel fibers deliver state information to both Purkinje cells and molecular layer interneurons, whereas a climbing fiber delivers negative reward. Purkinje cells calculate to emit appropriate actions called a policy, whereas molecular layer interneurons calculate temporal difference error from state-value information specified by excitatory postsynaptic currents at parallel fiber-molecular layer interneuron synapses. Furthermore, long-term synaptic plasticity at parallel fiber-Purkinje cell synapses as well as parallel fiber-molecular layer interneuron synapses, and

short-term synaptic plasticity at parallel fiber-molecular layer interneuron synapses are implemented to update internal parameters. To validate our model, we conducted simulation of Pavlovian delay eyeblink conditioning, and reproduced characteristic pause of Purkinje cells as conditioned responses. We also examined a machine learning benchmark known as a mountain car. These results support the hypothesis that the cerebellum could act as a reinforcement learning machine, and suggest that further research on not only Purkinje cells but also other neurons in the cerebellar cortex is necessary for better understanding of functional roles of the cerebellum.

Disclosures: R. Kuriyama: None. **H. Yoshimura:** None. **R. Hoashi:** None. **T. Yamazaki:** None.

Poster

213. Cerebellum: Climbing Fibers and Learning

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 213.18

Topic: E.02. Cerebellum

Support: NIH Grant R01 NS045193
NIH Grant R01 MH115750

Title: Different context-dependence of acquisition and extinction of sensory associative learning in freely-moving mice

Authors: *M. KISLIN, Y. CHEN, J. W. SHAEVITZ, S. S.-H. WANG;
Princeton Univ., Princeton, NJ

Abstract: Associative sensory conditioning is typically done in restrained animals, making it difficult to probe responses to the same stimulus in different behavioral states. To test whether associative learning generalizes beyond original context, we used machine vision-based pose tracking in freely-moving mice to study delay tactile startle conditioning (DTSC)[1], in which mice learn to withdraw from a conditional stimulus (CS; tone) paired repeatedly with an unconditional stimulus (US). The US was either a nose tap (US-tap) under head restraint on a running wheel, or optogenetic activation of channelrhodopsin-2 in Purkinje neurons of lobule IV/V (US-opto). During training, mice were either head-restrained or allowed to move freely in an arena. In both contexts, CS-US pairing caused a gradual acquisition of conditioned responses (CRs) over 6 daily sessions.
We investigated CS- and US-opto-evoked responses in freely-moving mice by tracking animal poses using SLEAP[2] to decode posture dynamics and quantify whole-body responses. CS/US-tap training led to CRs in head-restrained mice (context A), and CS/US-opto training led to CS responses in freely-moving mice (context B). Learning rates reached CR probability of 0.63 ± 0.16 (mean ± SD) in all conditions. Thus initial acquisition generalized across the two contexts. Extinction was done by presenting CS-only trials either in the original training context (AAA

and BBB-paradigm or “cis-extinction”) or in the other training context (ABA and BAB-paradigm or “trans-extinction”). For both wheel training and arena training, cis-extinction was effective, leading to a 58.0 ± 11.5 % reduction of CR rate (mean ± SD compared with session 6 of wheel training, CS + US-tap). However, trans-extinction led to moderate decrease in CR rate in the original “cis”-context (30.0 ± 14.1% reduction). Retraining led to rapid reacquisition of “cis”-CRs within 20 trials.
The context-specificity of extinction, as well as the rapidity of retraining, suggests that extinction has a more limited scope than with initial acquisition. Using two-photon microscopy under head restraint and miniscopes in freely-moving mice, we imaged neural activity in lobule IV/V Purkinje neurons. In dendrites showing learning-related increases in CS responsiveness, cis-extinction causes decreases in CS responsiveness and reacquisition restores CS responsiveness. We are now measuring the effects of trans-extinction on neural signals.
References:
1. Yamada, T. et al. Nature 569, 708-713 (2019).
2. Pereira, T.D. et al. Nat Methods 19, 486-495 (2022).

Disclosures: **M. Kislin:** None. **Y. Chen:** None. **J.W. Shaevitz:** None. **S.S. Wang:** None.

Poster

213. Cerebellum: Climbing Fibers and Learning

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 213.19

Topic: E.02. Cerebellum

Support: NIH grant NS116883
NIH grant NS105839

Title: The Influence of Experience on Cerebellar-Dependent Sensorimotor Learning

Authors: *T. WANG, R. IVRY;
Dept. of Psychology and Helen Wills Neurosci. Inst., Univ. of California, Berkeley, CA

Abstract: There is considerable debate focusing on how experience impacts cerebellum-dependent sensorimotor learning. On one hand, the cerebellum has been viewed as a rigid system that responds in a fixed manner. Alternatively, it has been argued that the learning rate is modified in response to recent errors (Herzfeld et al., 2014) or that the system flexibly switches between context-dependent motor repertories (Heald et al., 2021). Motivated by neurophysiological evidence, we propose a novel model in which cerebellar-dependent sensorimotor adaptation is modulated by experience, without assuming a change in learning parameters or context-dependent memory. This model captures three core observations: 1. Movement prediction is coded by a population of Purkinje cells (PC), with each cell tuned to a preferred error direction (Herzfeld et al., 2015). Thus, we assume that experiencing an error in one direction will suppress the synaptic efficacy of PCs tuned to that direction. When an opposite

error is experienced, the model predicts slower learning in response to the second error due to persistent suppression, a form of anterograde interference. 2. Our model assumes no context-dependent learning and no modulation of the learning rate during training. As such, it predicts that learning should not depend on the specific sequence of experienced errors. To test this, we used perturbed visual feedback that was not contingent on the participant's movement ("clamped feedback"), varying the consistency or variability of the error. At odds with context-dependent models and learning-rate modulation models, adaptation was insensitive to both manipulations. Moreover, no spontaneous recovery or savings is observed in implicit adaptation. 3. It has been hypothesized that complex spikes (CS) carry part of the memory in sensorimotor learning, with a higher CS firing rate observed during movement preparation if the preferred error of the PC appeared in the last trial (Junker et al., 2018). We assume that this CS activation directly suppresses single spike activity during movement preparation, producing a "fast" learning process on top of the slow process induced by the LTD. Consistent with this hypothesis, we found a large single-trial adaptation in response to random perturbations, while it showed a forgetting rate of approximately 0.5, precluding significant accumulation of learning across trials. In contrast, a "slow" process allows learning to accumulate across trials in response to a fixed perturbation. Both processes exhibit a similar non-linear response to error size, suggesting that they arise from a common source, attributed in our model to the influence of CS.

Disclosures: **T. Wang:** None. **R. Ivry:** Other; RI is a co-founder with equity in Magnetic Tides, Inc..

Poster

213. Cerebellum: Climbing Fibers and Learning

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 213.20

Topic: E.02. Cerebellum

Support: MOST 111-2321-B-A49-003
MOST 108-2321-B-010-010-MY2
MOST 108-2410-H-010-010-001
MOST 107-2410-H-010-010-001
VGHUST 109-V1-2-2
UST X108UST05
International Collaboration Project of Brain Science & AHRC 111Q20089Y

Title: Low-frequency cerebellar rTMS reduces the effect of visuomotor learning with reinforcement learning

Authors: *Y. TSAI[1], S.-H. N. LIN[2], C.-P. LIN[2], L.-H. CHANG[2];
[1]Natl. Yang Ming Chiao Tung Univ., Taipei city, Taiwan; [2]Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan

Abstract: Reinforcement learning (RL) and error-based learning (EL) are recognized as two distinct learning processes underlying visuomotor learning. However, a recent study suggested that the cerebellum might also contribute to the process of reinforcement learning (Miall et al., 2016). Furthermore, applying inhibitory neuromodulation, low-frequency repetitive transcranial magnetic stimulation (LF rTMS), over the cerebellum has been found to reduce the performance of error-based visuomotor learning (Lien et al., 2022). Therefore, we tested the hypothesis that the cerebellum also accounts for reinforcement learning. To investigate the relationship between reinforcement learning and the cerebellum, we developed the pursuit rotor task (PRT) with reinforcement learning and applied the inhibitory LF rTMS to the midline cerebellum of young and healthy participants (N=31) to transiently disrupt the cerebellar function. The quality of visual feedback and reward has been used to dissociate the EL and RL conditions (Izawa and Shadmehr, 2011). For the EL-PRT session, which was companied by high-quality visual feedback, the real-time visual feedback was provided during tracking, while for the RL-PRT session, which had low-quality visual feedback but a reward was given, the visual feedback was decreased and a score table was provided as a reward feedback after each trial. The participants first underwent an EL-PRT session for a baseline, followed by a single session of rTMS, and finally an RL-PRT. In the rTMS session, the participants received either the LF rTMS or Sham rTMS for about 10 minutes. There were two indexes to assess the performance of the PRT. The first was Mean Deviation (MD) which derived the learning efficiency and the second was Tracing Tendency (TT) which demonstrated the implicit preference for motor control when tracing the target. The learning efficiency in the RL-PRT session decreased after intervention for the LF group while there was no significant difference for the Sham group (learning rate: $p<0.001$; initial rate: $p<0.05$); furthermore, a significant between-group difference ($p<0.05$) was also found after the intervention. For the tracing tendency, after the intervention, the LF group significantly drew inside the circular track when compared to the Sham group, especially in the early period of learning ($p<0.05$). Our results revealed that the cerebellar LF rTMS influenced both indexes of the reinforcement learning condition, suggesting that the process of reinforcement learning is contributed to the cerebellum. These results gave the insight to explore more about the cerebellum and reinforcement learning in the future.

Disclosures: **Y. Tsai:** None. **S.N. Lin:** None. **C. Lin:** None. **L. Chang:** None.

Poster

214. Basal Ganglia Physiology and Movement

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 214.01

Topic: C.03. Parkinson’s Disease

Support: NIH Grant K23NS097576
NINDS Grant P50 NS123103-01

Title: The effect of levodopa on behavior and cortical activity during response inhibition in patients with Parkinson’s disease

Authors: *R. JAFARI DELIGANI[1], E. OPRI[1], Y. N. AL-MADANI[2], N. C. SWANN[3], S. MIOCINOVIC[1];
[1]Neurol., Emory Univ., Atlanta, GA; [2]Morehouse Sch. of Med., Atlanta, GA; [3]Human Physiol., Univ. of Oregon, Eugene, OR

Abstract: Parkinson's disease (PD) can alter response inhibition control resulting in impulsive behaviors. Dopaminergic medications including levodopa improve motor function, yet may worsen impulsivity. Much is still unknown about how PD medications affect response inhibition and underlying cortical activity. We tested two hypotheses: 1) motor performance relates to disease severity and improves on levodopa, and 2) levodopa worsens response inhibition and this is related to prefrontal cortical activity. We used a cued Go/NoGo (GNG) task where a left or right cue is presented on screen followed by a Go signal requiring a button press. On 25% of trials, a NoGo signal is given instead which mandates withholding the planned response. Prior to the GNG task, subjects performed a cued Go task to assess motor performance without the same need for inhibition. We measured median response times (RT) and percent error trials (wrong button, omission, premature and NoGo responses) in the off and on levodopa state (fixed order). Cortical activity was recorded using a 64-channel EEG. Disease severity was assessed with Unified Parkinson's Disease Rating Scale (MDS-UPDRS). We report results for 12 subjects (4 women, age 62.8±5.7 years, disease duration 9.8±4.0 years; all right-handed). In both medication conditions, RT was significantly longer during GNG (off med 431±87 ms; on med 450±125 ms) compared to Go task (off med 280±54 ms; on med 280±92 ms) ($p<0.001$; paired t-tests). However, RT did not change from off to on levodopa state for either task, even though clinical motor exam improved significantly (43±12 to 22±11 points on MDS-UPDRS). Off medications, disease severity positively correlated with Go RT (=0.6, $p=0.003$) but not with GNG RT. The total errors did not differ between the tasks and did not change from off medication (Go 5.1±5.5%; GNG 3.6±3.6%) to on medication state (Go 7.4±5.4%; GNG 5.2±5.7%). However, NoGo response errors increased significantly from off medication (1.2±1.3%) to on medication state (2.0±1.8%) ($p=0.03$). In three patients the NoGo error rate was more than 4% (or 17% of NoGo trials) on levodopa. Preliminary EEG analysis suggests a beta band power increase following NoGo signal compared to Go signal in the channels overlying the prefrontal cortex in both medication conditions. We conclude that disease severity relates to motor performance on a simple task, but not on a more complex task likely because response inhibition employs prefrontal cognitive cortical circuits in addition to the motor circuit. Levodopa does not improve motor responsiveness, but it worsens impulsivity, and this effect may be more pronounced in some subjects.

Disclosures: R. Jafari Deligani: None. **E. Opri:** None. **Y.N. Al-Madani:** None. **N.C. Swann:** None. **S. Miocinovic:** None.

Poster

214. Basal Ganglia Physiology and Movement

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 214.02

Topic: C.03. Parkinson's Disease

Title: Altered functional connectivity during resting-state EEG in patients with Parkinson's disease and freezing of gait

Authors: T. J. BOSCH[1], A. I. ESPINOZA[2], ***A. SINGH**[1];
[1]Univ. of South Dakota, Univ. of South Dakota, Vermillion, SD; [2]Univ. of Iowa, Univ. of Iowa, Iowa City, IA

Abstract: Though the underlying neural correlates of motor symptoms in Parkinson's disease (PD) have been previously explored, the freezing of gait (FOG) phenomenon observed in PD is still poorly understood. Resting-state electroencephalography (EEG) and functional connectivity analyses can be used to characterize intrinsic neural network activity in different disease states. We utilized graph theory measurements (strength and clustering coefficient) to explore functional connectivity in 83 PD (42 PDFOG+ and 41 PDFOG-) and 42 age-matched healthy controls. The phase lag index was computed for each electrode pair in different frequency bands, but we focused our analysis on the theta frequency band and selected the midfrontal region for the nodal analysis. We converted connectivity matrices to weighted graphs. Our results showed increased global strength and increased global clustering in PD patients compared to healthy controls, but no global differences were observed between PDFOG+ and PDFOG-. However, decreased nodal clustering was observed in PDFOG+ over midfrontal regions in the theta-band compared to PDFOG-. Furthermore, FOG scores were correlated with midfrontal theta clustering. These findings suggest the involvement of midfrontal theta oscillations in FOG symptoms in PD and demonstrate the sensitivity of graph metrics to characterize dysfunctional network activity in PDFOG+. Finally, alterations in midfrontal theta networks extend our previous findings on PDFOG+.

Disclosures: T.J. Bosch: None. **A.I. Espinoza:** None. **A. Singh:** None.

Poster

214. Basal Ganglia Physiology and Movement

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 214.03

Topic: C.03. Parkinson's Disease

Title: Examining the influence of movement tremor on motion-dependent motor learning

Authors: ***K. FORAY**[1], W. ZHOU[3], W. M. JOINER[2];
[2]Dept. of Neurobiology, Physiol. and Behavior, [1]Univ. of California, Davis, Davis, CA;
[3]Neurobiology, Physiol. and Behavior, Univ. of California Davis, Woodland, CA

Abstract: In addition to rigidity and slowness of movement (bradykinesia), patients with Parkinson's Disease (PD) often experience tremor—involuntary, rhythmical movements of the body. Subsequently, this rapid, rhythmic contraction and relaxation of muscles impairs motor

control and learning. For example, PD patients are unable to quickly adjust to environmental changes that require adapting motor output based on new, incoming sensory information (i.e., error feedback). Here, we were interested in the extent tremor affects the ability to adjust motor commands in response to novel movement dynamics. As a first step we trained healthy subjects to use a robotic manipulandum to move a screen cursor between two targets. Following a baseline period, reaching arm movements were perturbed by a velocity-dependent force-field during training. On these force-field trials, the robot perturbed the hand motion with forces that were proportional in magnitude and perpendicular in direction to the velocity of hand motion. There were two groups of healthy subjects, and each group experienced a different level of tremor (Magnitudes of either 0 or 3N, at an angle of 45 degrees and frequency of 10 Hz) in addition to the force-field perturbation. We used error clamp trials to assess the feedforward adaptive changes in motor output. Motor compensation to the force-field perturbation was quantified on these trials with an adaptation coefficient: the linear regression of the measured lateral force profile on each error clamp trial onto the ideal force profile required for full force-field compensation on that trial. Previous studies have shown that learning to compensate for the movement perturbation is the result of two concurrent learning processes: one process that responds quickly to movement errors, but has poor retention and another that responds slowly to movement errors, but retains the learning well from one trial to the next. We hypothesized that as the "noise" (i.e., amount of induced tremor) increased, the learning rate would decrease, specifically due to an impaired fast learning process. Preliminary results suggest that participants in the higher noise group demonstrated a reduced force profile after training compared to groups with less noise. Rate of decay between groups were similar, suggesting that the slow process was unimpaired across conditions. Our results provide preliminary evidence that the involuntary tremor plays a significant role in motor learning impairments, but this reduced ability may be due to deficits in specific learning mechanisms.

Disclosures: **K. Foray:** None. **W. Zhou:** None. **W.M. Joiner:** None.

Poster

214. Basal Ganglia Physiology and Movement

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 214.04

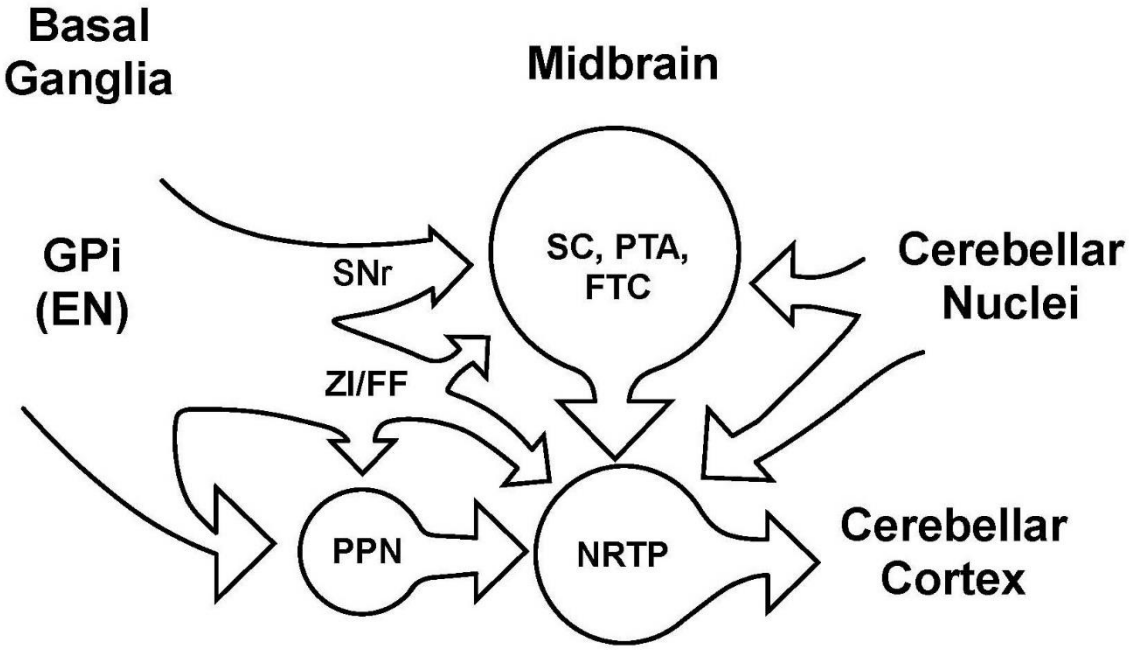
Topic: C.03. Parkinson's Disease

Support: Barrow Neurological Foundation
NINDS Grant 5R01NS44592

Title: Nucleus reticularis tegmenti pontis: a bridge between the basal ganglia and cerebellum

Authors: *A. GIBSON[1], M. PONG[2], K. M. HORN[3];
[1]Barrow Neurolog. Inst., Phoenix, AZ; [2]A.T. Still Univ., Mesa, AZ; [3]Chamberlain Univ., Phoenix, AZ

Abstract: Patients with Parkinson's disease (PD) can have akinesia and bradykinesia relieved by treatment with L-dopa. The restoration of movement indicates that aberrant basal ganglia (BG) output interferes with other, intact, brain regions that control movement. Two major brain regions involved in movement control are motor cortex and cerebellum. BG output from the internal segment of the globus pallidus (GPi) does not project directly to either region. GPi projects to the thalamus, which, in turn, projects to cortex. Thalamic lesions that interrupt this pathway can relieve tremor associated with PD but have little effect on symptoms such as akinesia and bradykinesia. In this presentation, we use anatomical methods to trace several pathways in the cat that connect BG output to the cerebellum. One major GPi projection is to cells surrounding the superior cerebellar peduncle (pedunculopontine nucleus, PPN). PPN has bilateral projections to nucleus reticularis tegmenti pontis (NRTP). NRTP has massive inter-connections with the cerebellum. Studies of NRTP function demonstrate profound effects on eye movements. Nonetheless, NRTP connections to the cerebellum are not limited to eye movement areas, and NRTP has the potential to affect movements of the entire body. We show that even slight stimulation (100ms, 5μa pulse train) of the cat NRTP can pause a reach in mid-flight. Cerebellar output has both ascending and descending projections. Classic studies indicate that interrupting the ascending projections to thalamus can alleviate tremor due to cerebellar damage, but other movements are not affected. Interrupting the descending projections of the cerebellum produces deficits in limb movements, including gait deficits. We propose that movement deficits remaining after thalamic lesions in PD patients are mediated by cerebellar projections that do not pass through the thalamus. NRTP receives input from many other regions of the brain, including motor cortex; NRTP probably plays an important role in control of most movements.



Disclosures: **A. Gibson:** None. **M. Pong:** None. **K.M. Horn:** None.

Poster

214. Basal Ganglia Physiology and Movement

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 214.05

Topic: C.03. Parkinson's Disease

Support: NIH Grant K23NS097576
NIH Grant P50 NS123103-01

Title: Behavioral performance and cortical activity during a motor response task in patients with Parkinson's disease

Authors: *J. RAYYAN[1], E. OPRI[2], F. ISBAINE[3], R. J. DELIGANI[2], N. LAXPATI[3], R. E. GROSS[3], N. AU YONG[3], S. MIOCINOVIC[2,1];
[1]Biomed. Engin., Georgia Tech. and Emory Univ., Atlanta, GA; [2]Neurol., [3]Neurosurg., Emory Univ., Atlanta, GA

Abstract: Parkinson's disease (PD) is a movement disorder characterized by difficulties in initiating movement and slowness of movement (bradykinesia). It is not well understood how PD affects the function of the premotor and motor cortex in the control of movement. Specifically, it is unknown how movement planning and initiation are dysregulated in PD and how these functions relate to clinical motor symptoms. We tested motor function in patients undergoing subthalamic deep brain stimulation (DBS) surgery using a delayed response task. We hypothesized that task performance would be proportional to the severity of disease and that response times (RT) and error rates would be higher in the more impaired hand. We also hypothesized a greater decrease in oscillatory beta band power in patients with faster RT. We recorded motor cortex activity using a temporary subdural electrocorticography (ECoG) electrode. Preoperative MDS-UPDRS motor exam score defined disease severity. Intraoperatively, patients performed 45 trials during a levodopa off state. A trial started with a central fixation box on a monitor followed by a left or right cue and then a 'go' signal requiring a button press. We calculated median RT and percent error (premature response, omission, or wrong button) in total, and for the right and left cues in relation to more and less impaired hand (based on preoperative clinical exam). Twelve patients were tested, but 2 were excluded because of greater than 50% errors. Ten patients were included in the analysis (3 women, age 62±6 years, disease duration 11±5 years). Average RT on correct trials was 381±134 ms and an average error rate was 8.4±7.2%. Disease severity (preoperative MDS-UPDRS score) was significantly positively correlated with RT (R^2=0.54; p=0.02), but not with total errors (R^2=0.23; p=0.16). Disease duration and age at surgery did not correlate with RT or errors. RT and errors were statistically comparable in the more impaired hand compared to less impaired hand (p=0.94, p=0.67, paired t-test; 5 patients were more impaired on the right). Similarly, right and left RT and errors were also comparable (all patients were right-handed). Preliminary motor cortex ECoG signal analysis indicated that onset of beta power desynchronization with respect to 'go' signal correlated with RT. We conclude that patients with more severe disease respond slower in a delayed-response task, but behaviorally this effect does not lateralize to the more impaired or dominant hemisphere. Because RT may be related to ECoG beta band activity, future studies will

focus on defining electrophysiological changes between the hemispheres and on modulating cortical activity with DBS.

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Poster

214. Basal Ganglia Physiology and Movement

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 214.06

Topic: C.03. Parkinson's Disease

Support: DFG 424778381 TRR 295

Title: Electrophysiological biomarkers of Parkinson's disease: evidence from a longitudinal study

Authors: *J. ZHANG[1], C. SANDER[2], T. HENSCH[3], U. HEGERL[4], G. SCHOMERUS[3], V. WITTE[1], A. VILLRINGER[1], V. NIKULIN[1];
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Abstract: Previous studies have repeatedly shown that Parkinson's disease (PD) is characterized by abnormal cortical PAC (phase-amplitude coupling) between the beta band (13–30 Hz) phase and the amplitude of broadband gamma (50–150 Hz). Yet, all the previous studies have been performed with a cross-sectional design. Given that elevated PAC has also been observed in some healthy elderly participants (Zhang et al., 2021), it is important to prove that elevation of cortical PAC develops along with the development of PD. Here we took advantage of a longitudinal study design, where we aimed at investigating the development of PAC from preclinical stage to fully developed PD. In order to do so, we analyzed a dataset based on a large cohort (N=~3000 participants) LIFE study (Leipzig Research Center for Civilization Diseases). The participants had two visits which took place on average 6.5 years apart. In total, we identified a group of six participants who were healthy at the first visit (t_0=0, age of 72.5±4.37 years, 3 females) but reported having developed clinically diagnosed PD at the second visit (t_1=6.5 years later, age of 79±4.28 years). Using multichannel resting-state EEG measurement (20 minutes), we computed the modulation index (MI) to quantify the strength of PAC. We found that in the *t1* condition, the subjects showed more pronounced beta-gamma PAC at the motor cortex (C3 and C4) in comparison to the t_0 condition which was confirmed statistically with Wilcoxon signed-rank test (p=0.0156). Our results thus demonstrate that the development of PD is indeed associated with an elevation of PAC. Combined with our previous study (Zhang et al., 2021) showing that the elevated PAC may also be present in healthy elderly participants, we suggest that PAC might be useful for the early detection of PD.

Reference: Zhang J., Idaji M. J., Villringer A., Nikulin V. V. (2021). Neuronal biomarkers of Parkinson's disease are present in healthy aging. NeuroImage 243:118512. 10.1016/j.neuroimage.2021.118512

Disclosures: **J. Zhang:** None. **C. Sander:** None. **T. Hensch:** None. **U. Hegerl:** None. **G. Schomerus:** None. **V. Witte:** None. **A. Villringer:** None. **V. Nikulin:** None.

Poster

214. Basal Ganglia Physiology and Movement

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Topic: C.03. Parkinson's Disease

Support: Parkinson's Foundation Postdoctoral Fellowship for Basic Scientists Award PF-PRF-839073
NIH Grant R01NS9580

Title: M4-mediated cholinergic transmission across dorsal striatum subregions is reduced following dopamine loss

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Abstract: A proper balance between dopamine (DA) and acetylcholine (ACh) in the dorsal striatum is critical for normal motor function, while imbalances are associated with striatal-based movement disorders such as Parkinson's disease (PD), where the loss of SNc dopaminergic neurons leads to a depletion of DA in the striatum. ACh can signal through both nicotinic and muscarinic receptors. For muscarinic receptors, M4 has the highest expression in the striatum, particularly in direct medium spiny neurons (dMSNs), and is the primary metabotropic subtype involved in DA transmission modulation and related motor behaviors in the basal ganglia. However, how M4-mediated cholinergic transmission occurs at the synaptic level across dorsomedial (DMS) and dorsolateral (DLS) striatum subregions is still unclear, as well as its alterations following DA loss. Combining brain slice electrophysiology and two-photon imaging of ACh release, we examined M4-mediated cholinergic signaling onto dMSNs across striatal regions with normal DA levels and after DA partial and complete depletion in a mouse model of PD induced by low or high doses of 6-hydroxydopamine respectively. While we found regional differences in M4-mediated transmission between the DMS and DLS, overall, the strength of M4-mediated transmission was reduced following DA loss, with the DLS being more sensitive than the DMS following partial DA depletion, and both regions being equally affected with severe DA lesion. Regional differences in presynaptic ACh release were responsible for differences in cholinergic transmission across regions in the healthy striatum, however, a reduction in the strength of postsynaptic M4 signaling accounted for the impaired cholinergic transmission in the DA-depleted striatum. Clearance of ACh was also distinct across subregions,

but not strongly affected after DA lesion. Taken together, our findings reveal a differential M4-mediated cholinergic transmission across dorsal striatal regions, which is sequentially impaired with the progressive DA loss that takes place in PD, positioning cholinergic system, and in particular M4-signaling, as a promising therapeutic target for restoring DA-ACh balance.

Disclosures: B. Nielsen: None. **C. Ford:** None.

Poster

214. Basal Ganglia Physiology and Movement

Location: SDCC Halls B-H

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Program #/Poster #: 214.08

Topic: C.03. Parkinson's Disease

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CMRPD1K0512
EMRPD1K0481

Title: Significance of corticostriatal glutamate in L-DOPA-induced dyskinesia in 6-OHDA-lesioned Parkinson's disease mice model

Authors: *Y.-T. HUANG, Y.-W. CHEN, J.-C. CHEN;
Grad. Inst. of Biomed. Sci., Chang Gung Univ., Taoyuan, Taiwan

Abstract: Long-term treatment of L-dopa in Parkinson disease's patients would induce dyskinesia (L-dopa-induced dyskinesia, LID), however, the underlying mechanism of remains unclear. In this study, we aim to determine the role of cortico-striatal glutamate in the striatum during the development of LID. We first established LID in unilateral medial forebrain bundle 6-OHDA-lesioned mice via measurement of AIMs (abnormal involuntary movements). Next, to explore the role of pre-synaptic and post-synaptic glutamate in the expression of LID, we used glutamate transporteragonist, LDN 212-320 and NMDA antagonist, amantadine to treat the LID mice, our results found these acute drug treatments significantly decreased the L-dopa-induced AIMs. The expression level of pERK and pNR2B significantly decreased in the drug-treated LID mice compared to controls. In this context, we applied optogenetics to manipulate the striatal glutamate activity in LID mice. Unilateral 6-OHDA-lesioned vGluT2-Cre mice were infected with Halorhodopsin (AAV9- EF1α-DIO-eNpHR3.0-mCherry) in the dorsolateral striatum with optical fiber implanted into the same region. After four weeks of viral infection, mice were treated with L-dopa for two weeks followed by AIMs measurement. One day after the AIMs test, mice received optical stimulation (NpHR, 532 nm green lazer; 15 mW intensity) during the L-dopa treatment. Our data showed that photo-inactivation of striatal glutamate terminals significantly reduced the amount of L-dopa-induced axis AIMs. Also, photo-inactivation of cortical - glutamate neuron in the M1 region significantly reduced the amount of L-dopa-induced AIMs. Next, mice were infected the NpHR in the M1 with fiber implanted into dorsolateral striatum followed by L-dopa treatment for 2 weeks, the photo-inactivation significantly reduced

the L-dopa-induced AIMs. After behavioral tests, we monitored the expression of TH and p-NR2B signals and found the expression of TH significantly decreased in both striatum and SNpc of the 6-OHDA-lesioned side. Further, levels of p-NR2B in the photo-stimulation in dorsolateral striatum of 6-OHDA-lesioned side was decreased compared to non photo-stimulation mice. Overall, our current findings indicate that cortico-striatal glutamate neural activity/signaling is responsible for the expression of LID.

Disclosures: **Y. Huang:** None. **Y. Chen:** None. **J. Chen:** None.

Poster

214. Basal Ganglia Physiology and Movement

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Topic: C.03. Parkinson's Disease

Support: DoD Grant (award #W81XWH-19-1-0757)

Title: Tracking Extracellular Dopamine Dynamics Following 6-OHDA Lesion of the Nigrostriatal Track

Authors: *A. CENTNER[1], N. CHAMBERS[4], S. COHEN[5], M. L. TERRY[2], M. COYLE[3], R. MCMANUS[6], W. NAVARRETE[7], M. SALVATORE[7], C. R. BISHOP[3];
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Abstract: Parkinson's disease (PD) is a neurodegenerative disease characterized by progressive striatal (STR) terminal degeneration and loss of dopamine (DA) neurons of the substantia nigra (SN). This process can begin 5-10 years prior to the presentation of motor symptoms, thus it is important to understand the timeline of neuronal and terminal loss, when motor symptoms emerge, and ultimately when and whether protection or reversal is possible. Interestingly, patients in early phases of PD present with asymmetric DA loss, therefore contralateral compensation in DA neurotransmission is an important variable to consider when evaluating time course of disease progression. Our recent data have shown that STR DA tissue levels ipsilateral to lesion are reduced in 6-OHDA rats at day 7 without significantly more loss at day 28, however, 6-OHDA lesioned rats showed continual ipsilateral SN DA loss from day 7 versus day 28. In contrast, STR DA tissue levels in the contralateral hemisphere of lesioned rats has been shown to significantly increase from day 7 to day 28, adding to the question of contralateral compensation. We have also demonstrated that although baseline extracellular STR DA levels are similar between sham and DA-lesioned rats, STR potassium-evoked DA release is

significantly reduced in lesioned rats as early as 7 days post-lesion with severe motor deficits emerging at day 7 that remains 28 days post-lesion. In the present study, we examined the changes in extracellular DA with *in vivo* microdialysis in the STR and SN at 7 and 28 days following a unilateral medial forebrain bundle (MFB) 6-OHDA or sham lesion in rats. In addition to a unilateral 6-OHDA or sham lesion, animals were fitted with microdialysis cannulae in STR and SN ipsilateral or contralateral to lesion in a between-subjects design. Given our initial results, we expect reduced STR DA release to persist at day 28 post-lesion. Furthermore, given evidence showing reduced DA levels in the SN, we could expect a small reduction in extracellular DA within the SN from day 7 to day 28. Importantly, evaluating DA dynamic changes over the course of terminal and cell loss can provide additional insight on optimal therapeutics for mitigating motor deficits for various stages of disease progression.

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Poster

214. Basal Ganglia Physiology and Movement

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 214.10

Topic: C.03. Parkinson’s Disease

Support: R01 DC017718-01A1

Title: Speech and orofacial-ralated modulation of subthalamic neuronal activity in Parkinson's patients

Authors: *Z. JOURAHMAD[1], J. I. BERGER[1], A. H. ROHL[1], K. JOHARI[2], F. TABASI[1], J. D. W. GREENLEE[1];
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Abstract: Parkinson’s disease (PD) is a chronic progressive disorder of the central nervous system, with prominent motor symptoms. Disturbances in speech - including impairments of voice, articulation, prosody, and intelligibility - are commonly presented alongside motor symptoms. Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is an accepted treatment in PD to ameliorate motor symptoms. The effects of STN-DBS on speech are much more varied, with studies reporting DBS-related speech decline in as many as 32% of patients. Previous studies have shown differential modulation of STN neural firing during speech and limb movement tasks. However, it is not clear whether STN modulation in response to speech tasks could purely relate to orofacial motor movement, rather than speech per se.
To examine this, we recorded 46 single- and multi-unit neuronal clusters from STN in 10 individuals (2F; 8M) between 56 and 74 years of age undergoing DBS implantation in bilateral STN for treatment of idiopathic Parkinson’s disease. The study was approved by University of

Iowa Institutional Review Board and all participants provided informed consent prior to participation. Participants completed sentence repetition, syllable repetition, and non-speech orofacial movement tasks (i.e. opening and closing mouth, sticking out tongue). Neuronal unit activity was collected from Alpha Omega microelectrodes implanted in the STN. Data were sorted using wave_clus to isolate single and multi-unit clusters. Absolute firing rates and rate modulation indices were calculated around the two behavioral tasks (speech and orofacial movement) and used to indicate task-related modulation. Preliminary analyses indicate diverse patterns of modulation in neuronal firing rates in STN for speech versus orofacial movement, with more clusters showing modulation by speech compared to orofacial movement, as well as an overall greater modulation of neuronal firing rates for speech vs. orofacial movement. These data suggest that modulation of STN during speech is not driven solely by orofacial movement, as we observe speech-specific modulation. Uncovering the role of STN in speech production as an effort to understand the speech network architecture can help with discovering novel treatment targets in speech disorders, and finding better treatment targets to avoid surgical complication during brain procedures.

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Poster

214. Basal Ganglia Physiology and Movement

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Title: GABAergic and glutamatergic innervation of the motor thalamus in normal and parkinsonian monkeys: A 3D Electron Microscopic Analysis

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Abstract: The thalamus is a major target of basal ganglia outflow to the cerebral cortex. In primates, the parvocellular part of ventral anterior nucleus (VApc) and the centromedian nucleus (CM) receive projections from the sensorimotor territory of the internal globus pallidus (GPi). Previous anatomical, neurochemical and electrophysiological studies suggest that the information flow through the basal ganglia-thalamocortical motor loop is altered in MPTP-treated parkinsonian monkeys. To determine if these changes may result from disruption of the synaptic connectivity of the basal ganglia-receiving regions of the motor thalamus, we used the

Single Block Facing/Scanning Electron Microscopy (SBF/SEM) 3D electron microscopic approach to reconstruct the synaptic innervation of distal and proximal dendrites of thalamocortical neurons of VApc and CM in control and parkinsonian monkeys. Putative GABAergic terminals from the reticular thalamic nucleus (RTN) or the GPi were differentiated from glutamatergic corticothalamic terminals by their ultrastructural features. Data obtained so far from 2 control and 2 parkinsonian monkeys demonstrate: (1) the average density of corticothalamic terminals (~1.2 term./μm^3) in contact with small-sized distal dendrites of VApc neurons is 5-10 times higher than the density of putative GABAergic terminals from GPi (~0.1 term./μm^3) and RTN (~0.2 term./μm^3), (2) The density of cortical terminals in contact with distal dendrites of CM is ~5 times lower than that in VApc, (3) In both VApc and CM, the extent of cortical innervation of individual distal dendrites is highly variable ranging from 0.5-1.8 term./μm^3 in VApc and 0-0.4 term./μm^3 in CM, (4) In contrast to distal dendrites that are preferentially targeted by putative cortical terminals over GABAergic boutons, the innervation of proximal dendrites in much sparser and accounted for preferentially by putative GPi and RTN GABAergic terminals in both VApc and CM, (5) No significant difference in the pattern of innervation of VApc and CM neurons was found between control and parkinsonian monkeys. In light of evidence that MPTP may be neurotoxic for CM neurons, most likely through mitochondrial dysfunction, three-dimensional reconstruction of individual dendritic mitochondria is in progress to quantify changes in dendritic mitochondrial network morphology and complexity in the CM of parkinsonian monkeys. Together, our quantitative 3D EM imaging data set the foundation for a deeper understanding of the underlying synaptic microcircuits that regulate function of the basal ganglia-receiving thalamus in normal and parkinsonian states.

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Poster

214. Basal Ganglia Physiology and Movement

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Topic: C.03. Parkinson's Disease

Support: The Defence Advanced Research Projects Agency (DARPA) Grant HR001118S0041-BAA-FP-014

Title: Chronic motor cortical electrocorticography reveals negative correlation between slow wave and beta activity during deep NREM sleep in Parkinson's disease

Authors: *M. ANJUM[1], C. SMYTH[1], S. RAVI[1], T. DIXON[1], P. STARR[1], T. DENISON[2], S. LITTLE[1];
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Abstract: In Parkinson's disease (PD), sleep dysfunction is directly linked to disease progression and to worsening of daytime motor symptoms (Nassan et al., 2021). Treating sleep disturbances in PD with precisely targeted therapies, including targeted Deep Brain Stimulation (DBS), therefore has the potential to improve motor and neuropsychiatric PD symptoms. In this study, we recorded chronic cortico-basal ganglia local field potentials from patients with PD (PWP), remotely in their own homes. We evaluated for an interaction between beta rhythms and slow wave activity (Mizrahi-Kliger et al., 2020) as well as the impact of DBS on cortical signals during deep non - rapid eye movement (NREM) sleep (N2 and N3). We recruited four patients with PD, implanted with Summit "RC+S" sensing-enabled DBS devices with chronic, bilateral, cortical and subcortical (either subthalamic or globus pallidus interna) electrodes. We recorded bilateral local field potentials overnight from M1 cortex for each participant for two consecutive nights with DBS (conventional high frequency; amplitude = 3.075 ± 0.65 mA) stimulation ON (8.61 ± 0.93 hours) and OFF (7.38 ± 2.25) respectively. Recordings were bandpass filtered (0.5-100 Hz), divided into 30-second epochs, decomposed into the frequency domain and normalized by total power. Epochs were labeled according to sleep stage using a portable polysomnogram (DREEM2) headband. A linear mixed-effect (LME) model using all 4 subjects (accounting for the dependency between left and right hemispheres and stimulation conditions within patients) showed an overall negative fixed effect of beta (12-32 Hz) on delta (1-4 Hz) power during N2 and N3 sleep (β = -1.83; p-value = 0.004). LME models with subjects as random effects revealed a negative fixed effect of ON/OFF stimulation condition on cortical low alpha (8-10 Hz; β = -1.47; p-value = 0.05), low beta (12-15 Hz; β = -1.93; p-value = 0.01) rhythms and a trend for increased delta (1-4 Hz; β = 1.06; p-value = 0.27). These results demonstrate that motor cortical beta power negatively correlates with slow wave (delta) during deep NREM sleep in PWP. We also found an effect of DBS modulation during deep sleep in PD on cortical alpha and low beta rhythms. Targeted DBS therapies that modulate physiological and pathological sleep rhythms have the potential to improve sleep symptoms in PWP.

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Poster

214. Basal Ganglia Physiology and Movement

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Topic: C.03. Parkinson's Disease

Support: UNC Physician Scientist Training Program, Department of Medicine

Title: Frontal midline theta power in Parkinson's disease

Authors: *M. SKLEROV[1], J. RIDDLE[2], E. CAMPBELL[1], D. JACKSON[3], F. FROHLICH[4]; [1]Univ. of North Carolina Sch. of Med., Chapel Hill, NC; [2]Psychiatry, [3]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [4]Psychiatry, Univ. of North Carolina, Chapel Hill, NC

Abstract: Objective and rationale: Parkinson's disease (PD) is a common neurodegenerative disease a with a complex set of symptoms, including numerous non-motor symptoms with poorly understood mechanistic underpinnings and limited available treatment, such as autonomic dysfunction and depression. Electroencephalography (EEG) is a highly accessible non-invasive tool that can be developed to improve targeting for non-invasive brain stimulation in research and clinical care. EEG is understudied in PD. Frontal midline theta (FMT) is a characteristic theta frequency oscillation (4-8 Hz) localized to midline frontal cortex, and is detectable using EEG. The amplitude of FMT oscillations is associated with metabolism in the anterior cingulate cortex (ACC), which is preferentially affected by PD pathology and is a major node for networks implicated in PD depression and autonomic dysfunction. It is unknown whether FMT oscillations, which are representative of dorsal ACC (dACC) function, are detectable during the resting-state in PD. We hypothesize that reduced amplitude of FMT oscillations would be indicative of impaired dACC function and would correlate with non-motor symptoms of PD.**Methods and results**: We are recruiting people with PD from the Neurology clinic at the University of North Carolina to undergo resting-state EEG and validated self-report questionnaires assessing symptoms of depression and autonomic dysfunction. Participants are free of other neurologic disease that may provide an alternative explanation of their symptoms, or which may cause abnormalities on EEG. Initial results show that of the 7 participants that have completed resting EEG in the study thus far, 3 have clearly identifiable FMT patterns on resting state EEG. The percentage of participants with detectable FMT is similar to what is reported in people without PD. We will next investigate the relationship between FMT amplitude with symptoms of depression and autonomic dysfunction.**Conclusions**: FMT, a characteristic EEG activity signature, was found to be present in PD with similar prevalence compared with reports from people without PD. Investigations of correlations between FMT power and non-motor symptoms of PD are ongoing. Establishing the link between FMT (and other EEG-based signatures) and symptoms in PD will enable the design of targeted brain stimulation treatments using TMS to modulate and restore the pathologically impaired network rhythms.

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Poster

214. Basal Ganglia Physiology and Movement

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Title: Enhanced delta oscillations in the basal ganglia of dopamine-depleted awake mice

Authors: *J. E. RUBIN[1], A. ARISTIETA[2], J. PARKER[1], R. S. PHILLIPS[3], T. C. WHALEN[4], A. H. GITTIS[2];
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Abstract: Parkinson's disease (PD) and animal models of PD feature enhanced oscillations in various measures of neural activity in the basal ganglia (BG). Past research has emphasized the intensification of 13-30 Hz beta oscillations in parkinsonism. In recordings from awake, dopamine-depleted mice analyzed with a novel computational approach to detect spike oscillations embedded in noise, however, we find a significant increase in oscillations in the delta band (0.5-4 Hz), which we determine to result from the loss of D2 dopamine receptor activation. Moreover, this delta oscillation intensity outperforms firing rate, irregularity, bursting and synchrony as a marker of dopamine loss and akinesia. We find that delta oscillations in the substantia nigra pars reticulata (SNr) lead oscillations in motor cortex (M1) and persist under M1 lesion, while oscillating SNr neurons segregate into two phase-shifted clusters. To investigate the origin of these coherent SNr oscillations, we consider two computational approaches. First, a model of spiking in the SNr network suggests that changes in the level of ongoing activity in the globus pallidus external segment (GPe), which sends GABAergic projections to the SNr, can transition the SNr into a low-frequency oscillation mode based on tuning of its extracellular chloride concentration and hence of the GABA reversal potential in SNr. Second, a model of the BG indirect pathway network that incorporates our recordings from dopamine-depleted mice, including prevalent but disorganized delta oscillations in GPe, captures with surprising accuracy the emergence of phase-shifted delta-band oscillation clusters in GPe. Finally, we test predictions from these models by measuring the effects of optogenetic activation of GPe on delta oscillations in GPe. Together, these results call for increased attention to delta oscillations in the BG as an indicator of parkinsonism and demonstrate how delta oscillations can potentially emerge within and propagate through BG nuclei.

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Poster

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Title: Prodromal phase alpha synucleinopathy-induced motor circuit dysfunction in vivo

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Abstract: Inclusions of aggregated α-synuclein (αSyn), called Lewy bodies (LBs), are pathological hallmarks in patients with synucleinopathy disorders, including Parkinson's disease (PD) and dementia with Lewy bodies (DLB). These abnormal deposits of αSyn lead to a rapid decline in cognition and motor performance. Despite remarkable advances in our understanding of the spread of αSyn pathology, the key circuit biomarkers and associated mechanisms through which αSyn aggregates perturb neural circuit dynamics remain largely unknown. In this study, we injected preformed fibrils (PFF) of αSyn in deep layers of mouse primary motor cortex (M1), and monitored early-stage propagation effect across the translaminar cortical circuit. Using high-density electrophysiology, high-speed two-photon (2P) Ca^{2+} imaging, and in vivo intracellular electrophysiology, we dissected the mechanisms in which αSyn pathology effects cortical microcircuits in self-initiated locomoting mice. We observed a gradual translaminar progression of αSyn pathology across M1 within 3 months of PFF seeding. Under this pathological window, we found cortical excitatory neurons across all layers increased their phase entrainment to beta LFP, with significant effects occurring within 4 weeks post-induction. Strikingly, this increased preference to broadband beta LFP occurred most prominently within the superficial layers of M1, namely L2/3. Moreover, we discovered the emergence and alteration in stochastic bursts of beta LFP waveforms - termed "beta events", lasting ~50 ms. Analysis of beta event temporal structure, backed by biophysical modeling and intracellular recordings, suggests that beta events reflect dendritic spikes in L5 pyramidal neurons. Altered beta event amplitudes preceded disruption in spiking preference of cortical excitatory neurons during αSyn spread. Importantly, bursts of beta events were prominent in sham controls and during the early stages post PFF seeding, but gradually decreased in PFF-treated animals as a function of Lewy body spread. Together, these findings suggest that beta events modulate the output of L5 neurons by shifting from a burst to rate code. 2P-Ca^{2+} of superficial excitatory neurons further revealed a gradual expansion of cortical ensemble architecture leading to a loss of sparse representation and engagement of network hyperexcitability with a time course that matched the beta LFP change over weeks. We surmise that a shift of cortical space in M1 L2/3 potently controls the generation of beta events in L5 neurons and provides a pathophysiological biomarker for prodromal phase synucleinopathy.

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Poster

214. Basal Ganglia Physiology and Movement

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 214.16

Topic: C.03. Parkinson's Disease

Title: Pathological alpha synuclein causes cognitive and molecular deficits in the PFF model of synucleinopathies

Authors: *M. MILLETT[1], N. E. CHAMBERS[2], A. TADJALLI[3], H. CHEN[4], D. NABERT[4], K. HUTCHINSON[4], A. HEUBERGER[4], S. BARSOUM[4], P. WAGNER[4], M. S. MOEHLE[5]; [2]Pharmacol. and Therapeut., [1]Univ. of Florida, Gainesville, FL; [3]Univ. of Toronto, Toronto, ON, Canada; [4]Univ. of Florida - Gainesville, FL, Gainesville, FL; [5]Pharmacol. & Therapeut., Vanderbilt Univ., Gainesville, FL

Abstract: Pathological Alpha Synuclein Causes Cognitive and Molecular Deficits in the PFF Model of Synucleinopathies.
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DisclosuresM. Millett: None. **N. E. Chambers:** None. **A. Tadjalli:** None. **H. Chen:** None. **D. Nabert:** None. **K. Hutchinson:** None. **A. Heuberger:** None**. S. Barosoum:** None. **P. Wagner:** None **M.S. Moehle:** None.
AbstractThe abnormal aggregation of alpha synuclein (aSyn) into insoluble cytoplasmic inclusions, termed Lewy bodies, is a pathological hallmark of several neurodegenerative disorders that affect millions worldwide and are a major cause of morbidity. Current treatment for the most prevalent synucleinopathies, Dementia with Lewy Bodies (DLB) and Parkinson's Disease (PD), are primarily symptomatic. Cognitive dysfunction is a common feature of synucleinopathies but the effects of pathological aSyn on disease relevant circuits remain largely unexplored. To investigate the impact of Lewy pathology on cognitive behaviors and cortical circuitry, we utilize an aSyn pre-formed fibril (PFF) model that causes the corruption of endogenous aSyn into pathological inclusions. PFF injected mice were subjected to comprehensive behavioral assessment to determine cognitive domains altered by corrupted aSyn. Notably, striatal PFF injection caused impaired short-term memory and executive function deficits within a 2 trial Y maze test and altered anxiety behaviors in robust behavioral assays of anxiety. Following behavioral evaluation, mouse brains were harvested for downstream electrophysiology and molecular analyses. Electrophysiology from acute brain slices of PFF injected mouse brains yielded significant decreases in excitability of layer 5 prefrontal cortex (PFC) pyramidal neurons and enhanced long-term depression in the PFC. Using expansion microscopy and RNAscope we uncovered potential mechanisms for the observed alteration in electrophysiology and behavior of PFF injected mice. Cumulatively, our data establishes distinct behavioral, circuitry, and molecular pathways that result from corruption of aSyn. These findings have identified specific behavioral pathways that could potentially serve as etiological therapeutic targets and warrant further investigation.

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Poster

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Topic: C.03. Parkinson's Disease

Support: K23NS080912
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NINDS R25: NS079188
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Title: Effects of STN DBS on Quantitative Sleep EEG in Parkinson's Disease

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Abstract: Disorders of sleep-wake patterns are common non-motor symptoms of Parkinson's disease (PD). Quantitative sleep EEG (sleep qEEG) markers, including scalp-slow wave (SW) density and sleep spindle density, are altered in PD and are related to cognition. Further, sleep dysfunction leads to impaired quality of life, so management of sleep complaints is essential. Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is superior to best medical therapy for treating motor symptoms in PD and has been demonstrated to improve sleep in patients with PD. We hypothesize that improvement in sleep qEEG markers might contribute to the beneficial effects of STN DBS on sleep. To explore the mechanism underlying this improvement, we will leverage an existing sleep dataset originally collected to evaluate macro sleep architecture in PD. Twenty participants, with unilateral (N=18) or bilateral (N=2) STN DBS, age 61.4 ± 8.9 years (mean ± SD), 75% male, duration of disease 10.1 ± 4.2 years, and median duration since DBS of 450 days, underwent two non-consecutive nights of laboratory-based nocturnal polysomnography (PSG): one night with DBS off and one night with DBS on. The two studies were performed within 4 weeks of each other. PSG-derived sleep EEG from this dataset will be manually inspected, with rejection of movement and electrical artifacts. Then, we will compute SW density and spindle density during non-rapid eye movement sleep stages N2 and N3 using the following parameters: For SW: 1) frequency filter= 0.16-1.25 Hz , 2) duration= 0.8-2 seconds, 3) amplitude threshold = 75^{th} percentile, and for spindles: 1) frequency filter = 9-15 Hz, 2) duration 0.5-2 seconds, and 3) amplitude= 75^{th} percentile . This novel work in progress, evaluating the effects of STN DBS on sleep qEEG may enhance our understanding of

the pathophysiology of sleep disorders in PD, provide strategies to optimize the clinical benefits, and guide novel treatments for sleep disorders.

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Poster

214. Basal Ganglia Physiology and Movement

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Topic: C.03. Parkinson's Disease

Support: ERC Grant 646886 - SynChI

Title: Striatal cholinergic cell assemblies in Parkinsonism and Levodopa-induced dyskinesia

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Abstract: Parkinson's disease (PD) is a debilitating hypokinetic movement disorder caused by the loss of dopaminergic neurons in the substantia nigra. Dopamine replacement therapy with L-3,4-dihydroxyphenylalanine (L-DOPA) greatly improves the motor symptoms of PD. However, treatment with L-DOPA causes excessive involuntary movements, namely L-DOPA induced dyskinesia (LID), in a majority of patients within a few years of use. In PD, depletion of dopaminergic inputs to the striatum, the main input structure of the basal ganglia, causes changes to the firing patterns of striatal spiny projection neurons (SPNs), which make up the majority of striatal cells. SPNs have also been repeatedly implicated in the development of LID, with recent work emphasizing the involvement of the indirect pathway SPNs in this phenomenon. Another group of striatal cells of interest are the cholinergic interneurons (CINs). While these cells constitute only a tiny fraction of striatal cells, they are the major source of acetylcholine in the striatum, and their photoinhibition reduced motor deficits in a 6-hydroxydopamine (6-OHDA) lesioned mouse model. Moreover, they have been recently shown to exhibit aberrant burst-pause activity in acute striatal slices from dyskinetic mice. Nonetheless, *in vivo* evidence for such alterations are lacking. Here we aim to study the large-scale spatiotemporal dynamics of CIN cell assemblies in control and 6-OHDA lesioned mice before and after treatment with L-DOPA and induction of LID, using endoscopic *in vivo* calcium imaging in freely moving mice. We began by collecting baseline data from parkinsonian double transgenic mice expressing GCaMP6f in a sparse population of striatal cells comprising mainly of SPNs, before and after treatment with L-DOPA. We were able to show a significant increase in the rate of Ca^{2+} events as opposed to a decrease in their amplitudes in 6-OHDA lesioned mice compared to control. During LID, striatal

cells exhibited a marked increase in both the rate and amplitude of Ca^{2+} events compared to parkinsonian and control states. These results are in line with the existing evidence. Next, we will compare these results with data collected from double transgenic mice expressing GCaMP6f selectively in choline acetyltransferase expressing cells. We will characterize the estimated firing dynamics of molecularly identified CINs during LID and demonstrate the timeline of the development of activity changes in these cells during each session and across sessions. The findings of this study will help shed the light onto the actual role of CINs in the pathophysiology of LID and possibly pave the way to new interventions aimed at alleviating it.

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Poster

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Topic: C.03. Parkinson's Disease

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Title: Movement-related activity of the internal segment of the globus pallidus in the Parkinsonian monkey

Authors: *D. KASE[1], A. ZIMNIK[2], Y. HAN[1], S. BACHA[1], D. HARSCH[1], M. R. RICHARDSON[3], R. S. TURNER[1];
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Abstract: Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to non-human primates (NHPs) leads to dopaminergic denervation and symptoms highly similar to those of idiopathic Parkinson's disease. Although the neurophysiologic correlates of MPTP-induced parkinsonism have been studied extensively in NHPs at rest, little is known about those correlates during voluntary movement. More specifically, we set out to determine if parkinsonian motor impairments are associated with abnormalities in the movement-related activity of neurons in the globus pallidus internus (GPi, primary output nucleus of the basal ganglia skeletomotor circuit). We used a simple choice reaction time reaching task that hemiparkinsonian NHPs were able to perform, albeit with slowed reaction times and movement durations. We studied the peri-movement activity of GPi neurons under neurologically-normal and parkinsonian states (n = 182 and 192, respectively).
We found that peri-movement changes in GPi firing rate were equally common pre- and post-MPTP. Those across-trial mean responses, however, were smaller in magnitude (-27.5%) and

longer in duration (+138.9%) than the changes observed pre-MPTP. Trial-by-trial analyses showed that the MPTP-induced prolongation of mean response durations was attributable to a combination of increased trial-to-trial variance in the timing of response onsets and an increase in the native duration of responses after accounting for trial-to-trial jitter in response timing. These influences of MPTP were common in responses that occurred at a consistent time across trials (i.e., were "time-locked") following the appearance of the task go-cue and also in responses time-locked to movement onset. Interestingly, the effects of MPTP on the latency of response onset differed between categories of time-locking. Responses that were time-locked to movement began markedly earlier prior to movement onset following MPTP administration. In contrast, MPTP did not affect the latencies of responses that were time-locked to go-cue appearance. In addition, MPTP increased the fraction of responses that were go-cue locked (+19%) and reduced the fraction that were movement locked (-23%).
In conclusion, we found multiple abnormalities in GPi peri-movement activity that may contribute to the motor impairments observed post-MPTP. The timing of task-related activities was relatively normal in the early reaction time period between go-cue appearance and onset of movement-locked activity. It remains to be determined what causes of the subsequent gap in time between this unchanged time course and the delayed onset of movement post-MPTP.

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Poster

215. Hand Control and Dexterous Manipulation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 215.01

Topic: E.04. Voluntary Movements

Support: NINDS NS107714
NINDS NS122333

Title: A method to study prehension in primates

Authors: *A. R. SOBINOV, C. M. GREENSPON, E. V. OKOROKOVA, S. J. BENSMAIA; Univ. of Chicago, Chicago, IL

Abstract: A hallmark of primate behavior is the exceptional ability to dexterously grasp and manipulate objects. Investigation of the neural mechanisms that support manual dexterity has been hindered by technical challenges. First, tracking the time-varying posture of the hand is difficult given its many articulated segments and the susceptibility of individual segments to occlusion. Second, characterizing the contact forces exerted on objects with sufficient precision is challenging given the exquisite sensitivity and spatial resolution of the sense of touch. Third, contact forces propagate down the limb in the form of joint torques and alter muscle activity. Measurements of limb state and object interactions is thus critical to understanding the neural

mechanisms that mediate manual object interactions.
With this in mind, we have developed an experimental apparatus to overcome these challenges. First, the apparatus allows us to place an object anywhere in the workspace. Second, the objects size and orientation can be systematically manipulated. Third, the object is sensorized with thousands of pressure-sensitive elements that allow us to measure the forces exerted at each point of contact. Fourth, hand and arm movements are tracked using eight high-speed cameras, which output is then processed using machine vision algorithms to track the positions of 34 markers on the limb in 2D for each camera. The positions of the markers are then triangulated based on the 8 planar estimates (on from each camera) and used to obtain time-varying joint angles using a musculoskeletal model of the limb. Finally, we solve an optimization problem in a physics engine to adjust the object's position with respect to the hand to reproduce the pattern of exerted forces. Having reconstructed contact forces, applied to the corresponding contact locations on the hand, and reconstructed the posture of the entire limb, we can then calculate the torque at each joint using inverse dynamics. Together, these measurements provide a characterization of primate manual behavior with exceptional precision, which opens the door to an unprecedented investigation of the neural mechanisms of manual dexterity.

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Poster

215. Hand Control and Dexterous Manipulation

Location: SDCC Halls B-H

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Program #/Poster #: 215.02

Topic: E.04. Voluntary Movements

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JSPS KAKENHI 18K19767
JSPS KAKENHI 20H05489
JSPS KAKENHI 20H05714
JSPS KAKENHI 18H04082
JSPS KAKENHI 18K13378

Title: Quantitative comparison of corticospinal tracts in humans based on diffusion fiber tractography

Authors: *N. USUDA[1,2], S. K. SUGAWARA[1], H. FUKUYAMA[3], K. NAKAZAWA[2], K. AMEMIYA[3], Y. NISHIMURA[1];
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Abstract: Corticospinal (CS) tract (CST) plays important role to control voluntary limb movements. The cortical origin and the proportion of CSTs in the cortical areas are well documented in monkeys, but it remains unclear in humans. In this study, we aimed to evaluate the origin of CST and estimate the proportion of CSTs among the cortical areas and spinal segment in the humans based on diffusion-weighted image (DWI).Thirty healthy volunteers participated in 3T MRI experiments. T1-weighted image and DWI were measured covering through the whole-brain to the cervical cord. T2-weighted image was scanned covering the cervical cord. The VOIs of spinal levels were defined based on T2-weighted image. To define the seed areas of the CS tractography, we reviewed anatomical and electrophysiological previous studies that searched the degenerated cells in cortical areas following spinal cord injury and investigated the functional mapping using intraoperative cortical electrical stimulation. Based on these evidences, primary motor cortex (M1), dorsal premotor cortex (PMd), ventral PM (PMv), supplementary motor area (SMA), pre-SMA, primary somatosensory cortex (S1), Brodmann area 5 (BA5), BA7, caudal cingulate zone (CCZ), posterior rostral cingulate zone (RCZp), and anterior RCZ (RCZa) were decided as the candidate of cortical origin of CST. In addition, we defined frontal pole (FP) as a negative control. We delineated CS streamlines from the candidate areas with negative control area and quantified the density of streamlines originated from each cortical area and the areas with a significantly higher density than a negative control area, FP was defined as the origin of CST. Then we calculated the proportion of CS streamlines in the defined cortical origin of CST and in each spinal segment.The results showed the CS sub-bundles from nine cortical areas; M1, PMd, PMv, SMA, Pre-SMA, S1, BA5, CCZ and RCZp were significantly denser than that from FP. The proportion of CS streamlines arose from nine cortical areas were M1 (mean ± SD = 49.71 ± 1.61%), PMd (16.33 ± 1.37%), PMv (11.02 ± 0.90%), SMA (5.14 ± 0.36%), preSMA (2.46 ± 0.26%), S1 (11.06 ± 0.91%), BA5 (0.88 ± 0.09%), CCZ (1.70 ± 0.30%), and RCZp (1.70 ± 0.34%), respectively. The number of terminals CS streamlines gradually decreased from the rostral to caudal spinal segments, but the intrasegmental proportion of CS streamlines from nine cortical areas were maintained through the cervical cord. These results showed that 75% of the human CS streamlines arose from the lateral surface of the frontal lobe, which may contribute to the dexterous and flexible voluntary limb control in humans.

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Poster

215. Hand Control and Dexterous Manipulation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 215.03

Topic: E.04. Voluntary Movements

Support: 11

Title: Multimuscle TMS motor mapping during finger dexterity training in young healthy volunteers

Authors: *M. NAZAROVA[1], A. ASMOLOVA[2], M. MAKAROVA[2], A. SUKMANOVA[2], P. NOVIKOV[2], P. SUNDARAM PATEL[3], V. NIKULIN[4];
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Abstract: Finger dexterity is one of the unique abilities of humans. There is some evidence that selective muscle relaxation is one of the major challenges both in people learning a motor skill, as well as in patients with conditions compromising hand dexterity. We suggest that the adequate excitability profiles among neighboring regions in somatopically organized brain areas may be one of the markers of such ability. Recently we have demonstrated that it is possible to trace interactions among motor cortical representations (MCRs) of different upper limb muscles using navigated transcranial magnetic stimulation (nTMS) as the MCR overlaps are highly reliable. Using nTMS motor mapping we further hypothesized that the MCR overlaps between the muscles trained to contract independently would decrease.24 young male volunteers have been enrolled in the study (18-36 y.o.). On the first and last days, volunteers underwent multi-muscle nTMS motor mapping with a grid of about 250 points. Between TMS sessions participants were training independent thumb and little finger abduction using EMG biofeedback from the abductor pollicis brevis (APB) and ADM (abductor digiti minimi) muscles for 10 days. Independent muscle contraction, hand dexterity (9-hole peg test (9HPT)), and finger maximum voluntary contraction (MVC) were accessed. TMS data were processed using TMSmap software. Training of the independent finger abduction was successful in all the volunteers, and the finger-movement independence increased almost twice (APB - from 16% to 29%, ADM - from 16% to 29% of MVC), the last day success rates and the changes correlated moderately between the muscles. MVC changes were less consistent. Hand dexterity, reflected by 9-HPT, improved significantly both for right and left hands. No link was observed between resting motor threshold and behavioral changes. Strikingly, we have not found a decrease in the overlap between the MCRs of the target muscles. On the contrary, we rather observed a significant increase in the areas and overlaps of all the investigated muscles. To conclude, finger independence training was successful in all the volunteers, and the skill has partly transferred to the untrained left hand, while the MVC did not change significantly and was not associated with dexterity. Our data do not support the initial hypothesis since the overlaps between MCRs of the trained muscles increased. We plan to address this question further by training the voluntary relaxation of just one of the investigated muscles. We also plan to probe how cerebellum-mediated inhibition affects the ability to perform voluntary muscle relaxation and cortical somatopical balance.

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Poster

215. Hand Control and Dexterous Manipulation

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Program #/Poster #: 215.04

Topic: E.04. Voluntary Movements

Support: MAIA, European Union's Horizon 2020, grant agreement No 951910

Title: Contribution of the medial and lateral human posterior parietal cortex to grasping execution and reprogramming

Authors: *R. BREVEGLIERI[1], S. BORGOMANERI[2,4], M. FILIPPINI[1], A. TESSARI[3], C. GALLETTI[1], M. DAVARE[5], P. FATTORI[1];
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Abstract: The dexterous control of grasping relies on the cooperative activation of different brain areas. In the parietal cortex, two grasp-related areas cooperate to orchestrate grasping actions: dorsolateral area AIP and dorsomedial area V6A. Single-cell recordings in monkeys and fMRI studies in humans have suggested that both these areas specify grip size and wrist orientation. To investigate the causal role of putative human homologous of anterior intraparietal area (phAIP) and putative human homologous of area V6A (phV6A), we stimulated with transcranial magnetic stimulation (TMS) these areas while participants were performing grasping movements (unperturbed grasping). rTMS over phAIP disrupted the wrist orientation, whereas stimulation over phV6A disrupted grip size encoding. In a small percentage of trials (25%), an unexpected reprogramming of grip size or wrist orientation was required (perturbed grasping). In these perturbed trials, rTMS over phV6A and over phAIP both impaired the reprogramming of grip size and wrist orientation. These results represent the first direct demonstration of a different encoding of grasping parameters by two grasp-related posterior parietal areas.

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Poster

215. Hand Control and Dexterous Manipulation

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Program #/Poster #: 215.05

Topic: E.04. Voluntary Movements

Title: Visual feedback improves the efficiency of anticipatory force control even when object properties are not salient

Authors: *S. BETHERS[1], J. BLAND[2], M. DAVARE[3], M. MARNEWECK[2];
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Abstract: Anticipatory force control for dexterous manipulation has historically been understood to rely on visual cues of object properties when they are salient and sensorimotor memories associated with previous experiences with the same or similar object. Visual input can be useful in providing knowledge of multiple object properties including size, mass distribution, surface texture and density. Knowing these features in advance enables anticipatory force control for efficient and dexterous interactions with objects. Visual cues are not always giving accurate information on underlying properties, particularly in the case of mass and mass distribution. In these instances, knowledge of object properties can be inferred from sensorimotor memories. In this experiment, we question whether anticipatory force control that relies on sensorimotor memories is modulated by visual feedback of the hand of the object, even when these visual cues are incongruent with an underlying key property. Right-handed human participants (N = 48) learned to minimize tilt of a symmetrically-shaped object with an asymmetrical mass distribution that switched between the left and right side in blocked trials. Using a between-subjects design, we removed visual feedback of the hand and object at reach onset or grasp (or both) to determine its timed contribution to anticipatory force control in manipulating an object without salient object properties. Across trials, anticipatory force control was similarly successful, quantified as an appropriate compensatory torque at lift onset that counters the external torque of an object, irrespective of the timing and availability of visual feedback. However, the way in which anticipatory force control was achieved, and its efficiency, varied depending on the timed availability of visual feedback. Visual feedback at either grasp *or* reach onset resulted in a significantly tighter coupling of grip to lift force generation compared to when subjects performed the task in the dark. The grip-lift force coupling significantly improved over subsequent blocks of trials in all conditions, but more remarkably under conditions with visual feedback at grasp or reach onset. Altogether, these results support the idea that visual feedback, irrespective of its timing, improves the efficiency of learning anticipatory force control for dexterous manipulation, even when key object properties are not salient. Task success irrespective of the availability of visual feedback reinforces the remarkable adaptability to maintain skilled manipulation under conditions with heightened uncertainty with compensatory strategies such as slowed generation of force.

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Poster

215. Hand Control and Dexterous Manipulation

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Program #/Poster #: 215.06

Topic: E.04. Voluntary Movements

Title: Muscle patterns associated with different thumb postures during piano performance

Authors: *S. A. WINGES, J. KRAWITZ;
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Abstract: Prevention and treatment of injury for musicians can include learning and selecting postures that reduce strain while still supporting performance over relatively long periods of time. Novices have yet to learn these optimal postures. For pianists, postures such as maintaining a neutral wrist position are typically prescribed, while more precise instructions for specific piano keystrokes are more variable across instructors/schools of piano performance. This study examined muscle activity associated with two different instructed thumb postures for playing the piano. The specific hypothesis was: Shifting the thumb posture to a more vertical "standing" position would result in less muscle activity than a more horizontal posture at thumb contact. We suspect that the shifted thumb contact point we propose will result in a thumb/hand posture that will allow pianists to better utilize their hand structure to create a stable segment that can transfer force derived from the forearm movement to the key and reduce the need for thumb muscle activity. Surface EMG was recorded from six muscles of the right and left arms from five piano students. Each student played two short pieces and a set of exercises using their preferred posture followed by instructed "standing" or "side" thumb postures. Intrinsic and extrinsic thumb muscles exhibited changes in average magnitude of muscle activity with opposite trends with respect to thumb posture. Individual differences in patterns of muscle activity associated with differences in thumb postures were observed.

Disclosures: S.A. Winges: None. **J. Krawitz:** None.

Poster

215. Hand Control and Dexterous Manipulation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 215.07

Topic: E.04. Voluntary Movements

Support: National Science Foundation BCS-1827752.

Title: Improving finger force interdependence through visuomotor training

Authors: *E. TAGOE[1,2], Y.-H. WU[2], M. SANTELLO[2];
[1]Mechanical Engin., Northern Arizona Univ., Flagstaff, AZ; [2]Sch. of Biol. and Hlth. Syst. Engin., Arizona State Univ., Tempe, AZ

Abstract: Many manual activities, e.g., manipulating tools or playing a musical instrument, require dexterous control of movement and forces of individual fingers. Humans' ability to control individual fingers has been extensively studied. This work has revealed a tendency for unintended finger movement or force by the 'non-instructed' finger(s) in response to movement or force generated by the 'target' finger. This phenomenon, known as *'enslaving'*, arises from central and peripheral constraints. Although it has been shown that finger movement enslaving can be reduced by training, the extent to which finger force enslaving (EN) might be reduced by

training is unknown. To address this gap, we designed an experimental protocol to train subjects (n = 7; 3 females; age: 22 - 25 years; right-hand dominant) to exert force with their right middle finger while minimizing the exertion of forces by non-instructed fingers. The middle finger force was mapped to the position of a cursor that had to be moved to a target. All finger forces were mapped to the cursor position such that force exerted by non-instructed fingers would cause a lateral trajectory deviation that interfered with reaching the target. In the first session (day 1), subjects performed 12 pre-training trials (PRE), followed by 108 training trials (TRA) and subsequently 12 post-training trials (POST). On day 2, subjects performed 12 trials of the same task to assess whether training effects, if any, were retained 24 hours post-training (RET). Every 18 trials of the TRA block, the gain of the non-instructed finger force mapping to cursor position was increased or maintained based on the subject's performance metrics from the previous 18 trials. We quantified EN as the force contribution by non-instructed fingers relative to the total force exerted by all fingers. One subject, who had the lowest EN magnitude across all subjects, exhibited no EN reduction despite being exposed to a high gain during the TRA block. In contrast, three of the remaining six subjects exhibited a reduction in middle finger force EN when comparing POST vs. PRE trials (-25 to -49%; average: -36 ± 12%). Importantly, however, all of these six subjects exhibited a statistically significant reduction in EN when comparing RET vs. PRE trials (-3 to -77%; average: -32 ± 25%; $p < 0.05$). These preliminary data suggest that central and peripheral neuromuscular constraints limiting individuated finger force control can be modified through a subject-specific adjustment of visuomotor training difficulty. Ongoing work is addressing the neural correlates underlying the improvement in independent finger force control and potential clinical applications.

Disclosures: **E. Tagoe:** None. **Y. Wu:** None. **M. Santello:** None.

Poster

215. Hand Control and Dexterous Manipulation

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Program #/Poster #: 215.08

Topic: E.04. Voluntary Movements

Support: NSF Grant BCS-1827752

Title: Context-dependent brain dynamics during planning and execution of dexterous manipulation

Authors: *C. BLAIS[1], Y.-H. WU[2], J. M. FINE[4], M. SANTELLO[3];
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Abstract: It is widely accepted that two distinct neural systems - the dorsomedial and dorsolateral pathways - coordinate reaching and grasping. However, research on the neural correlates of reach-to- grasp has been entirely based on tasks that constrain digit placement to

occur at the same points on an object over repeated manipulations (constrained grasping, CON). However, we have shown that CON grasping relies on the retrieval of sensorimotor memory of grasp forces to execute a near-identical motor plan across multiple lifts. In contrast, when grasp contacts are not constrained (unconstrained grasping, UNCON), online monitoring of digit placement becomes necessary for the modulation of digit forces to compensate for trial-to-trial variability in digit placement. Although this theoretical framework has been supported by kinematic, kinetic and neuromodulation approaches, the neural dynamics underlying planning and execution of UNCON grasping remain unknown. To address this gap, we asked twenty-two naïve right-handed volunteers (27 ± 3.51 years; 9 females) to perform a CON and an UNCON grasping task while 64-channel EEG was recorded. Visual cues were given to the subjects to allow them to plan for CON or UNCON grasps. Performance of dexterous manipulation was defined as subjects' ability to exert a compensatory torque at object lift-off (T_{com}) to compensate for the external torque caused by an added mass at the base of the object. Structural MRIs were collected to allow for accurate source analysis of EEG signals. Critically, our protocol was designed to ensure that T_{com} was similar in both grasp contexts, which was validated. We found that neural systems involved differed between CON and UNCON: Relative to object contact (execution), several nodes within the dorsomedial path (PMd, aSPL, mIPS, SPOC; all p's < 0.05) were differentially activated, these differences emerging as early as 40 ms post-contact and lasting throughout the object lift (~1000 ms post-contact). Importantly, significant differences were also found in the neural activity in these and other regions prior to reach onset, i.e., planning, and during reaching. Neural activity in the dorsomedial path during planning (aSPL, SPOC; ~200-900 ms) and reaching (PMd, aSPL, mIPS, SPOC; ~150-1000 ms), as well as in the ventromedial path during planning (PMv ~250-1000 ms) and reaching (PMv ~200-1000 ms), was significantly different as a function of grasp context (all p's < 0.05). Consistent with our previous work, our findings confirm that control of CON and UNCON grasping execution engages the same neural network in different ways not only during execution but also during planning.

Disclosures: C. Blais: None. **Y. Wu:** None. **J.M. Fine:** None. **M. Santello:** None.

Poster

215. Hand Control and Dexterous Manipulation

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Program #/Poster #: 215.09

Topic: E.04. Voluntary Movements

Support: NIH T32-NS082128-06

Title: Distinct neural activity for controlling the speed of ballistic contractions at low force levels

Authors: *J. KIM[1], S. DELMAS[1], Y. CHOI[1], J. HUBBARD[1], M. WEINTRAUB[1], B. YACOUBI[1], F. ARABATZI[2], E. A. CHRISTOU[1];

[1]Applied Physiol. & Kinesiology, Univ. of Florida, Gainesville, FL; [2]Physical Educ. & Sports Sci. at Serres, Aristotle Univ. of Thessaloniki, Serres, Greece

Abstract: Ballistic contractions are characterized by the intention to exert force with maximal velocity. According to Freund and Budinger (1978), who examined the relation between the speed of the fastest possible voluntary contractions and their force amplitude, the duration from the onset of force to peak force (TPF) was approximately constant for all amplitudes. Given that the EMG burst duration also remained approximately constant, they proposed the speed-control hypothesis, which suggests that the central nervous system regulates the velocity of the contraction to remain approximately constant. This creates a linear relation between the rate of force development (RFD) and force, which reduces the variables that the nervous system (NS) needs to control. Thus, during ballistic contractions, our NS needs to only consider the amplitude of force. However, this hypothesis has only been tested with force levels ranging from 20-80% maximum and six subjects. Here, we performed a study that examined the relationship between RFD and force amplitude ranging from 2 to 85 %MVC and the underlying structure of the EMG burst in 18 young adults. Participants exerted their fastest TPF for 50 trials in each of 7 randomly assigned force levels (2, 5, 15, 30, 50, 70 and 85 %MVC). They received visual feedback of their performance after each trial. To characterize the relation of TPF and force amplitude, we quantified the TPF for the 10 trials that best matched the targeted force. Like previous studies we found a linear relation between TPF and force level from 15 to 85 %MVC, indicating a slower contraction speed with force level. However, the contraction speed below 15 %MVC deviated from the hypothesized relation between force and RFD. Specifically, the contraction speed was slower at 2 and 5 %MVC than 15 %MVC. Indeed, the contraction speed at 2 %MVC was like the one observed for 50 %MVC. Thus, our results suggested a distinct organization for the lowest force levels, which was supported from our findings in the structure of the associated muscle activity burst. The slowing in contraction speed from 15 to 2 %MVC was associated with a decreased normalized power in muscle activity from 13-30 Hz, suggesting a neural effect. In contrast, the slowing of the contraction speed from 15 to 85 %MVC was only associated with the muscle activity amplitude, suggesting a mechanical effect. These findings provide novel evidence that the speed-control hypothesis fails to explain the contraction speed of very low force levels (≤5 %MVC), which appear to be controlled by a unique neural drive.

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Poster

215. Hand Control and Dexterous Manipulation

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Topic: E.04. Voluntary Movements

Support: DARPA-L2M program W911NF1820264
NSF CRCNS Award 2113096
NIH R21-NS113613 to F.J.V.-C

Title: Rigid robotic transformations can approximate the kinematics of soft fingers with 'bones'

Authors: *N. MATHARU[1], J. E. LAO[1], A. IWAMOTO[1], A. HELTON[1], T. FANELLE[1], A. ERWIN[2], F. J. VALERO-CUEVAS[1];
[1]Dept. of Biomed. Engin., [2]Div. of Biokinesiology and Physical Therapy, USC, Los Angeles, CA

Abstract: The ability to model and control soft bio-inspired robotic hands would enable a new class of manipulation applications because softness, by passively conforming, decreases the precision required for control (Brock and Valero-Cuevas, Physics of Life Reviews, 2016). Here we tested how well traditional rigid robotic transformations can approximate the kinematics of a semi-soft robotic finger (i.e., rigid `phalanges' embedded in soft material). We made one tendon-driven, semi-soft finger with a length of 13 cm and three 4 cm phalanges (plus a `metacarpal' for mounting) with 0.15 cm of silicone between serving as the `joints'. Then, this semi-soft finger was compared against a ground-truth, rigid 3-link planar hinged finger.
Tendons were routed per the N+1 design (Valero-Cuevas, Fundamentals of Neuromechanics, 2016) where N is 3 degrees of freedom, in which tendons cross, and therefore affect multiple joints. Motors pulled on tendons with seven activation sets to drive the finger to different flexion-extension positions. The resulting finger endpoints were measured at each position using the DeepLabCut motion tracking software (Mathis et al., Nature Neuroscience, 2018). To test the validity of the linear rigid robotic transformations for our semi-soft finger, we calculated a linear regression relating endpoint locations to the seven tendon excursion sets.
The proportion of variance explained by the regression for the semi-soft finger was 69% (R2=0.688), compared to 100% for the rigid-finger. The average discrepancy between the predicted and observed finger endpoints for the 7 positions was 2.1 (± 1.9) mm for the semi-soft finger and 0 (± 0) mm for the rigid finger.
Our results indicate that while kinematic prediction of fingertip endpoints for the semi-soft finger did not follow the linear rigid robotic transformations as closely as that of the rigid finger, they were approximated well with about 69% of the variance explained. These results are encouraging as they show that there may be a way to combine the ability of semi-soft fingers to passively conform to objects grasped, with the numerous effective control methods developed for hinged rigid kinematics chains.

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Poster

215. Hand Control and Dexterous Manipulation

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Program #/Poster #: 215.11

Topic: E.04. Voluntary Movements

Support: National Science Foundation BCS-1827752

Title: Performance of dexterous object manipulation is enhanced by grasp force modulation

Authors: *Y.-H. WU, M. SANTELLO;
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Abstract: In the past four decades, the coordination of digit forces responsible for controlling two-digit precision grip has been extensively studied. This research has mostly focused on the sensorimotor mechanisms underlying object slip prevention (reviewed in Johansson & Flanagan, 2009), whereas only a few studies have addressed how the central nervous system (CNS) simultaneously attains two goals involved with dexterous hand-object interactions: object slip prevention and manipulation, i.e., control of object orientation and position (pose) (Fu & Santello, 2014; Fu, Zhang, & Santello, 2010). To understand how digit forces are coordinated to both prevent object slip and control object pose, we asked subjects (n = 16, 8 males; mean age: 23.88 ± 5.50 years) to grasp and lift an instrumented inverted-T shape object using two digits (thumb and index fingertip) while preventing it from tilting. We systematically changed the object's mass distribution and the vertical distance between thumb and index fingertip (digit offset) to elicit a large space of feasible digit force solutions. We analyzed digit forces and torques using grasp analysis developed in robotics (*Manipulation−Grasp Force Decomposition, MGFD*; Kerr & Roth, 1986; Yoshikawa & Nagai, 1991). *MGFD* mathematically decouples digit forces into grasp force (F_G) responsible for object slip prevention and manipulation force (F_M) responsible for controlling object pose. Although F_G decreased with increasing digit offset ($t_{2685} = -22.26$, p < 0.0001), its magnitude remained two to three times greater than necessary to prevent object slip. Remarkably, this excessive grasp force was instrumental in increasing the robustness of manipulation robust, as indicated by the increase in the relative volume of manipulation solutions ($t_{2149} = 9.50$, p < 0.0001) and smaller manipulation performance error, i.e., peak object tilt ($t_{2152} = -3.75$, $p = 0.0002$). Furthermore, the space of manipulation solutions was larger at the onset of object manipulation than during hold ($t_{3233} = -2.70$, $p = 0.0069$), underscoring the transition from anticipatory (memory-based) to reactive (feedback-based) control. In sum, *MGFD* was instrumental in disentangling the roles of digit forces dedicated to object slip prevention versus pose control and has the potential for providing further insights into neural control of dexterous manipulation. The causal relation between grasp and manipulation forces is currently being investigated.

Disclosures: Y. Wu: None. **M. Santello:** None.

Poster

215. Hand Control and Dexterous Manipulation

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Topic: E.04. Voluntary Movements

Support: Brain and Spinal Cord Injury Research Trust Bridge Funds, Center for Respiratory Research and Rehabilitation (CRRR) and the Trauma, University of Florida (SV)

Title: Practice of random patterns may outperform sequence learning in manual force control

Authors: *F. BANIASAD, A. AVILA, C.-L. CHIANG, S. VAHDAT;
Dept. of Applied Physiol. & Kinesiology, Univ. of Florida, Gainesville, FL

Abstract: Background: Controlling the level of hand force is crucial for performing daily activities. Although repeated practice of a specific sequence of movements results in greater performance gains compared to practice of random patterns in learning movement kinematics, the benefits of random vs sequential practice in learning precise force control is not well understood. Here, we developed a motor learning paradigm that aims to achieve precise manual force control using a magnetic resonance imaging-compatible force transducer and compared performance gains obtained over 10 days of practice in random vs sequential force profiles.
Method: 48 healthy adults participated in 2 experimental sessions (Day1: D1, and D10), during which they practiced in two visually guided force production tasks using their right hand (14 blocks each) to track: i) a predefined force profile (sequence task), or ii) unpredictable force profiles (random task). Participants were divided in 3 groups: sequence/random groups that performed 8 additional daily sessions of sequence/random task between D1 and D10, and a control group that did not receive any additional training. Performance was evaluated by measuring the error between the produced and target force profiles during the sequence and random tasks. To evaluate motor memory, we obtained 3 additional testing conditions (3 blocks of sequence task each) on D1 and D10, but with altered feedback or end-effector: No-cursor condition (visual feedback of hand force eliminated), No-target condition (visual feedback of target force profile eliminated), and Left-hand condition (generalization to left hand).
Result: Our results show that compared to the control group: 1) Both sequence and random groups performed with significantly less errors ($p < 0.001$) in the sequence task on D10, 2) The random group performed with significantly less errors ($p < 0.001$) in the random task on D10, 3) The sequence group performed with significantly less errors ($p < 0.001$) during the No-cursor testing condition on D10, 4) The random group performed with significantly less errors ($p < 0.01$) during the No-target testing condition on D10, and, 5) Both sequence and random groups performed with significantly less errors ($p < 0.01$) during the Left-hand testing condition on D10.
Conclusion: Although long-term learning of a specific sequence of force results in greater cognitive memory of the task (as evident in No-cursor condition), practicing unpredictable random force profiles can ultimately result in superior motor performance that exceeds the cognitive benefit (as evident in No-target condition). Our results provide guidelines for learning hand force control.

Disclosures: F. Baniasad: None. **A. Avila:** None. **C. Chiang:** None. **S. Vahdat:** None.

Poster

215. Hand Control and Dexterous Manipulation

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Topic: E.04. Voluntary Movements

Support: Knight Campus Undergraduate Scholars award

Title: Electromyographic Markers of Widespread Motor Inhibition During Stopping

Authors: *I. J. MILLS, M. FISHER, I. GREENHOUSE;
Univ. of Oregon, Eugene, OR

Abstract: Evidence in humans and animals indicates the presence of a neural mechanism for reactive behavioral stopping with widespread inhibitory effects on the motor system. Transcranial magnetic stimulation studies have quantified the anatomical spread and extent of motor system inhibition during stopping, but this method only provides temporal snapshots of motor system excitability. Here, we tested the hypothesis that electromyography (EMG) can provide a more continuous marker of widespread motor system inhibition during stopping. Twenty subjects (9 male, 21.4 ± 0.92 years old) performed a novel version of the bimanual anticipatory response inhibition (ARI) task. In this task, participants timed simultaneous index finger lifts to the moment when rising bars intersected a target line. On stop trials (33%), the bars stopped rising before the target at a delay determined by individual task performance. This served as a stop signal, and participants were instructed to withhold their finger lifts on these trials. Participants maintained a constant force (tonic) contraction in the abductor digiti minimi (ADM) muscles, measured with surface EMG, throughout the task. We predicted a decrease in ADM EMG amplitude during successful stopping, relative to going and failed stopping, which would serve as a marker of widespread inhibition. Participants succeeded at stopping on 48.0 ± 2.4% of stop trials, and stop signal reaction time (SSRT), an estimate of the duration of the stopping process, was 305 ± 52 ms. Index finger lift time averaged across hands was 19 ± 9 ms relative to the target. Mean ADM EMG amplitudes for successful and failed stop trials were calculated using the SSRT epoch, i.e. 0 to 305 ms relative to the stop signal, and for Go trials using the epoch 0 to 305 ms following the average stop signal onset (derived from stop trials). Contrary to our hypothesis, tonic ADM EMG amplitudes did not differ between failed stop, go, and successful stop trials or between hands, and there was no interaction (all p's > 0.3). However, we visually identified suppressed ADM EMG during failed stop trials at 140 to 200 ms following the stop signal, suggesting an EMG marker of widespread motor system inhibition can be detected even during the execution of a failed stop response.

Disclosures: I.J. Mills: None. **M. Fisher:** None. **I. Greenhouse:** None.

Poster

215. Hand Control and Dexterous Manipulation

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Program #/Poster #: 215.14

Topic: E.04. Voluntary Movements

Title: Revisiting the 'point of no return' using electromyography during the stop signal task

Authors: *M. FISHER, H. TRINH, J. O'NEILL, I. GREENHOUSE;
Univ. of Oregon, Eugene, OR

Abstract: The ability to reactively stop movements is an important feature of the motor system to ensure safety in dynamic everyday environments. Previous investigations using the stop signal task have sparked the debate over whether there exists a "point-of-no-return," after which a rapid, stimulus-driven response cannot be successfully stopped. Here, we revisit this question using electromyography (EMG) during the performance of the stop signal task in two experiments to assess the putative point-of-no-return at the level of the muscles. EMG offers fine temporal resolution of muscle-level physiological changes associated with inhibitory control processes. The first experiment (n = 24, 10 female, 24 ± 4 years) included a simple stop task performed with either the right or left index finger in separate task blocks, with EMG collected from the first dorsal interossei (FDI). The task in the second experiment (n = 28, 10 female, age 24 ± 4 years) involved a choice between the right index and pinky fingers, with EMG collected from the right FDI and right abductor digiti minimi (ADM). In both experiments, visual stimuli were presented on a screen, and participants responded with button presses. GO stimuli were presented for .5 s, and STOP stimuli were presented on 33% of trials, each at a variable stop signal delay, adjusted according to individual task performance. Participants were instructed to respond as quickly as possible to GO stimuli and to attempt to stop in response to the STOP stimulus. To evaluate the approximate point-of-no-return, we calculated mean EMG amplitudes during the 100 ms epochs preceding the STOP stimuli and found these amplitudes were greater during failed stop trials than successful stop trials (i.e. stop trials containing partial EMG bursts in the absence of a button press). This difference between failed and successful stop trials was present for experiment 1 (left, p=0.05; right, p=0.01) and experiment 2 (index, p=0.01; pinky, p=0.001), indicating that EMG reliably differentiates failed from successful stopping prior to stop signal onset, regardless of whether or not a response choice is involved. Our findings strongly suggest EMG can detect a point-of-no-return.

Disclosures: M. Fisher: None. **H. Trinh:** None. **J. O'Neill:** None. **I. Greenhouse:** None.

Poster

216. Action and Sensation During Reaching Movements

Location: SDCC Halls B-H

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Program #/Poster #: 216.01

Topic: E.04. Voluntary Movements

Title: Proprioception during joint motion driven by muscle contraction or interaction torque

Authors: *P. R. HEIDARI, N. MANGOS, O. CODOL, J. A. PRUSZYNSKI, P. L. GRIBBLE;
Western Univ., London, ON, Canada

Abstract: Proprioceptive sense is believed to be more sensitive during movement that is self-generated than during movement that is passively guided. Although humans' day-to-day movements are rarely passive, they do often involve motion that is not driven directly by muscle contraction. For example, when reaching towards the periphery on the ipsilateral side of the arm, the torques driving motion of the forearm relative to the upper arm are produced primarily by active contraction of elbow extensor muscles. But when reaching towards the contralateral periphery, elbow extension is driven mostly by motion of the upper arm about the shoulder joint. In this case, interaction torque—rotational force that acts at one joint due to motion of an adjacent limb segment about another joint—is the primary driver of elbow motion. Proprioception is known to play a critical role in compensating for interaction torque during multi-joint limb movement, and deafferented patients have marked deficits in this aspect of motor control compared to their healthy counterparts. This observation is seemingly at odds with the widely-held belief that proprioceptive sense is poor during motion that is not driven by active muscle contraction, and suggests that proprioceptive acuity might be preserved during motion that is driven by interaction torque. In the present study, we quantified proprioceptive acuity at the elbow joint while participants were midway through reaching movements in which elbow extension was primarily driven passively by interaction torque, or in other movements, actively by elbow muscle torque. We delivered equally sized and timed perturbations to the elbow joint during motion and tested participants' ability to correctly sense the direction in which the elbow was perturbed (flexion or extension). The sensitivity of participants' responses to the actual direction of the perturbation differed depending on whether the perturbation was delivered during interaction torque-driven motion or active muscle torque-driven motion. Specifically, we found that participants had superior perceptual acuity when joint motion was driven by interaction torque, suggesting that proprioceptive sense is preserved during this type of motion.

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Poster

216. Action and Sensation During Reaching Movements

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Program #/Poster #: 216.02

Topic: E.04. Voluntary Movements

Support: JSPS-JP16H06566

Title: High gain of reflexive manual response to the peripheral visual motion can be explained by decoded translational self-motion

Authors: *H. GOMI;
NTT Communication Sci. Labs, NTT Communication Sci. Labs, Kanagawa, Japan

Abstract: We frequently extend our arms toward external objects during body movements. Previous studies have proposed that the reflexive manual response induced by a large field visual motion during reaching movement (named MFR: manual following response, Saijo et al. 2005; Gomi et al. 2006) is considered as a compensatory adjustment against the estimated self-motion from visual motion, as extensively examined for the reflexive ocular response induced by visual motion (OFR: ocular following response, Miles et al. 1998; Angelaki & Hess 2005). Despite the similarity of spatiotemporal frequency tunings of MFR and OFR for the full-field visual stimuli, spatial integration characteristics of MFR was greatly different from that of OFR: MFR is highly sensitive to the peripheral visual field with the low spatial frequency stimuli, compared to the OFR (Gomi et al. 2013). To explore the mechanisms of spatiotemporal frequency tuning and spatial integration characteristics of MFR, we have examined whether the convolutional neural network (CNN) decoder trained to estimate self-motion from the image sequences can explain these specificities of MFR. The visual image sequences (90x60 deg; 60 Hz) and 6-DoF velocities were recorded by the head-mount camera system (ZED-mini) during 30 human natural movements (mainly walking outside and inside), then the CNN was trained to decode the 6-DoF velocities from the image sequences. Both of the rotational output in vertical direction and translational output in transversal direction decoded by the learned CNNs exhibited tuning peaks at low-spatial and high temporal frequencies for contrast grating visual motions with various spatial and temporal frequencies. In addition, the translational output decoded by the learned CNN was robust to the large center-mask reducing the low spatial frequency grating visual motion while the vertical-axis rotational output decreased with center-mask size. Interestingly, these features are respectively similar to the tuning specificities of MFR and OFR for the different center masks. The high sensitivity of the transversal motion on the visual periphery may be ascribed to the visual statistics: the lower peripheral vision frequently receives the near field view, on which visual motion consistently represents the self-motion. These features obtained from the synthetic modeling of the self-motion coding from visual motion support the idea that the manual following response, MFR, is produced by the estimated transversal self-motion from the visual motion, rather than the rotational self-motion.

Disclosures: H. Gomi: None.

Poster

216. Action and Sensation During Reaching Movements

Location: SDCC Halls B-H

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Program #/Poster #: 216.03

Topic: E.04. Voluntary Movements

Title: Proprioceptive control of a tool.

Authors: *W. G. DARLING[1], B. I. ZUCK[1], J. ADHIKARI[2], L. MIKHAIL[2];
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Abstract: Using a tool is known to affect conscious perception of a limb's length (e.g., Martel et al. 2019 Sci Reports 9:5517) and hand motion (e.g., Musseler and Sutter 2009 Consc Cogn 18:359). However, it is not known whether minimal tactile/proprioceptive experience with a never seen tool is sufficient to allow proprioceptive control of tool movements to proprioceptive and visually specified targets. We investigated accuracy of such movements using a 30 cm rod held in the right hand of blindfolded subjects to reach with a single movement to touch tool endpoint and midpoint to the stationary and moving index-tip of the left hand, to the ear lobes, and to visually specified external targets. We also assessed transfer of proprioceptively specified tool characteristics to the left hand for reaches to the right and left ear lobes. Subjects were instructed to execute a single smooth movement and not to make corrective movements if they missed the target. If corrective movements occurred, they were eliminated in the analysis by measuring errors at the end of the first movement. Thus, we focused on use of proprioception to control initial hand-held tool movements, rather than on conscious perceptual processing of proprioceptive inputs. Mean and variable 3D distance errors were computed for reaches to proprioceptive targets. Errors in reaches to visually specified targets were computed in a spherical coordinate system with a shoulder origin. Movements with the tool held in the right hand to proprioceptive targets with the tool endpoint were accurate as mean and variable distance errors averaged about 2 cm. In contrast, mean distance errors were much larger when pointing with the tool midpoint, averaging about 5.5 cm ($p < 0.001$), but variable distance errors were similar to those when pointing with the endpoint ($p > 0.05$). Left hand movements of the tool endpoint and midpoint to the ear lobes had similar errors to the right hand movements ($p > 0.05$), indicating excellent transfer of proprioceptive information for control of left hand movements. Radial distance errors were large for visually specified targets with undershoot of close targets and overshoot of far targets, but direction errors were small, therefore being consistent with previous reports of large sensorimotor transformation errors in reaching to visual targets (Soechting and Flanders 1989 J Neurophys 62:595). Overall, our results show that proprioceptive information about length of a never seen tool can be used to accurately control movements of the tool endpoint to proprioceptive targets. but movements to visually remembered targets are less accurate due to approximations of the sensorimotor transformations.

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Poster

216. Action and Sensation During Reaching Movements

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 216.04

Topic: E.04. Voluntary Movements

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Title: Visual perturbation type affects movement planning in the absence of proprioception

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Abstract: Proprioception is critical for providing the information necessary for the nervous system to efficiently coordinate movement. Previous studies with individuals deprived of proprioception due to an acquired large fiber sensory neuropathy revealed movement deficits, possibly due to an inability to update internal models of movement properly. Such individuals tend to use vision to partially compensate for the absence of proprioception in order to make accurate reaching movements. However, it remains unclear whether online responses to visual perturbations rely on proprioceptive feedback. While Bard et al. (1999) reported that corrective movements in response to a small target displacement were unimpaired in a deafferented individual, Sarlegna et al. (2006) observed abnormal corrections in response to large target displacements. With respect to a cursor displacement (i.e., the visual representation of hand position), Bernier et al. (2006) showed that a deafferented individual could adapt to the perturbation. We hypothesized that an individual with chronic peripheral deafferentation (GL; 70 years old; right hand dominant; 40 years since onset of peripheral deafferentation) may respond more efficiently to target jumps because there is a direct visual representation of the perturbation that is unrelated to limb position, unlike in cursor jumps. Proprioceptive cues regarding limb position are absent regardless of the type of perturbation, but there are differences in what needs to be internally programmed. We designed two tasks on the Kinereach virtual reality motion tracking system to be completed with each hand separately. In the cursor jump task, GL had to move a cursor, representing the hand position, from a start circle to a target placed 15 cm ahead, as quickly and as accurately as possible. The cursor jump occurred in pseudorandom trials at movement onset and involved one of six lateral displacements. In the target jump task, the target could jump to one of six lateral locations pseudorandomly at movement onset. These paradigms did not examine adaptation to perturbation or its aftereffects; instead, the tasks were designed to understand the online corrective mechanisms used during unexpected visual perturbations. Results showed that target jumps produced larger movement deficits in movement accuracy and initial direction error during the first stage of movement than cursor jumps. GL may be programming for a specific final position during target jumps, and an initial posture during cursor jumps. Perhaps this difference in what needs to be programmed gives rise to the deficits.

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Poster

216. Action and Sensation During Reaching Movements

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Title: Functional organization of the lateral reticular nucleus for dexterous forelimb movement.

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Abstract: Dexterous forelimb movements require the exquisite coordination of dozens of muscles to achieve a desired goal. The precision of these behaviors is achieved in part through rapid online corrections of motor output throughout the course of a movement. While sensory feedback provides information critical for assessing movement outcomes, feedback delays suggest that rapid corrections are driven by a faster form of online control. The cerebellum is thought to perform computations necessary for these rapid online corrections by using internal copies of motor commands to predict movement outcomes. The lateral reticular nucleus (LRN) is a brainstem nucleus that receives ascending internal copy signals from the spinal cord and projects its output to the cerebellum. Little is known about how these internal copy signals are organized and processed in the LRN to facilitate coordinated movement. To address these questions, we: (1) perturb LRN circuits to evaluate the consequences on dexterous forelimb movement; (2) record the activity of LRN neurons during limb movements to evaluate the encoding of kinematic and task features; and (3) dissect the molecular and anatomical organization of LRN neuron subtypes. We found that both chronic and acute perturbation of LRN neurons disrupts the performance of dexterous forelimb movements, and we have developed machine learning-based approaches to identify kinematic features that contribute to behavioral phenotypes. Additionally, we find diverse responses of LRN neurons during the execution of forelimb movements, suggesting that there are distinct subsets of LRN neurons that encode and process different movement related features. Finally, using single cell RNA sequencing we have identified and characterized anatomically and molecularly distinct subtypes of LRN neurons. These subtypes are distributed into different anatomical subregions of the LRN, and potentially form the cellular basis for functionally distinct LRN subcircuits. Further genetic and functional dissection of these subcircuits will reveal how the LRN organizes information from a broad range of spinal internal copy pathways for the refinement of skilled movement.

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Poster

216. Action and Sensation During Reaching Movements

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Title: Dissecting the contribution of spinal interneurons to rhythmic and discrete forelimb movements

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Abstract: Animals generate a wide range of forelimb behaviors to meet diverse task demands. Interneurons in the cervical spinal cord play a central role in generating these behaviors, integrating descending motor commands and sensory feedback to produce coordinated patterns of muscle activity. Spinal interneurons are classified into various subtypes via their genetic identities and developmental provenance. The roles of many spinal interneuron subtypes in the lumbar spinal cord have been investigated in locomotor behaviors, but whether functions are conserved or diverge in the cervical spinal cord to accommodate the wide repertoire of forelimb motor behaviors remains unclear. Here, we hypothesized that: 1) genetically identifiable spinal interneuron subtypes give rise to similar neuronal circuit architecture between the cervical and lumbar cord and, therefore, serve similar functions in rhythmic forelimb and hindlimb movements; and 2) these circuits can be repurposed to control discrete goal-directed movements that are unique to the forelimb. To address these questions, we leveraged high-resolution mouse forelimb behavioral assays to study the contributions of key spinal interneuron subtypes in the generation of distinct types of forelimb behaviors: a string-pull task, requiring rhythmic goal-directed limb movements, and a joystick reaching task, requiring precise, discrete limb movements. We used an intersectional genetic and viral approach to perturb specific interneuron classes unilaterally in the cervical spinal cord, and behavioral phenotypes were monitored by recording electromyographic (EMG) activity of forelimb muscles and by quantifying forelimb kinematics. We further investigated a causal link between EMG recordings and the observed kinematics by developing a biomechanical model of the mouse forelimb. While interneuron perturbation revealed behavioral phenotypes in both the string-pull task and joystick center-out reaching task that were generally similar to phenotypes observed during hindlimb rhythmic behaviors, we identified specific differences in muscle recruitment and forelimb kinematics, suggesting that cervical spinal interneurons can play limb-specific roles. This work clarifies how genetically similar neural circuits across the neuraxis are differentially recruited to produce a wide range of motor behaviors.

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Poster

216. Action and Sensation During Reaching Movements

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Title: Forelimb movement refinement by distinct cerebellar output pathways

Authors: *O. WILCOX[1,2], A. R. THANAWALLA[2], J. JIANG[3], R. YUSUFI[2], A. I. CHEN[4], E. AZIM[2];
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Abstract: The cerebellum ensures the precise coordination of limb movements in part through the online adjustment of motor output. The cerebellar nuclei (CN) serve as the source of cerebellar output by sending axonal projections that ascend and descend to target diverse motor structures. Yet, how discrete output pathways influence forelimb movement and contribute to online motor refinement remains unclear. Leveraging mouse genetic tools and known ascending and descending CN projections, we used intersectional viral approaches to target cerebello-spinal (CN^{sp}) and cerebello-supraspinal (CN^{ssp}) pathways selectively. While CN^{ssp} neurons have been the focus of several studies, CN^{sp} neurons are a largely distinct group whose role in the online control of dexterous forelimb movement has been less explored. Thus, we concentrated our efforts on defining the functional connectivity and behavioral contributions of descending CN^{sp} neurons, drawing comparisons to the ascending CN^{ssp} neurons. Using anatomical and electrophysiological approaches, we found that CN^{sp} neurons project directly to cervical spinal interneurons and through this population rapidly influence motor neuron activity (within ~2-3 ms). Perturbation of CN^{sp} neurons results in distinct effects on forelimb muscle activity and reach kinematics when compared to CN^{ssp} neurons. Ongoing electrophysiological recording in behaving mice is identifying how the activity of neurons belonging to each CN output pathway correlates with features of limb movement. Together, this comparative approach reveals distinct

roles for pathways originating from the cerebellar nuclei and highlights the contribution of CN^{sp} neurons to rapid online adjustments of forelimb movement.

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Poster

216. Action and Sensation During Reaching Movements

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Title: Large-scale capture of hidden fluorescent labels for training generalizable markerless motion capture models

Authors: *D. J. BUTLER, A. P. KEIM, S. RAY, E. AZIM;
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Abstract: As deep learning has become a mature technology for extracting animal pose from images and video, markerless tracking methods have become widely adopted in behavioral neuroscience. Modern tracking methods can produce highly accurate results, sometimes with as few as 100 to 1000 labeled training images. Our approach builds on this influential work in markerless tracking and aims to overcome several key obstacles that remain, including poor robustness to changes in visual context and sparse labels. Although tracking models can in principle be applied to new experimental setups and visual environments not seen during training, in practice, even small changes, for example in lighting, camera position, or the behavior being measured, often cause accuracy to drop dramatically. Moreover, much of the movement data is discarded: only a handful of sparse landmarks are typically labeled, due to the inherent scale and accuracy limits of manual annotation. To address these issues, we developed an approach, which we term GlowTrack, for generating large training datasets that overcome the relatively modest limits of manual labeling, enabling deep learning models that generalize across experimental contexts. The key innovations are: a) an automated, high-throughput approach for generating hidden labels free of human error using fluorescent markers; b) a multi-camera, multi-light setup for generating large amounts of training data under diverse visual conditions; and c) a technique for massively parallel tracking of hundreds of landmarks simultaneously using

computer vision feature matching algorithms, providing dense coverage for kinematic analysis at a resolution not currently available. These advances yield versatile deep learning models that are trained at scale, laying the foundation for standardized behavioral pipelines and more complete scrutiny of animal movements.

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Poster

216. Action and Sensation During Reaching Movements

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Support: National Sciences and Engineering Research Council
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Canadian Foundation for Innovation

Title: Acute plasticity of online monocular visuomotor function

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Abstract: When interacting with our environment, visual information is typically available to both our dominant and non-dominant eye. Also, when performing upper-limb movements with the dominant hand, humans tend to be more effective in using visual information from their dominant eye to implement online trajectory amendments (Manzone et al., 2018: J Mot Behav). However, there are situations in which visual information is only available to our non-dominant eye (e.g., objects and people in our visual periphery, blind spot while driving, etc.). Further, it is not known if online control processes can acutely improve when visual information is solely available to the non-dominant eye. The current study investigated whether the visuomotor pathways associated with the non-dominant eye can be acutely trained. Specifically, we tested if the ability to make online corrections to ongoing upper-limb pointing movements using non-dominant monocular information can improve with practice. Participants planned reaching movements to a target located 30 cm from the start position, while wearing liquid crystal goggles. Vision was provided to both eyes before the movement and to one eye for another 20 ms early in the trajectory (i.e., when the limb reached 1 m/s; Tremblay et al., 2017: Exp Brain Res). To assess online control, an imperceptible target jump (i.e., minus 3 cm from the initial target) was introduced after movement onset on one third of trials. On the first day, participants were tested for eye dominance during a pre-test, which was based on their movement correction amplitude during target jump trials for each eye. Then, participants trained for 45 minutes using their non-dominant eye only (i.e., acquisition; with and without target jumps). Both eyes were tested again immediately after acquisition as well as 24 hours later (i.e., immediate and delayed post-tests). As hypothesized, participants exhibited larger movement correction amplitudes in the

post-tests compared to the pre-test when vision was available to the non-dominant eye. In terms of the dominant eye, its movement correction amplitude did not change significantly after the acquisition phase. Thus, it appears possible that visuomotor pathways associated with the non-dominant eye can undergo acute neuroplastic changes that yield improved online control mechanisms.

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Poster

216. Action and Sensation During Reaching Movements

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Topic: E.04. Voluntary Movements

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Title: Neural activity in primary motor cortex as a monkey performs a virtual balancing task

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Abstract: It is now cliché for neuroscientists to talk of reaching for a cup of coffee. Likewise, many tasks studied in motor neuroscience focus on the simple act of reaching. However, once we pick up the coffee, the task becomes more complex, requiring continual adjustments driven by sensory feedback. We understand remarkably little about the motor control of this and other sensory-driven tasks we perform daily. This work attempts to shine more light on this aspect of neural control, examining primary motor cortical (M1) activity as monkeys performed a sensory-driven, one-dimensional virtual balancing task.
The balancing task starts with monkeys using their hand to control a cursor, moving it to the center of a screen in front of them. Once the trial starts, the cursor's motion becomes unstable: at each time step, the horizontal cursor velocity is proportional to the sum of horizontal hand and cursor positions, referenced to the center of the screen. Left alone, the cursor would fly towards the edge of the screen. Therefore, the monkey's task was to counteract this instability by moving his hand opposite to the cursor. To be rewarded, the monkey had to keep the cursor within 5 cm of the center for 6 s.
For comparison, we interleaved balancing trials with simple reach-to-target trials. As expected, behavior during balancing trials was far more nuanced than the reach trials--each balancing trial was unique, despite being confined to left-right movements. In the neural activity, we found that

balancing had less variance and higher dimensionality than center-out reaching, perhaps corresponding to the fine, sensory-driven adjustments required to complete the task. Further, while the relationship between neural activity and arm movement was similar in the two tasks, we identified a neural signature corresponding to which of the two tasks the monkey was about to perform, suggesting a separate "neural preparation" prior to balancing.
As a field, motor neuroscience has focused on the neural activity behind relatively simple tasks. This balancing task offers a window into the neural activity underlying the generation of feedback-driven behaviors, offering a stepping stone towards understanding the complex tasks we perform in our daily lives.

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Poster

216. Action and Sensation During Reaching Movements

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Title: Emergent low-frequency oscillatory activity in cortico-cerebellar networks with skill learning

Authors: *P. FLEISCHER, A. ABBASI, T. GULATI;
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Abstract: The primary motor cortex (M1) controls skilled arm movement by recruiting a variety of targets in the nervous system, and it is important to understand the emergent activity in these regions as refinement of a motor skill occurs. However, our understanding of this activity in one of the fundamental projections of the M1, that which targets the cerebellum (CB), is incompletely understood. Skill and performance related patterns of neural activity could reveal markers for stimulation-based treatments for cortical damage such as that from stroke. To fill this knowledge gap, we chronically recorded the motor and cerebellar cortices in healthy male Long-Evans rats (n=5) which revealed several skill-related activity patterns. Here, we report that a low-frequency oscillatory (LFO) activity (1-4 Hz) emerges in the local-field potentials (LFPs) of M1-CB networks with motor skill learning during a reach-to-grasp task, as evidenced through significant increase in LFO power. M1 LFP and CB-LFP low-frequency coherence and trial by trial stability in spiking patterns in these areas also significantly increased as the reaching skill consolidated. When we analyzed consistency of spiking to both local and cross-area LFOs, we

found that the likelihood of spikes being phase-locked grew significantly with skill development [GT1] . This synchronization was also significantly more widespread in successfully executed trials than in failed trials. Finally, gaussian-process factor analysis (GPFA) of the spiking activity revealed significant differences between session-averaged factor trajectories' correlation to the individual successful and unsuccessful trials, with successful trail factors showing increased correlation. Together, this work suggests that coordinated LFO activity emerges in M1 and CB with reach skill learning and this is concomitant with conserved spiking patterns in each region. Our next steps are testing if this M1-CB activity can serve as a biomarker for motor recovery post-stroke. We have made preliminary recordings from stroke-induced rats that underwent recovery on the reach task. Our preliminary data from these experiments shows that LFO activity and LFO-spike synchronization increase as motor recovery occurs, suggesting that LFO activity in M1-CB networks may prove to be a useful biomarker of stroke recovery and target of neuromodulatory therapies.

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Poster

216. Action and Sensation During Reaching Movements

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Topic: E.04. Voluntary Movements

Support: JSPS KAKENHI 18H05287
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Title: Chemogenetic activation of convergent inputs to the spinal motoneurons enhances motor outputs in monkeys

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[1]Neural Prosthetics Project, Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan; [2]Section of Viral Vector Develop., Natl. Inst. For Physiological Sci., Okazaki, Japan

Abstract: Convergent inputs from descending motor pathways and spinal reflex circuits to the spinal motoneurons (SpMNs) serve a wide range of motor functions such as voluntary limb control and spinal reflex. Damage of these pathways induces motor paralysis and the amount of remaining pathway is associated with residual motor function after spinal cord injury or stroke. Thus, the activation of the preserved pathways may facilitate functional recovery. One of the feasible tools activating input sources to the SpMNs simultaneously is the chemogenetic technology, designer receptors exclusively activated by designer drugs (DREADDs) which afford remotely reversible control of neuronal activity. In order to establish the activation of convergent inputs to the SpMNs using DREADDs, we injected retrograde viral vector (Gq-DREADD) unilaterally into the cervical enlargement (C7-C8) targeting forearm motoneurons in four macaque monkeys. The histological experiment was performed in two monkeys to confirm

the retrograde labeling after Gq-DREADD injection. The number of labeled neurons was highest in the contralateral primary motor cortex (M1) to the spinal injection side. The labeled neurons were also observed in the dorsal premotor, supplementary motor area, red nucleus, and reticular formation as well as ipsilateral spinal interneurons and propriospinal neurons. Then, the chemogenetic activation was conducted in other two monkeys. Under anesthesia, we recorded the cell activity from the contralateral M1 and muscle activity from ipsilateral forelimb muscles to the Gq-DREADD injection side, and then compared spontaneous activity before and after intramuscular injection of selective agonist deschloroclozapine (DCZ) for muscarinic-based DREADDs. Spontaneous firing rates in several M1 neurons and activity of most of forelimb muscles were robustly increased after DCZ injection, and peaked at ~2 h and 4-6 h after injection, respectively. The increased spontaneous activations in the M1 neurons and muscles continued at least 6 hours after injection, and washed out in 24 hours. These increased spontaneous activations were not observed after vehicle injection. Overall results demonstrate that the chemogenetic activation of the convergent inputs to the SpMNs facilitates spinal motor outputs, suggesting the potential application to enhance motor functions and accelerate functional recovery after neuronal damage.

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Poster

216. Action and Sensation During Reaching Movements

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Topic: E.04. Voluntary Movements

Title: Deep reinforcement learning strategies for musculoskeletal control predict motor cortex activity during novel limb movements

Authors: M. ALMANI, ***S. SAXENA**;
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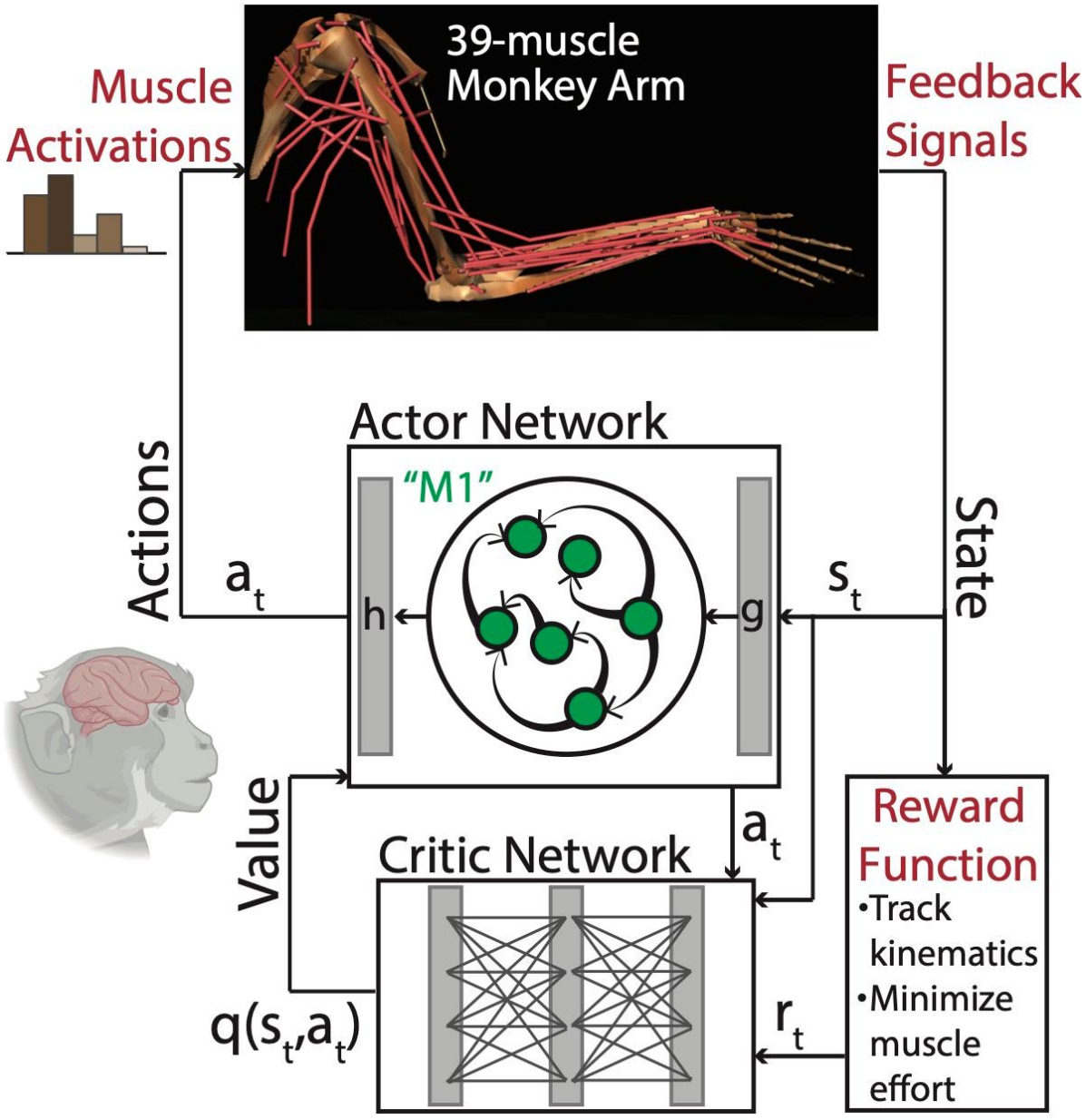
Abstract: Goal-driven networks trained to perform a task analogous to that performed by biological neural populations are being increasingly utilized as insightful computational models of motor control. The resulting dynamics of the trained networks are then analyzed to uncover the neural strategies employed by the motor cortex to produce movements. However, these networks do not take into account the role of sensory feedback in producing movement, nor do they consider the complex biophysical underpinnings of the underlying musculoskeletal system. Moreover, these models can not be used in context of predictive neuromechanical simulations for hypothesis generation and prediction of neural strategies during novel movements. In this research, we adapt state-of-the-art deep reinforcement learning (DRL) algorithms to train a controller to drive a developed anatomically accurate monkey arm model to track experimentally recorded kinematics. We validate that the trained controller mimics biologically observed neural strategies to produce movement. The trained controller generalizes well to unobserved conditions

as well as to perturbation analyses. The recorded firing rates of motor cortex neurons can be predicted from the controller activity with high accuracy even on unseen conditions. Finally, we validate that the trained controller outperforms existing goal-driven and representational models of motor cortex in single neuron decoding accuracy, thus showing the utility of the complex underpinnings of anatomically accurate models in shaping motor cortex neural activity during limb movements. The learned controller can be used for hypothesis generation and prediction of neural strategies during novel movements and unobserved conditions.



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Poster

216. Action and Sensation During Reaching Movements

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Title: Mouse sensorimotor cortex reflects complex kinematic details during reaching and grasping

Authors: *H. A. GRIER[1], M. T. KAUFMAN[2,3];
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Abstract: Neural recordings in non-human primates have shown that activity in sensorimotor cortex correlates with the kinematic and postural details of reach to grasp movements. We characterized this relationship in the mouse. To obtain a diverse set of forelimb postures and movements, head-fixed mice performed unimanual sound-cued reaches to grasp water rewards from two spout targets (based on Galinanes et al. 2018). We collected high speed camera data and tracked 15 points on the proximal and distal limb using DeepLabCut. 3D triangulation allowed extraction of 25 angular degrees of freedom (DoFs) from skeletal relationships between markers. These variables showed that our task evoked complex kinematics: cross-validated dimensionality reduction (PCA) of single-trial joint angle time series revealed dimensionalities in the range of 6-11 across mice. To relate these variables to neural activity, we recorded from layer 2/3 excitatory cells of contralateral forelimb S1 (S1-fl) and M1 (Caudal Forelimb Area, CFA) using 31 Hz two-photon calcium imaging in GCaMP6f transgenic mice (337-635 cells/dataset). Deconvolved calcium events showed that a substantial fraction of cells in both areas were significantly modulated over time or across spout targets: 61-66% in S1-fl and 38-80% in CFA. To determine how these responses reflected forelimb kinematics, we used cross-validated ridge regression encoding and decoding models. We found that 40-44% of significantly modulated neurons in S1-fl and 51-54% in CFA encoded joint angles, velocities or accelerations. Joint angles were better decoded from both areas than velocities or accelerations, with proximal joints such as shoulder abduction, wrist rotation, and elbow flexion decoding at 74-78%, 74-76%, 60-65% variance explained respectively. Many distal joints also decoded well, with metacarpal flexions at 55-68% variance explained and the most distal phalanges flexions at 23-28%. In addition, optogenetic inhibition of CFA in VGAT-ChR2 mice blocked reach and grasp execution while S1-fl inhibition perturbed the movement less severely without precluding movement completion. These results suggest that cortical representations of reaching and grasping in the mouse are highly distributed across sensory and motor cortices, and that activity strongly reflects the details of forelimb and digit positions spanning many angular DoFs.

Disclosures: **H.A. Grier:** None. **M.T. Kaufman:** None.

Poster

216. Action and Sensation During Reaching Movements

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 216.15

Topic: E.04. Voluntary Movements

Support: National Sciences and Engineering Research Council of Canada
Canadian Foundation for Innovation
Ontario Research Fund

Title: Nice catch: Tactile facilitation during actual and expected object reception

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Abstract: During a reach-and-grasp action, the processing of external tactile events is typically suppressed (e.g., Juravle et al., 2010: Behav Brain Res; Manzone et al., 2018: Behav Brain Res). In contrast, when receiving or catching an object, the object is acquired but the central and peripheral processes contributing to tactile suppression are much less evident. Without reaching towards the object, tactile information may instead be facilitated as the object approaches in anticipation of contact and the utilization of tactile information. Therefore, the current study sought to explore the time course of tactile processing when receiving an object. Participants (n = 16) sat at a table with their right arm resting on the surface and with their hand and fingers in a position to receive an object. The object (~34 mm diameter plastic dowel) was placed on top of a carriage of a custom-built linear slide which was attached to a closed-loop belt controlled by a stepper motor. Participants performed a reception task in which the object moved 26 cm and contacted their fingers. Further, participants were presented with tactile stimuli (2 ms constant current pulses) to their index finger at four different time points relative to the object's movement. The stimulus intensities were above or below a pre-determined resting perceptual threshold. The tactile stimulus could be presented before the object moved (Pre condition), early into the movement (Early condition), around the middle of the movement (Middle condition), or around the end of the movement but before object contact (End condition). After each trial, participants indicated whether or not they felt the stimulus. Additionally, in a fifth condition the dowel only travelled 22 cm, so it did not contact the participant, but the tactile stimulus was still presented at the same time as the End condition (End-no contact condition). Because the object contacted the fingers on 80% of the trials, participants had the expectation of object contact even on the End-no contact trials. When compared to the Pre condition, tactile perceptual thresholds were reduced in the Early, Middle and End-no contact conditions (cf. no systematic difference in the End condition). The two key conclusions that can be made from these results are that: 1) tactile information is facilitated when an object is coming toward the receiving hand (i.e., mere

expectation of contact leads to facilitation), and 2) backward masking may contribute to tactile perception during reception because object contact shortly after the stimulation may have masked perception of the tactile stimulus (re: End condition).

Disclosures: D.M. Manzone: None. **L. Tremblay:** None. **R. Chua:** None.

Poster

216. Action and Sensation During Reaching Movements

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Topic: E.04. Voluntary Movements

Support: Hermann & Lilly Schilling Foundation
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Title: Reach and grasp deficits following thalamic lesions

Authors: *M. WILKE[1], H. J. EISENBERG[2], C. SCHMIDT-SAMOA[3], S. MAHDAVI[3], J. LIMAN[2], P. DECHENT[3], M. BAEHR[2];
[1]Cognitive Neurol., Univ. Med. Goettingen/German Primate Ctr., Goettingen, Germany; [2]Neurol., [3]Cognitive Neurol., Univ. Med. Goettingen, Goettingen, Germany

Abstract: While most concepts of goal-directed reach-grasp behavior emphasize cortico-cortical interactions, many of those direct connections are paralleled by indirect cortico-thalamic-cortical routes. Recent electrophysiological and lesion studies in monkeys and humans provide evidence for a critical involvement of higher-order thalamic nuclei such as central and pulvinar nuclei to reach-grasping while it is not clear which spatial or effector-specific information they contribute. We here examined reach-grasp errors in 30 acute stroke patients with circumscribed thalamic lesions and 40 age-matched healthy controls. Tasks included reach-grasps to small objects at different spatial positions and semi-standardized testing for optic ataxia. Reach-grasp errors were evaluated as a function of hand, visual field and fixation condition scored from videos and analyzed with mixed-design ANOVA's in SPSS. Individual lesions were registered to MNI space and mapped to the Morel atlas. MRI-based lesion-symptom mapping and fMRI functional connectivity analysis addressed the critical contribution of specific thalamic nuclei to reach-grasp errors and their effect on the cerebellar and cortical networks. Compared to the healthy controls, thalamic patients made significantly more reach-grasp errors when using the contralesional hand towards the contralesional space (main and interaction effects of group, hand and space, all $p < 0.01$). The thalamic group also exhibited significantly elevated optic ataxia scores, again most pronounced with the contralesional hand and space, pointing to a problem with visuomotor transformation. The functional connectivity analysis revealed lower connectivity strength between the cerebellum and pallidum in the patient group. The lesion contrast between patients with and without a contralesional reach-grasp deficit (defined by z-scores > 1.5) revealed bilateral clusters involving the ventral posterior (VP), central medial

(CeM) and anterior pulvinar nucleus. Our data indicate that reach-grasp deficits are frequent following lesions in the central and pulvinar thalamus and relatively infrequent with mediodorsal lesions. Furthermore, the previously reported grasp deficits with dorsal pulvinar lesions might be due to involvement of its anterior portion that primarily connects to somatomotor networks. Our results further suggest that in the study of visuomotor behavior, more emphasis should be placed on thalamo-cerebellar connections.

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Poster

216. Action and Sensation During Reaching Movements

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 216.17

Topic: E.04. Voluntary Movements

Title: Diversity and specialization of spinal and supraspinal-projecting V2a medullary neurons in the control of orienting behaviors

Authors: *G. USSEGLIO[1], E. GATIER[1], A. D'HUMIERES[3], E. TOSCANO[4], N. ZAMPIERI[4], J. BOUVIER[2];
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Abstract: The brainstem reticular formation (RF) is key for controlling motor actions. Indeed, reticular neurons interface multiple sensory and cognitive modalities upstream, with executory motor circuits downstream. However, how the RF orchestrates complex and adaptive movements is still unknown. In particular, what is the neuronal architecture responsible for the selection and mixing of unitary components of multi-faceted motor behaviors? We recently showed that a genetically-defined class of glutamatergic neurons in the medial reticular formation, the V2a neurons (Chx10-expressing), encompasses at least two distinct spinally-projecting subsets: a cervical-projecting subset whose activation controls head rotation movements and imposes a change of locomotor trajectory, and a lumbar-projecting subset that depresses locomotor speed *(Usseglio et al., 2020*). V2a reticular neurons thus comprise distinct functional subsets defined by projection and whose functional outcome may owe to the targeted cell type. Here, we reveal that V2a reticular neurons also control other motor components of orienting whose executive circuits are located in the brainstem, including snout and eye. Importantly, these functions are supported by brainstem-projecting V2a subsets rather than by collaterals of spinally-projecting ones. Secondly, we question the possibility that the divergent outcomes across muscular groups (e.g., ipsilateral excitation for the head and snout, bilateral inhibition for the hindlimbs) might reflect differences in V2a efferent connectivity at each executory module. Hence, using a rabies-based transsynaptic strategy on newborn mice, we document the identity of the postsynaptic cells of the V2a neurons at different brainstem and spinal cord levels. Our work highlights that V2a

neurons in the gigantocellular reticular nucleus control multiple orienting motor actions and can be subdivided in two macro-categories, anatomically and functionally, i) one that controls ocular, orofacial and respiratory movements through supra-spinal projections, and ii) another one that controls trunk and limb movements through spinal projections. Within each, a further specialization by projection site and postsynaptic targets supports the control of single motor actions. Altogether, our work places V2a neurons as important orchestrators of orienting movements. It also highlights that a specialization following a muscle-group connectivity framework may allow the expression of individual components of multi-faceted behaviors.

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Poster

216. Action and Sensation During Reaching Movements

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Topic: E.04. Voluntary Movements

Support: JST Grant JPMJSP2124
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Title: Continuous EEG decoding of the online correction during arm reaching movements

Authors: *K. NUMASAWA[1], T. KIZUKA[2], S. ONO[2];
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Abstract: Many of our movements are supported by unconscious motor control. As an example, we can correct rapidly and implicitly the arm trajectory deflected by the sensory perturbation during goal-directed movements. The mechanism of this online correction is based on the sensory prediction error between the efference copy and the actual feedback signals. The central nervous system (CNS) would process quickly and implicitly the feedback signal by estimating the sensory feedback from the efference copy. However, how the CNS generates the implicit motor correction is not enough understanding. Recent studies using noninvasive electroencephalographic (EEG) have demonstrated accurately continuous decoding of kinematics or muscle activity during movement from EEG signals. The continuous decoding technique mainly contributes to applying the brain-computer interface (BCI), while it is also expected to understand the neural mechanism associated with motor control. Therefore, we attempted to clarify how the CNS control the implicit online correction using the method of continuous decoding. The apparatus consisted of a digitized tablet to capture the hand movement and a monitor to display a visual stimulus. Participants wore a 20-channel wireless electrode cap for EEG measurement and were required to control a cursor on the monitor synchronized with a stylus. They sat facing the monitor with their heads positioned by a chin rest and were asked to

move the cursor from a start circle to a target. The distance between the start circle and the target was 20 cm, and a fixation cross was presented on the center of the monitor. Participants were required to fixate on the fixation cross during the experiment. The cursor was displaced to the left side from an actual cursor position in 1/3 of all trials, as a perturbation. We applied all continuous data that kinematics and EEG to the decoding algorithm to reconstruct hand position, cursor position, and hand velocity. In addition, we calculated the electrode contribution of scalp regions to decode hand kinematics. Our results showed that the hand position was decoded by the EEG signals despite the implicit online correction, and parietal electrodes covering the sensorimotor areas contributed to the online correction. These findings are important for understanding how the CNS generates implicit online correction. Furthermore, identifying a characteristic of brain activity during implicit motor control would contribute to creating an elaborate BCI system.

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Poster

216. Action and Sensation During Reaching Movements

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Title: Posture-dependent and velocity-dependent components of arm dynamics are reflected in muscle co-contraction during reaching

Authors: *A. S. KOROL[1], A. B. THOMAS[1], V. GRITSENKO[2];
[1]Neurosci., [2]Human Performance, West Virginia Univ., Morgantown, WV

Abstract: Reaching movements can be separated into two distinct actions 1) the postural action that involved supporting the limb against gravity and 2) the transport action that involves propelling and orienting the hand. This separation is reflected in the muscle activity patterns as static and phasic components of electromyograms (EMGs). These two components are not independent but are thought to be controlled separately by dedicated neural circuits that together produce joint torques (moment) that propel the arm and modulate limb stiffness. Our previous study has shown that the static and phasic EMG components underlie the gravity-related and velocity-related biomechanical actions of muscle torque (Olesh et al., 2017). Here we tested the hypothesis that the findings for the right arm generalize to the left (non-dominant) arm and to other movements in a different area of the workspace. To achieve these, we recorded EMG of 12

muscles and the kinematics of both arms of 10 right-handed participants doing reaching movements in multiple directions from two starting positions. Using principal component analysis, we have shown that the temporal profiles of the first principal component (PC1) of EMG and PC1 of posture-related muscle torque are highly correlated. We have also shown that the temporal profiles of the PC2 of EMG and PC1 of velocity-dependent muscle torque are highly correlated. These results confirm our primary hypothesis. Moreover, we have analyzed the changes in eigenvalues across movements using linear regression analysis. We have found that for multiple muscles, the eigenvalues change together across movements. For example, when a movement upward is associated with large gravity-related muscle torque at the shoulder, the anterior deltoid and teres major both have large PC1 eigenvalues, indicating a large contribution of these muscles to supporting the limb against gravity during reaching. Our overall results suggest that the redundancy problem of selecting which muscles to activate and when is solved by the nervous system by embedding biomechanics, i.e., by taking advantage of the synergistic biomechanical actions of specific muscle groups to generate muscle torques and the antagonistic biomechanical actions of other muscle groups to modulate limb stiffness.

Disclosures: **A.S. Korol:** None. **A.B. Thomas:** None. **V. Gritsenko:** None.

Poster

216. Action and Sensation During Reaching Movements

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Program #/Poster #: 216.20

Topic: E.04. Voluntary Movements

Title: Non-dominant hand has larger timing errors in muscle activity

Authors: *A. TAKAGI, S. ITO, H. GOMI;
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Abstract: Most people exhibit a strong preference in using one hand over throughout tasks of daily living. Many theories have been proposed to explain the lateralization in the hand's function. Some suggest that the brain's dominant hemisphere could be subject to less sensory noise during execution. Others point to the possibility of the non-dominant arm being specialized for stabilization or impedance control. However, existing hypotheses fail to explain why the dominant hand's advantage only consistently emerges during fast movements but not slow ones. We propose that the source of the dominant hand's advantage lies in its precisely controlled muscle activation timing. Unlike other theories, ours predicts increasing force variability at high speeds due to unintentional overlaps in muscle activity. This prediction was tested in 10 right-handed people (all male aged 32±4) who exerted periodic forces against a fixed handle by following the beats of a metronome. Participants had to flex and extend their wrist or elbow or shoulder joint in time with the beats of a metronome whose frequency was set to {3, 4, 5} Hz. The force and muscle activity time-series from six muscles were recorded for analysis. The dominant arm's force variability remained constant at all frequencies, but the non-dominant

arm's force became increasingly variable at higher frequencies. The source of this force variability cannot be explained by signal-dependent noise as both the magnitude and the standard deviation of the peak muscle activity increased by the same amount in both arms. Additionally, larger signal-dependent noise does not lead to larger timing errors. What was different was the precision in the timing of the peaks (two-way repeated measures ANOVA, $F(1,9)=10.5$, $p=0.01$). While the mean timing of the muscle peaks was the same in both arms, their standard deviation was twice as big in the non-dominant arm. This difference in timing precision was observed at the shoulder, elbow and wrist joints. While the source of the timing precision remains unclear, precisely timed muscle activity could be the key to dexterity.

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Poster

216. Action and Sensation During Reaching Movements

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Topic: E.04. Voluntary Movements

Support: NIH Brain Initiative Grant 5U19NS112959

Title: Unveiling the anatomical and molecular organization of spinal circuits controlling dexterous movement in adult mice

Authors: *S. PIMPINELLA[1], J. BRUSCH[1], M. GOULDING[2];
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Abstract: Unveiling the anatomical and molecular organization of spinal circuits controlling dexterousmovement in adult mice.Sofia Pimpinella, Jeremy Brusch, Martyn GouldingComplex nervous systems are built around movement with premotor interneurons (Pre-MN INs)playing a preeminent role in coordinating the activity of distinct motor pools that innervate specificmuscles. Efforts to date have been largely focused on functionally characterizing premotorinterneuron cell types at lumbar spinal levels that mediate simple highly stereotyped behaviourssuch as locomotion and spinal reflexes, often absent of detailed information about theirconnectivity. Here, for the first time, we are characterizing the premotor networks within thecervical spinal cord of adult mice to understand how different muscles are activated duringcomplex dextrous behaviours. To study the premotor (Pre-MN) circuitry of antagonist forelimbmuscle pairs at the elbow and wrist joints, we performed intramuscular injections of rAAV.oG(P15) and intraspinal injections (P49) of the pseudotyped Rabies virus (SADB19-dGmCherry/EnvA) in ChAT ^{cre}, Rosa26 $^{LSL\text{-}TVA}$ mice. Interestingly, we see no difference on theoverall spatial distribution of rabies labelled Pre-MN INs innervating forelimb flexor (biceps brachii)and extensor (triceps brachii) muscles. In undertaking a comprehensive description of presynapticsensory (periphery) and neuronal (spinal) inputs to the forelimb motor circuitry, we have tracedspinal and sensory pre-synaptic inputs to four cardinal pre-MN INs classes in

postnatal day 56(P56) adult mice. To enable the specific infection and spread of the rabies virus followingintraspinal injection, we combined distinct ventral IN population (V1, V2a, V2b and V3) Cre mouselines with R26 $^{LSL\text{-}HTB}$ conditional line. Preliminary results revealed differences in the dorsal andventral horn of the spinal cord. In particular, V1 presynaptic INs are located in the deep dorsallaminae (between laminae IV and VI), whereas other studies have shown that V2b INs alsoreceive inputs from neurons in the superficial dorsal horn. By contrast, V3 presynaptic INs arelocated in dorsal laminae III-IV. These studies are essential for defining the neuronal connectionsin the cervical spinal cord that drive the coordinated movements generated by the cervical motornetwork and will lead to a fuller understanding of how dextrous forelimb motor behaviours areshaped by sensory intraspinal and descending pre-synaptic inputs.

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Poster

216. Action and Sensation During Reaching Movements

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Topic: E.04. Voluntary Movements

Support: NIH Brain Initiative grant :5U19NS112959

Title: Generation of a multidimensional interactive atlas for the mouse cervical spinal cord

Authors: *J. BRUSCH[1], Q. SILVERMAN[1], A. TYSON[3,4], P. BAZZIGALUPPI[4,3], S. LENZI[3,4], M. CANTARELLI[4,3], R. ROWEKAMP[2], T. SHARPEE[2], M. GOULDING[1];
[1]Mol. Neurobio. Lab., [2]Computat. Neurobio. Lab., Salk Inst. for Biol. Studies, San Diego, CA; [3]MetaCell LLC, CIC Cambridge, MA; [4]MetaCell Srl, Cagliari, Italy

Abstract: The need for an annotated anatomical reference atlas that precisely identifies regions of interest and specific cell types in the spinal cord is underlined by previous mouse brain atlas models that have been generated by the Allen Brain Institute. The U19 Spinal Cord Circuit Project (Team SCC) are addressing this void by building a multidimensional spinal cord reference atlas that captures key anatomical and cellular features of the premotor cell types that populate the cervical spinal cord. The overall goal is the creation of a comprehensive, high resolution, and interactive atlas of the mouse cervical spinal cord. Using adult P56 mice as the reference dataset, a combination of intersectional genetics, in situ hybridization and immunohistochemistry techniques have been used on serial sectioned spinal cords to create precise 3D positional maps of four cardinal pre-motor interneuron (IN) populations and specific forelimb motor pools. These populations have then been fractionated into smaller genetically-defined subpopulations according to their developmental provenance. Particular molecular markers have been utilized to identify functional cell types (Renshaw cells, central canal cells and $ChAT^{+}$ C-bouton interneurons). In collaboration with MetaCell, a pipeline for incorporating the anatomical features of these neurons (3D position, morphology, connectivity and cell type

information has been developed that is supported by semi-automatic registration). In the first phase of map building, serially-sectioned spinal cords are normalized and registered into the reference 3D atlas. Next, we created a freely accessible web-platform where researchers can select multiple neuronal populations to see and compare INs' unique spatial distributions and coordinates in the 3D space as well as the neuronal populations' density in different segments of the cervical spinal cord. In addition to providing positional information for cervical motor neurons and pre-motor INs, the web platform will contain information about morphology, connectivity, cellular properties, gene expression profiles and function. By the end of the project, the web application and the reference atlas will be made freely available to the whole scientific community. Scientists will be able to contribute to the curation of the reference cord atlas and to incorporate datasets generated by other investigators or from other validated sources.

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Poster

216. Action and Sensation During Reaching Movements

Location: SDCC Halls B-H

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Program #/Poster #: 216.23

Topic: E.04. Voluntary Movements

Support: 1 U19 NS112959-01

Title: Transcriptomic profiling of ventral interneurons reveals molecular diversity across spinal segments

Authors: *P. OSSEWARD, N. MOORE, S. DRISCOLL, S. PFAFF;
GEL-P, Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Neural networks in the spinal cord are involved in motor control for a variety of behaviors, including reaching, grasping, and locomotion. While cervical and lumbar segments are key in regulating forelimb and hindlimb movements respectively, developmental studies indicate that the major cardinal classes of spinal neurons are arrayed across all levels of the cord. To examine possible segmental specialization of the cardinal classes, we profiled the transcriptomes of spinal interneurons from the mouse from individual cardinal classes using fluorescence-activated cell sorting. For each cardinal class analyzed, we found that the most prominent genetic division corresponded to Group-N vs Group-Z genetic signatures, previously shown to be related to timing of neurogenesis and axonal projection range. Further divisions

revealed populations of cells enriched with sets of Hox genes reflecting distinct spinal segments. These results indicate that many aspects of transcriptional diversity within a single ventral cardinal class are shared across classes and spinal segments.

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Poster

216. Action and Sensation During Reaching Movements

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Topic: E.04. Voluntary Movements

Support: University of North Georgia Presidential Semester Incentive Award

Title: The effect of short-term archery training on reaching movement performance and lateralized motor control.

Authors: O. BEYAZ[1], V. EYRAUD[2], S. AKPINAR[3], ***A. PRZYBYLA**[2];
[1]Physical Educ. and Sport, Nevsehir Haci Bektas Veli Univ., Nevsehir, Turkey; [2]Physical Therapy, Univ. of North Georgia, Dahlonega, GA; [3]Physical Educ. and Sport, Nevsehir Univ., Nevsehir, Turkey

Abstract: Lateralized motor control manifested as handedness and associated with hand preference can be modified through sensorimotor and task conditions or long-term training. For example, top-level college-age fencers who trained fencing extensively from 8-10 years of age showed modulation of hand preference favoring the dominant hand to less extent than the aged-matched non-athletes. This study aimed to investigate the effect of a short-term 12-weeks traditional archery intervention program on motor lateralization. This novice-level archery training required one hour of practice daily, three days a week. Healthy right-handed volunteers, 19-23 years old, were randomly assigned to either an experimental or control group. While the former group completed archery training, the latter did not perform any exercise. The outcome measures included kinematic parameters of a discrete hand reaching movement performance. For this purpose, each participant performed unimanual reaching movements with right and left hand to targets in the frontal space before and after the archery training period. Our results show that short-term archery training improved both hands' movement accuracy, precision, and efficiency. It was not the case for any hand in the control non-training group. Furthermore, while the training group improved reaching movements, the effects of lateralized motor control remained. We speculate that while the short-term archery training could improve hand reaching performance, it will not affect hand preference, given that the associated interlimb differences persist.

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Poster

216. Action and Sensation During Reaching Movements

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Topic: E.04. Voluntary Movements

Support: Academy of Pediatric Physical Therapy Research Grant 1

Title: Examining the role of vision in upper extremity movements in typically developing children

Authors: A. RICHARDSON, D. WANG, E. KOHL, ***R. HAWE**;
Univ. of Minnesota, Minneapolis, MN

Abstract: Introduction: When performing an action, vision is first used to search the environment to identify relevant information. Visual information is then used to select an action and generate a motor plan. During movement execution, vision is used to facilitate accuracy. These processes may be altered in children with hemiparetic cerebral palsy due to impairments in visuospatial attention and an increased need to visually monitor limb position to compensate for proprioceptive impairments. The objective of this study is to establish the role of vision during an upper extremity obstacle navigation task in typically developing children to later be used to assess the role of vision in children with hemiparetic cerebral palsy.
Methods: We designed a novel robotic task using the Kinarm Exoskeleton and gaze tracking. The task required children to move their upper extremity through 2-3 narrow openings to reach the final target. In one version of the task participants received visual feedback of their hand position. In a second version, participants did not receive any visual feedback of their hand position, though did get feedback of they hit obstacles in the task. Five typically developing children ages 9-15 completed the study. We examined number of errors, time to complete the mask, and location of gaze.
Results: With visual feedback, the average number of errors per participant was 0.8±0.8 errors. Gaze behavior showed a trend of saccades to the proximal (relative to hand position) edge of each opening prior to limb movement. Participants fixated on the openings and only looked at the visual feedback of the hand for an average of 13.51±6.4% of each trial. Participants visually monitored their hand as it passed through each opening, however, gaze was fixated on the next opening while the limb moved between openings.
Conclusion: Our results demonstrate that typically developing children need to visually monitor limb position only during portions of movement requiring high degrees of accuracy. Typically developing children use anticipatory gaze strategies in which they fixate on the next object of interest rather than their moving limb. We hypothesize that children with hemiparetic cerebral palsy will demonstrate different visual strategies, including increased visual monitoring of their limb position.

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Poster

216. Action and Sensation During Reaching Movements

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 216.26

Topic: E.04. Voluntary Movements

Title: Transcallosal Connectivity Differentially Influences the Effects of tDCS on Reaching Performance in Young and Older Adults

Authors: T. MUFFEL[1], A. MATUSCHE[1], P.-C. SHIH[1], B. KALLOCH[1], C. J. STEELE[2], A. VILLRINGER[1], ***B. SEHM**[1,3];
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Abstract: Background: The effects of transcranial direct current stimulation (tDCS) are variable and the factors contributing to this variability are not sufficiently understood. In particular, aging has been discussed as an influencing factor. Age-related changes in transcallosal structural connectivity (TCC) between the primary motor cortices have been implicated as a mediating factor suggested to underly sensorimotor performance declines in older adults. Additionally, different tDCS setups have been shown to elicit differential responses in the same individuals. While these factors are important mediators of efficacy, the relationships between age, TCC and setup-specific effects of tDCS have not previously been combined in a single assessment until now. **Objective:** This study aims to investigate whether TCC and age differentially mediate the effectiveness of 2 different tDCS setups on motor performance. **Methods**: 20 young (YA) and 20 older healthy adults (OA) performed a simple reaching task while receiving unilateral-anodal (ua-tDCS), bilateral-dual (bi-tDCS) or sham tDCS in a cross-over design. Age-related performance modulations in accuracy and speed and TCC fractional anisotropy (FA) differences were analyzed separately (ANOVA, t-tests) and combined in a multiple linear regression model. **Results**: TCC FA mediated tDCS effects age-specifically: higher individual FA was linked with greater movement speed during bi-tDCS in OA but ua-tDCS in YA. No such relationship was found for accuracy. **Conclusion**: Our results suggest that age-related differences in transcallosal connectivity contribute to differential effects of uni vs bilateral tDCS. Hence, transcallosal connectivity could serve as a biomarker to stratify patients to different tDCS protocols, thus contributing to more individually tailored stimulation protocols.

Disclosures: T. Muffel: None. **A. Matusche:** None. **P. Shih:** None. **B. Kalloch:** None. **C.J. Steele:** None. **A. Villringer:** None. **B. Sehm:** None.

Poster

216. Action and Sensation During Reaching Movements

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 216.27

Topic: E.04. Voluntary Movements

Support: NRF-2021R1A2C2011648
HY-202000000002753

Title: Double dissociations of the effects of visual feedback on motor and somatosensory cortices during visuomotor learning

Authors: *J. KIM[1], S. PARK[2], S. KIM[1,2,3];
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[3]Inst. for Basic Sci., Suwon, Korea, Republic of

Abstract: The primary motor and somatosensory cortices (M1/S1) are the main regions for human motor control. Their respective roles in regulating efferent motor commands and processing afferent sensory feedback and interaction have been widely investigated. Their functional dissociation during human visuomotor learning, on the other hand, is poorly understood. To investigate the neural basis of the dissociation, we designed an fMRI study in which individuals learned a new motor skill. To do this, we manipulated the visual feedback of a moving effector (i.e., a cursor) so that participants learned to reach a target under two alternating conditions: online cursor feedback is available or unavailable except when a target is reached. We used a surface-based GLM analysis instead of a volume-based GLM to separate the BOLD responses of M1 and S1, which are closely located. As a result, we found the higher response in M1 when the online feedback is available but lower when it is not, and vice versa in S1. Interestingly, we discovered a similar dissociation in visual cortices: stronger responses in early visual cortices with the online visual feedback and in late visual cortices with the restricted visual feedback, respectively. These findings support the idea that M1 and S1 distinctively interact with visual cortices depending on the level of visual feedback during visuomotor learning. The current work may contribute to development of novel rehabilitation strategies for patients with stroke by customizing visual feedback of motor performance.

Disclosures: J. Kim: None. **S. Park:** None. **S. Kim:** None.

Poster

216. Action and Sensation During Reaching Movements

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 216.28

Topic: E.04. Voluntary Movements

Support: NIH Grant 1U19NS112959-01

Title: Deciphering the spinal circuit architecture driving flexor and extensor coordination during rhythmic movements

Authors: *M. YAO[1,3], A. NAGAMORI[2], E. AZIM[2], T. SHARPEE[1,3], M. GOULDING[2], D. GOLOMB[4], G. GATTO[5];
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Abstract: The precise timing of flexor and extensor muscle activity during limb movement is largely driven by reciprocal inhibition. Previous work identified two subtypes of Ia inhibitory interneurons (INs) involved in reciprocal inhibition: a subset of V1 INs that predominantly inhibiting flexor motoneurons (MNs), and a subset of V2b INs that preferentially inhibit extensor MNs. Nevertheless, the spinal network architecture and dynamics that coordinate flexor and extensor muscle activity remain largely unresolved. To address this challenge, we analyzed a relatively simple rhythmic movement, the scratch reflex, which, being mostly driven by ankle oscillations, can be modeled as neural circuitry controlling a single joint. Using an intersectional genetic approach, we probed the changes in the pattern and rhythmicity of scratching that arise from perturbing three premotor IN populations. Activation of excitatory V2a INs increased the frequency of rhythmic scratching, whereas augmenting inhibition (V1 IN activation) induced an atonia-like state. Surprisingly, the removal of either excitatory (V2a) INs or inhibitory (V1 or V2b) INs slowed the rhythm. Simultaneously manipulating excitatory and inhibitory INs by targeting either the entire V2 lineage (V2a and V2b) or the V1 and V2a INs in combination revealed that the interactions among these INs is more complex than just a linear summation of functions. To better understand the network dynamics driving these changes, we constructed a biophysical rate model that represents these IN populations and flexor-extensor MNs that drive a one degree-of-freedom biomechanical joint model. We found that modeling V1 and V2b INs, either as mutually coupled flexor- and extensor-tuned Ia INs, or as mediating disynaptic inhibition between excitatory populations, resulted in a higher frequency rhythm following V1 or V2b IN ablation that is inconsistent with our experimental data. To resolve this discrepancy, it was necessary to introduce an inhibitory loop formed by two inhibitory IN pools, whose identity is yet to be clarified. We hypothesize that V1 and V2b INs function by disinhibiting this inhibitory loop, which directs the mutual inhibition between flexor and extensor MNs. This model reproduced the experimental results for both individual IN types and their non-linear cooperation dynamics. Stability analysis of the model suggests that feedback inhibition, and biomechanics are key to preventing the drift of the joint angle during rhythmic movement. Taken together, our findings suggest a novel functional architecture for the premotor networks coordinating flexor and extensor muscle activity during rhythmic behaviors.

Disclosures: M. Yao: None. **A. Nagamori:** None. **E. Azim:** None. **T. Sharpee:** None. **M. Goulding:** None. **D. Golomb:** None. **G. Gatto:** None.

Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 217.01

Topic: E.04. Voluntary Movements

Support: Vision: Science to Applications (VISTA)

Title: Prefrontal "visual" responses predict planned action in a head-unrestrained reach task

Authors: P. ABEDI-KHOOZANI, *V. NACHER, V. BHARMAURIA, H. ARORA, X. YAN, S. SUN, H. WANG, J. CRAWFORD;
York Univ., Toronto, ON, Canada

Abstract: Most areas of visuomotor cortex show visual responses that encode eye-centered target location, but the nature of visual coding in prefrontal cortex is less clear. We examined this question by recording single neurons from posterior dorsolateral prefrontal cortex (pDLPFC) in two monkeys trained to perform a head-unrestrained reaching paradigm. Animals touched one of three central LEDs at waist level while maintaining gaze on a central fixation dot and were rewarded if they touched a target appearing at one of 15 locations in a 40° x 20° (visual angle) array. Analysis of 509 neurons in two monkeys showed an assortment of target/stimulus, gaze, pre-reach and reach-related responses. Here we focused on analysis of 142 neurons that showed an early “visual” response, time-locked 80-180 ms after target onset. We used an offline algorithm to test which spatial model best fit the response field in this epoch. First, assuming that this response is mainly influenced by target position, we found that individual neurons showed variably preferences for target location relative to the eye (Te), head (Th), or space (Ts). At the population level Ts was preferred, with the target relative to the eye (Te) significantly eliminated. However, when we included motor models in the fits, we found that most of these neurons preferentially encoded gaze (4.7 %), head (37.5 %) or arm (27.5 %) motion parameters, specifically the future movement vector or final position. Similar fits were found in the first 50ms of this response. Based on these data, we conclude that the early pDLPFC target response in this task is not truly visual but is rather a visually-triggered motor set to prepare for the upcoming movement.

Disclosures: P. Abedi-Khoozani: None. **V. Nacher:** None. **V. Bharmauria:** None. **H. Arora:** None. **X. Yan:** None. **S. Sun:** None. **H. Wang:** None. **J. Crawford:** None.

Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 217.02

Topic: E.04. Voluntary Movements

Support: Vision: Science to Applications (VISTA)

Title: Effector-specific sensorimotor transformations in dorsolateral prefrontal cortex during a head-unrestrained reach task

Authors: V. NACHER, *P. ABEDI KHOOZANI, V. BHARMAURIA, H. ARORA, X. YAN, S. SUN, H. WANG, J. D. CRAWFORD;
York Univ., Toronto, ON, Canada

Abstract: Dorsolateral prefrontal cortex is associated with executive control and response selection, but the extent to which it is involved in effector-specific transformations is unclear. We addressed this question by recording 711 single neurons from posterior dorsolateral prefrontal cortex (pDLPFC) while two trained monkeys performed a head-unrestrained reaching paradigm. Animals touched one of three central LEDs at waist level while maintaining gaze on a central fixation dot and were rewarded if they touched a target appearing at one of 15 locations in a 40° x 20° (visual angle) array. Animals typically shifted gaze first, followed by sustained head movement and a reach (Arora et al. *J. Neurophys.* 2019). Analysis of 499 neurons in two monkeys showed an assortment of target/stimulus, gaze, pre-reach and reach-timed responses in pDLPFC. Most neurons could be described as falling into three main groups: 'Early" (increased firing rate during the target presentation / gaze onset), "Early-late" (sustained activity from target presentation through reach) and "Late" (peaking during reaches). Importantly, early gaze-related activity only occurred when followed by a reach (compared to 172 neurons also tested in no-reach controls). We then tested the spatially tuned neurons using a model-fitting procedure to determine their spatial codes (Keith et al. *J. Neurosci. Meth*. 2009). Early responses were often gain modulated by initial hand position (41%), gaze position (19 %), or initial head position (5 %), with some overlap between these modulations. Individual neurons showed a variety of preferred spatial codes (target, gaze, head, hand) but at the overall population level, early responses showed a preferential coding for head movement, whereas later responses showed a preference for coding hand movement. Overall, these data suggest a specific role for pDLPFC in eye-head-hand coordination where gaze signals appear to trigger posture-dependent head and hand control signals—and a more general role for this structure in cognitive-motor integration.
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Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 217.03

Topic: E.04. Voluntary Movements

Support: NSF Grant 1912557
NIH Grant EY033071

Title: Integration of Motor and Visual Information in the Activity of Parietal Area 5 Neurons During Locomotion

Authors: *I. N. BELOOZEROVA[1], W. U. NILAWEERA[2], G. VIANA DI PRISCO[3], V. MARLINSKI[4];
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Abstract: Area 5 of the posterior parietal cortex is part of the cortical "dorsal stream," the "Where?" pathway involved in the analysis of spatial properties of the environment for guiding movements. In our recent study in cats, we analyzed locomotion-related activity of groups of cortico-cortical neurons (CCs) in area 5 that project to the motor cortex (Beloozerova et al. 2022). We found that during walking on a flat surface, which does not require vision, 70% of CCs had low-rate firing activity (~2 spikes/s) strongly modulated with strides. During walking on the horizontal ladder, which requires visual control of strides, the firing rate of CCs in cortical layer V doubles, and the number of cells with 2 bursts per stride tends to increase. The group with 2 bursts per stride, thought to be related to movements of both left and right limbs, discharges in synchrony with 2 peaks of gaze shifts away along the surface. The group with 1 burst per stride, thought to be related to movement of one limb, and both groups in the upper cortical layers do not have a unified stride-related firing activity. Here we analyzed the change in the firing activity of individual CCs with no-, one-, and two-bursts per stride on the flat surface when the cat goes on the ladder. We identified three major groups: i) cells whose activity was dominated by visual information, ii) cells whose activity was mostly determined by limb movements, and iii) cells whose activity reflected both motor and visual information. The stride-related firing activity of only 5% (5/90) of CCs depended solely on visual input (Fig. 1A,B). In 16% of CCs, visual input shifted the peak in the firing activity to a different phase of the stride (Fig. 1C,D). 7% of CCs started to discharge 2 bursts instead of 1 (Fig. 1E). The stride-related activity of 14% of CCs depended mostly on motor input (Fig. 1F,G). The activity of 16% CCs was modulated by both motor and visual inputs (Fig. 1H-J). We concluded that during locomotion on a complex surface, area 5 CCs process and transmit to the motor cortex signals that integrate motor and visual information.

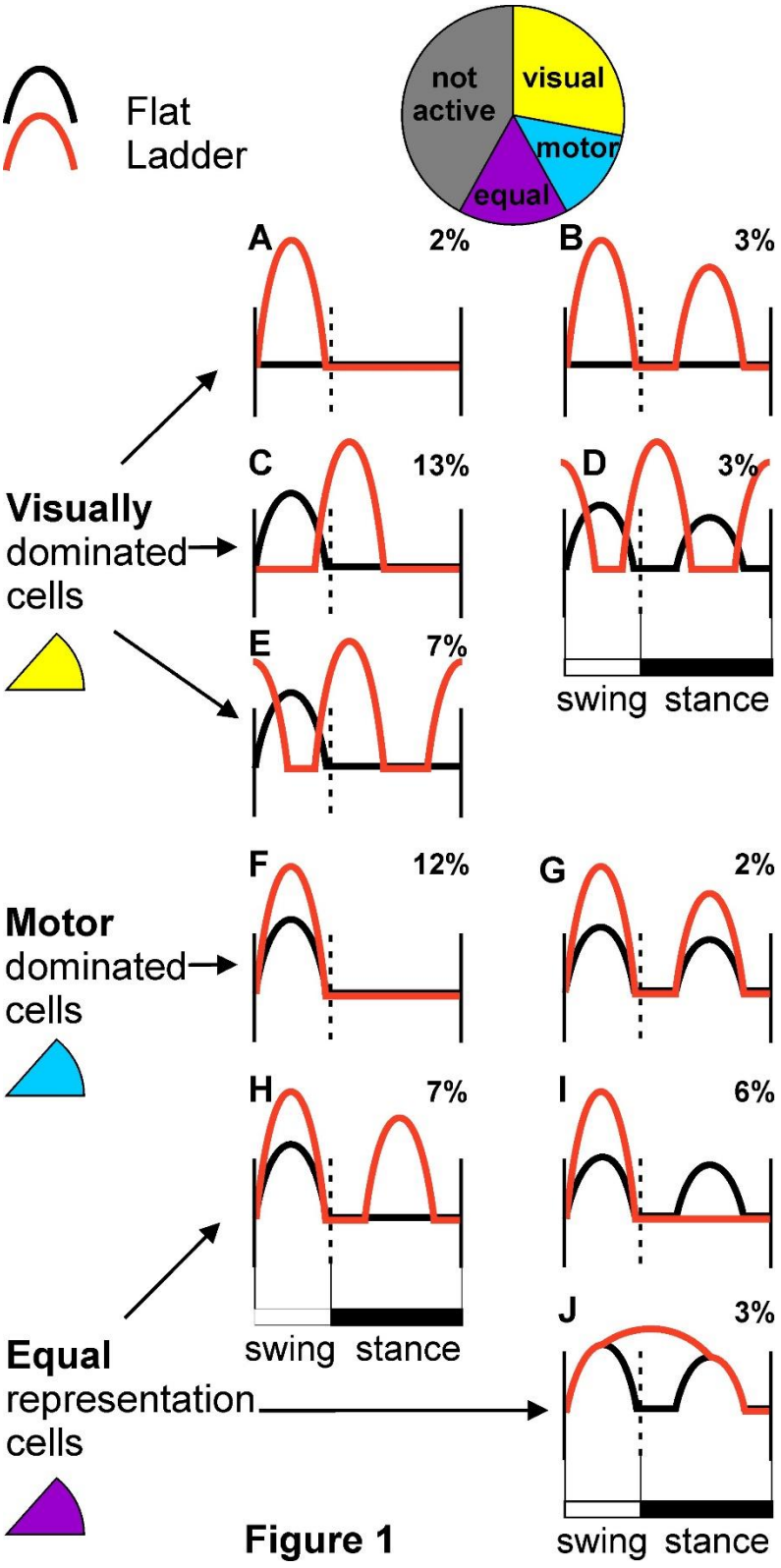

Figure 1

Disclosures: **I.N. Beloozerova:** None. **W.U. Nilaweera:** None. **G. Viana Di Prisco:** None. **V. Marlinski:** None.

Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 217.04

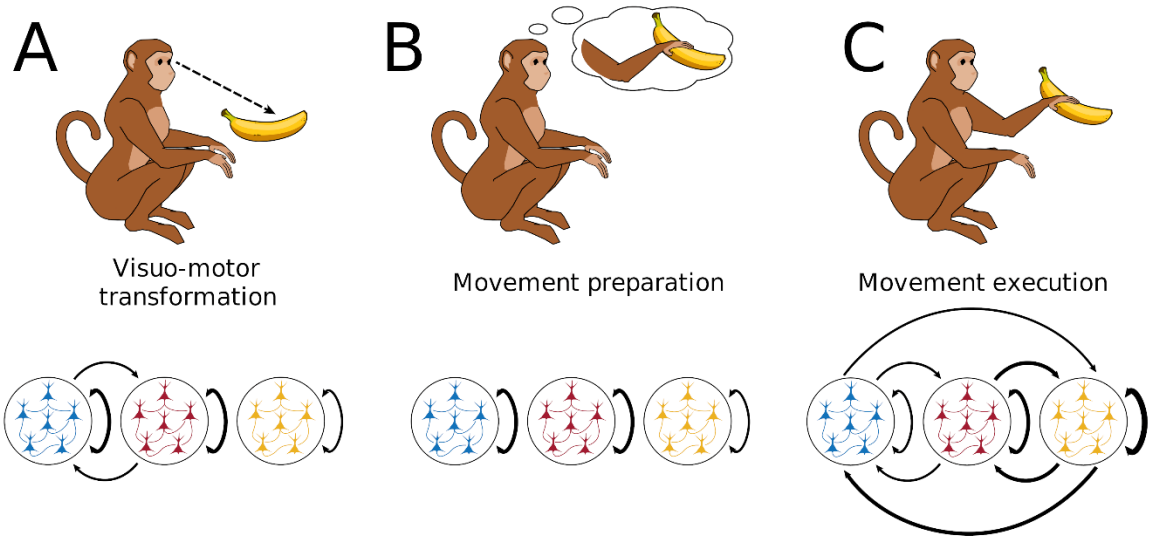
Topic: E.04. Voluntary Movements

Support: NSF Grant 1735095
NIH R01 MH121978
German Ministry of Education and Reserach FKZ 01GQ1903

Title: Information processing dynamics in neural networks of macaque cerebral cortex reflect cognitive state and behavior.

Authors: *T. VARLEY[1], O. SPORNS[2], S. SCHAFFELHOFER[3], H. SCHERBERGER,[3], B. DANN[4];
[1]Indiana University, Bloomington, Bloomington, IN; [2]Psychological and Brain Sci., Indiana Univ., Bloomington, IN; [3]Univ. of Gottingen, Gottingen, Germany; [4]German Primate Ctr., Goettingen, Germany

Abstract: One of the essential functions biological neural networks is the processing of information. This comprises processing sensory information to perceive the environment, up to processing motor information to interact with the environment. Due to methodological concerns, it has been historically unclear how information processing changes during different cognitive or behavioral states, and to what extent information is processed within or between the network of neurons in different brain areas. In this study, we leverage recent advances in the calculation of information dynamics to explore neural-level processing within and between the fronto-parietal areas AIP, F5 and M1 during a delayed grasping task performed by three macaque monkeys. While information processing was high within all areas during all cognitive and behavioral states of the task, inter-areal processing varied widely: during visuo-motor transformation, AIP and F5 formed a reciprocally connected processing unit, while no processing was present between areas during the memory period. Movement execution was processed globally across all areas with a predominance of processing in the feedback direction. Additionally, the fine-scale network structure re-configured at the neuron-level in response to different grasping conditions, despite of no differences in the overall amount of information present. These results suggest that areas dynamically form higher-order processing units according to the cognitive or behavioral demand, and that the information processing network is hierarchically organized at the neuron-level, with the coarse network structure determining the behavioral state and finer changes reflecting different conditions.



Disclosures: **T. Varley:** None. **O. Sporns:** None. **S. Schaffelhofer:** None. **H. Scherberger,:** None. **B. Dann:** None.

Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 217.05

Topic: E.04. Voluntary Movements

Support: Searle Scholar Award
Sloan Research Fellowship
Whitehall Research Grant Award
Chicago Biomedical Consortium with support from the Searle Funds at the Chicago Community Trust
Simons Collaboration on the Global Brain
NIH grant DP2 NS120847

Title: A hidden hierarchy among motor cortices

Authors: *S. SAVYA, M. AGRIOS, A. SAIKI, A. FORREST, H. SROUSSI, F. XU, A. MIRI; Northwestern Univ., Evanston, IL

Abstract: Anatomical, lesion, and stimulation studies over the past several decades have been interpreted through two competing theories of motor cortical organization: functional hierarchy and parallel control. Under functional hierarchy, premotor cortex influences movement primarily through projections to primary motor cortex in a feedforward manner. In parallel control, premotor and primary motor cortices interact through recurrent connections and each influence

movement through their own subcortical projections. We set out to more precisely resolve the interaction between premotor and primary motor cortices using contemporary methods that enable assessment of neural interactions on the timescale of synaptic communication. Here we examined interactions between the rodent analogs of premotor and primary motor cortex, the rostral forelimb area (RFA) and caudal forelimb area (CFA), respectively, using head-fixed mice engaging in a directional reaching task. We first used simultaneous optogenetic inactivation of RFA (CFA) and neural activity recording in CFA (RFA), along with electromyographic recordings from forelimb muscles. Consistent with functional hierarchy, we found that the inactivation of RFA produced a large and lasting change in CFA activity while inactivation of CFA had a smaller and briefer effect on RFA activity. In order to further probe hierarchical influence, we then simultaneously recorded neural activity in both areas. We used projection to latent structures and canonical correlation analysis to find modes of neural activity that are shared between RFA and CFA. Neural dynamics in RFA and CFA were found to be strikingly similar. When accounting for synaptic delay by lagging activity in one region from that in the other, no evidence of a feedforward bias was found. We posited that this apparent symmetry may stem from the limited temporal resolution of the smoothed firing rate trial averages used in this analysis. To better resolve functional interactions, we then used functional connectivity metrics like transfer entropy, Granger causality, and convergent cross mapping on spike times for pairs of neurons within and between both regions. Consistently across metrics, we found a balance of predictive influence in both directions (CFA to RFA and RFA to CFA), again consistent with parallel control. To reconcile these disparate results, we are currently building recurrent neural network models to reproduce perturbation effects and functional connectivity measurements. Our results will provide new insight into the manifestation of functional hierarchy at the level of single unit activity.

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Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 217.06

Topic: E.04. Voluntary Movements

Support: Jean Sheng
NSF Graduate Research Fellowship

Title: Subtle contact events guide ongoing movements during licking

Authors: *B. S. ITO, X. HUANG, B. KARDON, J. H. GOLDBERG;
Neurobio. and Behavior, Cornell Univ., Ithaca, NY

Abstract: During non-visually guided grasping, subtle contact events guide ongoing movements. For example, when holding a cup of coffee, we readjust our grip in response to fine details of a slip - an upward slip on the index finger may recruit a different response than a downward one on the pinky. To study how fine tactile information is used to guide ongoing movements, we created a novel lick paradigm in head-fixed mice. First, we use kilohertz frame-rate imaging and a deep neural network to track tongue kinematics in 3D as mice retrieve water from a spout. Next, we detected the offset of tongue-spout contact on the first lick (L1) within a lick bout and randomly displaced the spout to the left or the right before the onset of the second lick (L2). Spout displacement magnitude was calibrated so that mice would 'nick' the displaced spout with either the left or right side of the tongue during L2. We find that mice use this nick event to guide the immediately ensuing lick to the new target: left nicks guided the third lick (L3) left, and right nicks guided L3 right. These across-lick adjustments were not only aimed in the correct direction, but the magnitudes of these corrective responses were scaled appropriately depending on the tongue tip position at L2 contact: mice made larger corrections to the left or right on L3 if the tongue tip was farther away from the spout at L2 contact, and smaller corrections on L3 if the tongue tip was closer to the spout. Populations of neurons in anterolateral motor cortex (ALM) encoded nick position and lick direction fast enough to implement tactile feedback-guided corrections but, surprisingly, bilateral photoinhibition of ALM, tongue-jaw primary motor cortex (TJM1), or tongue-jaw primary somatosensory cortex (TJS1) from L2 contact to L3 retraction did not impair nick-guided corrections. Yet, unilateral photoinhibition of ALM and TJM1 impaired corrections to the contralateral side, revealing that although ALM/TJM1 could influence lick direction, contact-driven corrective responses are largely cortex-independent. Bilateral lesions of the fastigial nucleus also did not affect re-aiming on L3, suggesting these nick-guided corrections may not rely on cerebellum. Together, these data implicate yet-to-be identified subcortical circuits in implementing directionally specific contact-driven corrections.

Disclosures: B.S. Ito: None. **X. Huang:** None. **B. Kardon:** None. **J.H. Goldberg:** None.

Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 217.07

Topic: E.04. Voluntary Movements

Support: Leibniz Association: Collaborative Excellence - Neuro-Optogenetics (WGL SAW-2014-DPZ-1)
DFG CRC-889 "Cellular mechanisms of sensory processing"

Title: Pathway-specific optogenetic inhibition reveals causal modulation of parietal motor goal encoding via frontal-to-parietal projections in rhesus monkeys

Authors: *H. GUO[1], M. FORTUNA[1], J. HÜER[2], S. TREUE[2], A. GAIL[1];
[1]Sensorimotor Group, [2]Cognitive Neurosci. Lab., German Primate Ctr., Goettingen, Germany

Abstract: Context-dependent visuomotor transformations are associated with the frontoparietal network in the cerebral cortex of primates. It has been hypothesized that the dorsal premotor cortex (PMd) and the parietal reach region (PRR) coordinate their activities via reciprocal connections to select motor goals. Yet, if and how the activity in PMd causally influences neural dynamics in PRR during context-sensitive goal-directed action selection is unclear. Particularly, we tested the hypothesis that the PMd impact on PRR is stronger during non-standard mapping (anti-reach) compared to direct mapping (pro-reach) due to putatively higher levels of cognitive control. To address this question, neural dynamics of PRR neurons were studied in combination with pathway-selective (PMd → PRR) optogenetic silencing of PMd axons projecting to PRR, while monkeys performed a memory-guided, center-out, anti-reach task. To this aim, neurons in the PMd area of two rhesus monkeys were transduced with the inhibitory opsin eArchT3.0 delivered in an AAV5 vector, and driven by the CamKIIα-promoter (AAV2/5-CamKIIα-eArchT3.0-eYFP). While the subjects performed the task, continuous laser stimulation (532 nm λ, 330 ms pulse duration) was applied during the presentation of the visual cues that instructed the spatial target and the pro-anti task rule prior to a delay period for planning the movement. Stimulation trials were randomly interleaved with no-stimulation trials, and single-unit microelectrode recordings were performed simultaneously in the laser-stimulated neuropil, either in the transfected PMd or in PRR. Local laser stimulation in PMd resulted in reliable silencing of PMd neural activity. Laser stimulation in PRR decreased local field potentials and modulated neural responses in nearby neurons, supporting the view that inhibition of presynaptic activity from PMd was effective, including during the movement planning period which followed the laser stimulation. Time-resolved analysis of directional selectivity in PRR revealed that the causal influence of PMd on PRR is context-dependent, in that motor goal encoding at the PRR population level is delayed as a consequence of silencing PMd inputs exclusively during anti-reach trials. These results support the hypothesis that dynamic reorganization in PRR, as it is selectively needed for spatial remapping in anti- but not pro-reach trials, depends on the functional and direct input from PMd. Our findings strengthen the view that rule-based, goal-directed reaching partly builds on frontal-to-parietal causal modulation.

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Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 217.08

Topic: E.04. Voluntary Movements

Support: Ministry of Education, Culture, Sports, Science and Technology of Japan (18H05523, 19J21332, 21H04810)
Takeda Science Foundation

Title: Prefrontal neuronal activity related to storage and extinction of short-term memory during oculomotor n-back task in monkeys

Authors: *R. SAWAGASHIRA[1,2], M. TANAKA[1];
[1]Hokkaido University, Sch. of Medicine, Dept. of Physiology, Systems Neurosci. Lab., [2]Dept. of Psychiatry, Hokkaido Univ., Sapporo, Japan

Abstract: Working memory (WM) consists of multiple components, including storages of short-term memory and the central executive system that manages them (Baddeley and Hitch, 1974). Although neural correlates of short-term memory have been demonstrated, the neural mechanism of the central executive system remains largely unknown. We explored neuronal activity in the lateral prefrontal cortex (LPFC) in monkeys performing the oculomotor version of the n-back task that requires both storage and update of short-term memory.During central fixation, two to four peripheral visual cues were sequentially presented with a short delay between them (800 ms). In response to the offset of the fixation target, animals made a memory-guided saccade to one of the cue locations. When the fixation target was either a red triangle or a white cross (1-back condition), they were required to make a saccade to the most recent cue location. When the fixation target was either a blue square or a white star (2-back condition), they needed to generate a saccade to the location of the two previous cue. In three Japanese monkeys that were well trained for the task (correct rate > 80%), we found three types of task-related neurons (n = 159). Visual neurons (n = 41, 26%) exhibited a transient activity as the cue appeared in the receptive field (RF). Memory neurons (n = 36, 23%) displayed sustained activity following the cue in the RF, which lasted for one (1-back) or two (2-back) delay intervals. Extinction neurons (n = 17, 11%) showed a transient activity when the memory of a specific cue location became no longer necessary. Importantly, these neurons responded to cues at any location when the previous (1-back) or two previous (2-back) cue was presented at the specific location. To account for the delay period activity of each neuron, we performed a regression analysis using the generalized linear model incorporating visual, memory, extinction, stimulus number, and task rule components. The results showed that 22, 23, and 13% of neurons carried visual, memory, and extinction signals, respectively. Most of these neurons also modulated their activity according to the stimulus number (62%) or task rule (44%). Extinction neurons were often found in the ventral part of the LPFC and memory neurons in the dorsal part. So far, we have found several sites where electrical microstimulation successfully changes animal's choice in a manner predicted from the neuronal activity. These results suggest that three different types of neurons in the LPFC contribute to the maintenance and updating of short-term memory for goal-directed behavior. The extinction signal appears to be an important element of the central executive system.

Disclosures: **R. Sawagashira:** None. **M. Tanaka:** None.

Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 217.09

Topic: E.04. Voluntary Movements

Support: EC FETPROACT-16 732266
DFG RU-1847-C1

Title: Proactive planning of consecutive reaches in a sequential action selection task in the fronto-parietal reach network of rhesus macaques

Authors: *L. HANSMEYER, A. TRUNK, N. S. AGHA, A. GAIL;
Sensorimotor Group, German Primate Ctr., Goettingen, Germany

Abstract: Everyday tasks often require a sequence of actions. Knowing actions in advance, allows us to prepare for an upcoming action before the first one has been executed. Here we ask whether areas of the fronto-parietal reach network show proactive movement planning activity during ongoing action in preparation of a subsequent movement when selecting between two options. We trained rhesus macaques to perform a memory-guided sequential action selection task on a touchscreen. Animals made two consecutive reaches to cued targets in a defined order. Possible target locations were arranged on two horizontal lines, one for each reach. In each trial two possible target positions for each reach were shown in different colors, one being the target the other serving as a distractor. Color cues denoted the correct target before a go-cue. In proactive trials, both targets and distractors were shown in color from the beginning. In sequential trials, the target and distractor for the second reach were revealed only after the first reach. Proactive compared to sequential trials resulted in shorter reaction time towards the second reach (pro: 283±69ms (m±sd), seq: 469±116ms), suggesting that the animals proactively prepared the second movement if possible. We recorded neural activity in the primary motor cortex (M1), parietal reach region (PRR) and dorsal premotor cortex (PMd) and analysed their spatial selectivity with respect to the direction of the pending reaches. As a result, selectivity for the second reach target could already be observed during the reach to the first target in proactive trials, while it was only expressed after the first reach was completed in sequential trials. Using a SVM, we decoded the spatial position of the second reach target in time bins aligned to the touch of the first target separately in the three different brain regions and the two trial conditions. During sequential trials, the spatial position of the second reach target could only be decoded after the first target had been touched. In contrast, in proactive trials, the decoding performance starts increasing from chance level approximately 150ms before the first target is touched for signals in PRR and PMd. The decoding performance from signals in M1 is similar for proactive and sequential trials, meaning that the spatial position can only be decoded after the first reach has been completed. Our results suggest that reaches are planned in a proactive manner, making use of the information about following movements in an action sequence before the ongoing action has been completed, and that this information is available with comparable lead-time in parietal and premotor areas.

Disclosures: L. Hansmeyer: None. **A. Trunk:** None. **N.S. Agha:** None. **A. Gail:** None.

Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

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Program #/Poster #: 217.10

Topic: E.04. Voluntary Movements

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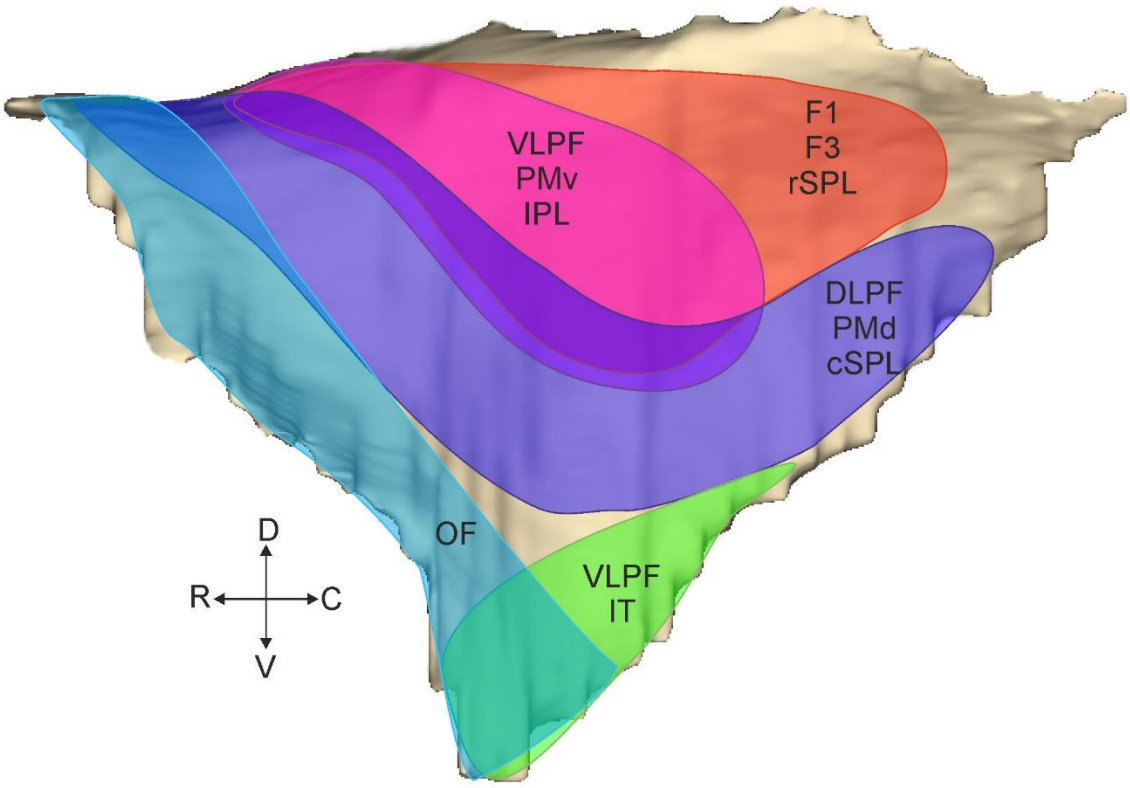
Title: Topography of claustral projections to the cortex and the striatum in the macaque brain

Authors: *G. LUPPINO, G. BALLESTRAZZI, D. BIANCHERI, G. COLUCCIO, E. BORRA; Dept. of Med. and Surgery, Univ. of Parma, Parma, Italy

Abstract: The claustrum is a telencephalic subcortical structure connected to almost the entire extent of the cortex and several subcortical structures including the basal ganglia. In the present study, we analyzed the origin of the claustral projections to different cortical and striatal regions to assess whether different parts of the claustrum project to cortical areas and striatal zones involved in specific corticostriatal circuits. Neural tracers were injected in different prefrontal, motor, parietal, and temporal areas (30 injections, 10 macaques) or in different striatal zones (6 injections, 4 macaques). Claustral projections to the cortex showed a topographic organization in which different parts of the claustrum appeared to project to areas involved in different large-scale cortical networks (see Figure). Specifically, a large dorsal, mid-caudal claustral sector was connected to the hand field of the primary motor cortex (F1) and to rostral superior parietal (SPL) areas. The rostral and ventral part of this sector also projected to ventral prefrontal (VLPF), ventral premotor (PMv), and inferior parietal (IPL) hand-related areas. A further oblique sector, running more rostrally and ventrally, up to the caudal part of the claustrum, was connected to dorsal prefrontal (DLPF), dorsal premotor (PMd) and caudal SPL areas. Finally, a ventral sector of the claustrum was connected to orbitofrontal (OF), VLPF and inferotemporal (IT) areas. Striatal tracer injections showed that hand-related zones of the motor putamen and of the pre-commissural putamen are targets of projections originating from the claustral sector connected with hand-related fronto-parietal areas. Furthermore, a tracer injection in the caudate head was a target of the rostral claustral sector connected also to the dorsal prefrontal and orbitofrontal cortex. The present data provide evidence for a gross topography of the claustral connectivity, which reflects the involvement of different claustral sectors in different large-scale cortical networks and basal ganglia circuits.



Disclosures: **G. Luppino:** None. **G. Ballestrazzi:** None. **D. Biancheri:** None. **G. Coluccio:** None. **E. Borra:** None.

Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

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Program #/Poster #: 217.11

Topic: E.04. Voluntary Movements

Support: 111 project (Base B16018)
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Title: Distinct roles for rat premotor cortex in World-Centered versus Self-Centered Planning

Authors: ***J. LI**[1], C. BAO[1], L. LI[2], J. ERLICH[1,2];
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Abstract: Planning can be carried out under different reference frames during navigation or orientation. Classic studies indicate that self-centered planning depends on the striatum, while world-centered planning depends on the hippocampus. Recent results suggest the frontal orienting field (FOF) in secondary motor cortex might play a role in integrating the two reference frames. To determine whether the FOF plays distinct roles in self- vs. world-centered planning, we developed two novel auditory memory-guided orienting tasks using an 8 port wall. In both tasks, rats are instructed to "fixate" in a start port using a visual cue. During fixation, one of two sounds indicates to the animal which port needs to be poked to get a reward is on that trial. After the sound, there is a silent memory period, followed by a go cue. In the self-centered "ego" task, sound A tells the animal to move to the left and sound B to the right, regardless of their start position. In the world-centered "allo" task, sound A tells the animal to move to the bottom-left port and sound B to the bottom-right port, regardless of their start position. We have recorded neural activity from three animals in each task, and found similarities and differences in the representation of planning in the two tasks. In both tasks, FOF activity represents the current position of the animal throughout the fixation period. However, in the "ego" task, the FOF uses a common neural code for planning left trials across start positions (and likewise for right trials). In the "allo" task, the code does not generalize across start positions, and planning activity is later and weaker than in the "ego" task. Preliminary optogenetics experiments support this finding: that the FOF plays distinct roles in the two tasks.

Disclosures: **J. Li:** None. **C. Bao:** None. **L. Li:** None. **J. Erlich:** None.

Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 217.12

Topic: E.04. Voluntary Movements

Support: NSF Grant 1912557

Title: Premotor area 6 firing during accurate stepping on a complex terrain

Authors: G. VIANA DI PRISCO[1], V. MARLINSKI[2], I. N. BELOOZEROVA[3];
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Abstract: The premotor cortex area 6 receives both visual and somatosensory information. Recent studies in walking cats showed that half of the neurons in the 6iffu region change firing activity before or while stepping over a barrier (Nakajima et al. 2019), and microstimulation within 6aα and 6aγ areas evoke stride phase-dependent modification of muscle activity (Fortier-Lebel et al. 2021). These data suggest that area 6 is involved in the planning and execution of stride adjustments on complex terrain. Here we tested the hypothesis that area 6 contributes to

controlling the accuracy of steps. We analyzed the firing activity of 201 neurons in areas 6aα and 6aγ of 2 adult cats as they walked on a flat surface, a task that does not require accuracy of steps, and along a horizontally placed ladder, a task that requires accurate paw placement. Flat 5 cm wide rungs of the ladder were spaced 25 cm apart, i.e., at half the average length of the cat's stride. We have previously shown that, aside from the greatly reduced variability of the stride's length, kinematics of locomotion on such a convenient ladder as very close to those on a flat surface (Beloozerova et al. 2010). When cats walked on the flat surface, 98% (196/201) of cells, including all but one of 33 pyramidal tract neurons (PTNs), were active. The average discharge of PTNs was 12.4 ± 9.7 spikes/s (mean ± SD), while that of cells whose axon projection was unknown (noIDs), was half that (5.3 ± 2.9 spikes/s; p=0.01, t test). The discharge of 86% of cells (168/196), including 84% (27/32) of PTNs, was modulated with the strides, i.e., the discharge was higher during some phase(s) of the stride and lower during other phase(s). Most cells (58%, 113/196) had one period of elevated firing per stride (1-PEF), while 28% (55/196) had two (2-PEF). There were more 1-PEF cells among PTNs (71%) than among noIDs (55%). For 1-PEF group, the average PEF duration was 68 ± 19% of the stride and for 2-PEF group the combined duration of the PEFs was similar, 64 ± 13%. In both groups, PEFs of different neurons were distributed evenly across the step cycle, and 60-70% of cells had a PEF simultaneously. When cats went on the ladder, firing activity of 41% of PTNs increased, to 23 ± 15 spikes/s, and that of 26% of noIDs rose as well, albeit not as much. 47% of PTNs retained the 1-PEF discharge pattern, occasionally shifting the preferred phase of the activity by ~20% of the cycle. The pattern of ~20% of both PTNs and noIDs changed from 1-PEF to 2-PEF, while that of ~10% changed oppositely. We concluded that premotor areas 6aα and 6aγ contribute to the accuracy of steps by increasing firing and occasional change of the phase or pattern of the discharge.

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Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

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Program #/Poster #: 217.13

Topic: E.04. Voluntary Movements

Support: NRF-2019M3E5D2A01058328

Title: Neural state of movement initiation in the mouse premotor cortex varies with the presence of temporal uncertainty

Authors: *S. CHAE, S.-P. KIM;
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Abstract: Motor cortical neurons show preparatory activity before movement. The final state of preparatory activity (preparatory end-state) predicts ensuing behavior (e.g., reaction time; RT), so preparatory activity has been assumed as a process of shaping proper end-state to produce

desired movements. Yet, it is unknown how task paradigm affects the shaping of preparatory end-state in motor cortex, even to make identical movement. Thus, we aim to test if the preparatory end-state varies with task paradigm, and how the end-state is shaped in relation to task paradigm.We analyzed firing activity of anterior lateral motor cortex (ALM) of mice during delayed-response task (CRCNS.org). 6 mice were trained to perform fixed delay task. Auditory cue was given at 3 or 12 kHz for 1.15s to inform water port locations (right or left, respectively). Mice licked right or left according to auditory cue after a 2s fixed delay. Also, 5 mice were trained to perform random delay task, a variant of fixed task in which delay length was randomly selected among 7 delay lengths across trials. We made two evidential observations that the preparatory end-state could be different between task paradigms. Given the same preparatory end-state, time taken to transfer from the end-state to movement state (RT) should be unchanged. But RT was significantly longer in fixed task. Also, amounts of direction information in the preparatory end-state showed positive correlations with behavioral accuracy and RT in fixed task, but not in random task. Then, we hypothesized that such differences would be related to presence of temporal uncertainty. In fact, behavioral accuracy and RT were positively correlated in fixed task, but not in random task, indicating that different strategies would be used for each task. We further hypothesized that in fixed task, the preparatory end-state is formed to maximize accuracy, while accompanying longer RT. However, in random task, preparatory activity quickly approaches a state to be ready to make licking, which persists until go cue. To test the hypotheses, we inferred preparation and movement subspaces from population activity such that activity on preparation space could determine activity on movement space when the first lick occurred. In fixed task, activity on the preparation space was shaped in direction of increasing accuracy but with larger cost in state transition to result in longer RT. In random task, activity on the preparation space quickly reached a state to make movement right after delay started and persisted until go cue. In conclusion, the preparatory end-state is shaped differently depending on presence of time uncertainty and in a way to be suitable for given task.

Disclosures: S. Chae: None. **S. Kim:** None.

Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

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Program #/Poster #: 217.14

Topic: E.04. Voluntary Movements

Support: NIH R01NS102259

Title: Movement preparation and re-preparation under uncertain conditions

Authors: *T. ARAKERI[1], R. C. MANJAREKAR[2], C. E. LARKIN[2], K. M. GOTHARD[3], A. J. FUGLEVAND[3];
[1]Neurosci., [3]Physiol., [2]Univ. of Arizona, Tucson, AZ

Abstract: In our daily lives we are constantly forming and correcting motor plans to interact with the external world accurately and efficiently. Often, we are required to prepare and execute movements with incomplete information about the movement goal. It is still unclear how the motor system prepares movements under different levels of uncertainty, and how this uncertainty affects the ability to re-prepare movements. Using a Forced Reaction-Time paradigm, a rhesus macaque was trained to produce center out reaching movements to one of two diametrically opposite potential targets. We manipulated the uncertainty about the target location using a coloring scheme that indicated the probability (p) of where the final target will appear. For example, if p=0.5, the target could appear at either of the potential locations with equal probability. Using this paradigm, we found that the subject was able to re-prepare movements faster with increasing levels of uncertainty (i.e., decreasing values of p). We similarly observed a significant reduction in re-preparation time when the initial plan was made using a predicted target location versus a visual target. These findings suggest that when movement preparation is driven by an uncertain source of information, it also leads to a greater degree of flexibility to re-prepare the movement when faced with unexpected changes in the movement goal. We have also collected preliminary data from dorsal pre-motor cortex in a monkey that shows reduced preparatory subspace occupancy (i.e., a reduction in the across-condition variance of the neural states) when movement preparation is driven by prediction of the target location when compared to an overt visual stimulus. Importantly, the subsequent movement dynamics under both conditions were essentially identical. The reduced occupancy in the preparatory subspace provides a potential explanation of how the motor system re-prepares movements faster with increases in the uncertainty about the movement goal.

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Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

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Program #/Poster #: 217.15

Topic: E.04. Voluntary Movements

Title: Optogenetic mapping of neuronal interactions in the motor cortex during goal-directed behavior

Authors: *A. FINKELSTEIN[1,2], K. DAIE[2,3], M. RÓZSA[2,3], R. DARSHAN[2], K. SVOBODA[2,3]; [1]Tel Aviv Univ., Tel Aviv, Israel; [2]Janelia Res. Campus/HHMI, Ashburn, VA; [3]Allen Inst. for Neural Dynamics, Seattle, WA

Abstract: Behavior-related neural dynamics is an emergent property of network connectivity. The network structure at the level of individual neurons, and its relationship to neural coding are largely unknown. Here we developed an optical method for *rapid* (500k pairwise connections / 30 minutes) mapping of effective connectivity *in vivo*. This method is based on 2-photon

optogenetic stimulation of individual excitatory neurons and simultaneous 2-photon volumetric calcium imaging of evoked responses in non-stimulated neurons ('effective connection'). We applied this method in anterior lateral motor cortex (ALM) in a novel behavioral task in which untrained mice performed multidirectional tongue-reaching for water rewards presented at multiple (up to 16) locations on a grid in front of the mouse face. A majority of ALM neurons were modulated by task variables with a subset of neurons (~25%) exhibited strong tuning to the reward location. Specifically, some neurons showed tuning to direction of the reward location with respect to the mouse face, whereas other neurons were selective for particular reward locations. We then mapped effective connectivity between 10,000,000 pairs of layer 2/3 neurons imaged in this task. Nearby neurons were more strongly connected and shared directional selectivity, revealing a fine-scale columnar architecture. By analyzing connectivity patterns with methods borrowed from network science, we discovered neurons that function as network hubs. Hub neurons had an unexpectedly high number of connections, weak directional tuning, and strong influence on neighboring neurons - suggesting that they may act as local conductors of the neural orchestra.

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Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

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Topic: E.04. Voluntary Movements

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Title: Exceptionally large rewards collapse task information in neural population activity

Authors: *A. L. SMOULDER[1], P. J. MARINO[3], N. P. PAVLOVSKY[3], E. R. OBY[4], S. E. SNYDER[5], W. E. BISHOP[6], B. M. YU[2], S. M. CHASE[1], A. P. BATISTA[3];
[1]Biomed. Engin., [2]Electrical Engin., Carnegie Mellon Univ., Pittsburgh, PA; [3]Bioengineering, [4]Biomed. Engin., [5]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [6]HHMI Janelia, Ashburn, VA

Abstract: Our performance typically improves as motivation increases. However, we often struggle when the payoff is exceptionally large, a phenomenon dubbed "choking under pressure." What is the neural basis of choking under pressure? Because monkeys choke under

pressure, like humans do, they can provide an opportunity to answer this question.
We studied choking under pressure in 3 rhesus monkeys as they performed a challenging reaching task, in which the reward available for a successful reach was cued at the start of each trial. We recorded neural population activity in motor cortex with 96-channel multielectrode arrays. All three animals choked under pressure: success rates increased as a function of reward to an extent but decreased for the highest level of reward. This gave rise to an "inverted-U" relationship between success rate and cued reward size.
How does the brain mediate the unintuitive inverted-U relationship between reward size and behavioral performance? We examined how reward size altered the neural activity before movement onset to determine if we could identify neural correlates of choking under pressure before action even occurs. We identified three main effects related to reward and reach direction information in the motor cortex.
First, we found that reward information was primarily organized along a monotonic "reward axis" of the neural population state space, as opposed to exhibiting the inverted-U characteristic of the success rates. The reward axis separated neural activity as a function of reward irrespective of upcoming reach direction.
Second, we found that the reward axis was nearly orthogonal to the linear 2D projection that best separates neural activity for reach directions ("target plane"). This indicates that different patterns of neural activation drove changes in the encoding of reward and reach direction information.
Third, higher reward cues drove average preparatory activity for different reach directions farther apart in the target plane, but the highest reward level pushed them back together. This expansion-then-collapse of target conditions was sufficient to yield an inverted-U in the accuracy of an offline discriminator of the reach direction as a function of reward.
In summary, increasing reward initially drives motor cortical preparatory activity in a manner that is more informative about the upcoming movement (as measured by decoding accuracy), but then as reward signals grow, less information about target location is present in the population response. We propose this expansion-then-collapse in reach information during preparation as one potential mechanism for choking under pressure.

Disclosures: **A.L. Smoulder:** None. **P.J. Marino:** None. **N.P. Pavlovsky:** None. **E.R. Oby:** None. **S.E. Snyder:** None. **W.E. Bishop:** None. **B.M. Yu:** None. **S.M. Chase:** None. **A.P. Batista:** None.

Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

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Program #/Poster #: 217.17

Topic: E.04. Voluntary Movements

Support: R01 NS053603

Title: Pharmacological manipulation of monoamines modulates the gain between M1 and motor output

Authors: *R. POWELL[1], X. MA[2], L. E. MILLER[3];
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Abstract: Our motor system can accurately control a remarkably wide range of force. Yet, it accomplishes this feat using noisy cortical neurons, each with rather limited bandwidth. One mechanism by which the motor system could effectively operate over this force range may be through peripheral gain control mediated by monoamines, including serotonin (5-HT), and norepinephrine (NE). Monoaminergic neurons originating from brainstem nuclei project diffusely to motoneurons at all levels of the spinal cord. There these monoamines act to facilitate voltage-sensitive ion channels, increasing motoneuronal excitability and amplifying descending synaptic input. We wondered whether this system might play a role in nonlinear relation between M1 activity and force or EMG identified in several earlier experiments. To test this hypothesis, we measured baseline neural activity from the hand representation M1 as a monkey completed power grasping and spring-loaded wrist tasks. We gave the monkey drugs to either augment or suppress levels of these monoamines and repeated the experiment after the drug reached approximately peak plasma concentration. We also recorded muscle activity (EMG), and force during both sessions to confirm that behavior was similar in the two experiments. Comparing the depth of modulation of discriminated neurons before and after the drug, we found when levels of 5-HT were suppressed via cyproheptadine, cortical modulation increased by 20% ($p < 0.001$). Following either caffeine or the selective serotonin reuptake inhibitor escitalopram, modulation decreased 19% ($p < 0.001$) and 15% ($p = 0.001$), respectively. During control sessions when the monkey received no drug, there was no significant change in modulation (3%, $p = 0.45$). We also observed these changes at the single-neuron level. When comparing changes in depth of modulation between well isolated units, after cyproheptadine, modulation increased 19%, $p = 0.001$. After caffeine and escitalopram modulation decreased 14% ($p = 0.006$) an 20% ($p < 0.001$). Finally, during control sessions there was no significant change in modulation (-0.7%, $p = 0.86$). Although there were small differences in EMG between sessions, they were much smaller than the changes in cortical modulation and did not change consistently in a direction that could have explained the neural change. These drug-induced changes in modulation were consistent across both motor tasks and provide evidence for the existence of gain control in the motor system and the role serotonin and norepinephrine play in this control.

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Poster

217. Prefrontal and Parietal Cortical Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

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Program #/Poster #: 217.18

Topic: E.04. Voluntary Movements

Support: Pennsylvania HRFG

Title: Evolution of neural dynamics in the motor cortex during reaching

Authors: *H. MAO, A. B. SCHWARTZ;
Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: A mechanistic framework for movement generation has gained interest in recent years. With this perspective, neuronal activity in the motor cortex is set to an initial state during movement preparation. Starting from this initial state, neuron-neuron interactions determine the subsequent patterns of neural activity, resulting in muscle activation and movement generation. This framework has been used to explain motor neural activity during the early stage of delayed reaching movements, but whether it extends to the remainder of the reach is largely unknown. Here we studied neural population dynamics in the motor cortex through the time course of reaching, and found that it evolves through the movement.

Two rhesus monkeys performed a standard center-out reaching task while single-unit activity was recorded from microelectrode arrays in the motor cortex. Firing rates were calculated for all recorded motor cortical units in each of 16 movement directions. Dimensionality reduction was performed to find a 6-dimensional space that captured about 70% variance of the neural population activity. Within this 6-D space, dynamical models were found in sequences of 150 ms overlapping windows during the reaching movement. The dynamics were characterized by eigenvalues of the model's coefficient matrix, and these eigenvalues were found to change through different stages of reaching. In particular, models constrained by rotational dynamics (used previously to explain reaching) did not fit the data in the latter part of the reach as the hand approached the target. These findings suggest that neural dynamics in the motor cortex are subject to change as movement proceeds.

Using a dimensionality reduction approach, we analyzed the same neural population data without an explicit dynamical model. Our approach revealed three pairs of latent components. The peaks of these components came in a sequential order, and they appeared to be aligned in time with behavioral landmarks of reaching - movement onset, peak movement speed, and movement offset. Moreover, the magnitude of each component was modulated by movement direction, and their preferred directions were largely constant over time. These results suggest that factors in addition to intrinsic neuron-neuron connectivity contribute to reach-related motor cortical activity. How these multiple factors are combined mechanistically for movement generation, is an open question awaiting further investigation.

Disclosures: H. Mao: None. **A.B. Schwartz:** None.

Poster

218. Neuroprosthetics: Decoding of Neural Activity and Neuromuscular Stimulation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 218.01

Topic: E.05. Brain-Machine Interface

Support: Kahn Foundation Grant N028634

Title: Continuous error detection during the control of two finger groups with intracortical brain-machine interfaces

Authors: *D. M. WALLACE[1], M. BENYAMINI[4], M. S. WILLSEY[2], P. G. PATIL[2], C. A. CHESTEK[3], M. ZACKSENHOUSE[4];
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Abstract: Summary: Brain-machine interfaces (BMIs) can restore function of paralyzed limbs to those suffering from spinal cord injury. However, BMI decoders are prone to prediction errors which can cause frustration or potential harm to the user of a physical system. We have previously demonstrated preliminary results detecting erroneous movements using intracortical BMIs and have evaluated its performance offline with non-human primates (NHPs) while moving 1 finger group (Benyamini et al., NER 2019). Here we demonstrate results in offline validation and real-time detection of erroneous movements in simultaneous and independent movements of two finger groups, and also present offline results demonstrating the representation of target distance error in the neural activity.
Methods: We implanted one NHP with a Utah microelectrode array in the hand area of primary motor cortex (M1) and recorded spike-band power (SBP) from 96 channels. The NHP was trained to perform a task where two finger groups (index and middle-ring-small fingers) were moved to match virtual targets presented on a screen. Online BMI control of the virtual fingers was performed using a Kalman filter trained on the 50 ms binned SBP activity (Nason et al., Neuron 2021). We then trained classifiers to detect when the decoded movements are erroneous based on a step-wise linear discriminate analysis using the SBP activity. Offline classifiers were evaluated according to their receiver operating curves, quantifying the tradeoff between true positive rate (TPR) and false positive rate (FPR), and in practical operation the FPR was restricted to 5% to prevent false corrections. Offline results include variance analysis and classifier validation for 4 days and preliminary results demonstrate ability for real-time detection of erroneous movements.
Results: Offline variance analysis of the SBP activity revealed that channels encode the distance to the target in addition to position and velocity, and that adding this distance explained significantly more ($p < 0.05$) variance than just position and velocity. Offline error detection results demonstrate a TPR of 37.57% ± 18.12% for both finger groups when FPR is thresholded to 5%.
Conclusions: Distance to target contributes significantly to the variance of SBP activity explained by finger position and velocity alone, and erroneous movements away from the target can be detected from SBP activity with good TPR while keeping FPR low. Correcting for errors continuously in closed-loop control provides the possibility for increasing decoder performance, and the ideal method for error correction in online control is currently being investigated as part of this work.

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Poster

218. Neuroprosthetics: Decoding of Neural Activity and Neuromuscular Stimulation

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Program #/Poster #: 218.02

Topic: E.05. Brain-Machine Interface

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University of Michigan Robotics Institute
A. Alfred Taubman Medical Research Institute

Title: Predicting finger movements in a brain-machine interface across task contexts

Authors: *M. J. MENDER[1], S. R. NASON-TOMASZEWSKI[1], H. TEMMAR[1], J. T. COSTELLO[2], D. M. WALLACE[3], M. S. WILLSEY[4], N. GANESH KUMAR[5], T. A. KUNG[5], P. G. PATIL[4], C. A. CHESTEK[1];
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Abstract: A key factor in the clinical translation of brain-machine interfaces (BMIs) for restoring hand motor function will be their robustness to changes in a task. This poses a challenge for applications such as functional electrical stimulation (FES), where the patient's own hand will be used to produce a wide range of forces in otherwise similar movements. We aim to investigate the impact of task changes on performance in a BMI first during control of a virtual hand and second during brain-controlled FES (BCFES). Two rhesus macaques were implanted with Utah microelectrode arrays targeted to the hand area of motor-related precentral gyrus. In a separate surgery, one monkey was implanted with 10 bipolar electromyography (EMG) electrodes in muscles of the wrist and fingers. The monkeys performed a 2-degree of freedom (DOF) task to control a virtual hand, using their index and middle-ring-small fingers to push doors in a manipulandum while neural activity, finger positions, and EMG were recorded. Context was changed by adding springs to the doors of each finger group or by altering wrist posture. In the real-time BMI task, intended movements were decoded from the recorded neural activity using a position/velocity Kalman filter and used to control the virtual hand in real time. We first found that the context changes introduced errors in offline kinematic predictions. When predicting finger movements in off-context trials using linear models trained on data from only normal trials, prediction mean-squared error (MSE) during off-context trials increased by 65.3% and 10.3% for position and velocity, respectively. Interestingly, context had little effect on real-time BMI control. This was tested in two ways: first by adding context changes to the manipulandum during the online task, and second by training one model on normal trials, another model on off-context trials, then using each model online. Off-context performance was equivalent to normal online performance for all metrics in 28 of 30 experiments across both methods. To begin studying the impact of context on BCFES, we also predicted muscle

activations in new contexts. MSE increased by 262% on average when predicting off-context muscle activation compared with predictions during normal trials. This suggests that the impact of context on BCFES may be larger than the impact on kinematic based applications like controlling a computer cursor, virtual hand, or prosthesis. Future work will evaluate this impact on BCFES and quantify the ability of FES to make precise movements across various contexts. To this end, we have developed an FES setup to control both wrist and 1-DOF finger movements simultaneously.

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Poster

218. Neuroprosthetics: Decoding of Neural Activity and Neuromuscular Stimulation

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Title: Characterizing improvements to finger movement predictions by artificial neural networks in real-time brain-machine interfaces

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Abstract: Brain-machine interfaces aim to restore motor function by decoding neural signals into movement commands. Linear decoders have demonstrated high performance in the past, but recent advances in machine learning have prompted investigation into the development of nonlinear decoders which may outperform current approaches. We present a novel neural network decoder (NN) to control finger prostheses using neural activity recorded from intracortical Utah arrays. An intention based retraining step, similar to that used in the recalibrated feedback intention-trained (ReFIT) Kalman filter, was used to produce an improved decoder, the ReFIT NN (RN). Utah arrays were implanted in motor cortex of two male rhesus

macaques, trained to perform a two-dimensional finger task acquiring random targets. Using a low power proxy for spiking rate, spike-band power (SBP), a five-layer feed-forward neural network decoder (NN) was trained to predict finger velocities from SBP. The NN was used to predict finger velocities online and then further refined by the ReFIT re-training step to produce the RN decoder. The RN and NN were both compared to the ReFIT Kalman Filter (RK; Nason et al. 2021) in closed-loop settings and NN was compared to ridge regression (RR) in offline settings. Over multiple days of testing, comparisons between the NN/RN and RR/RK decoders were made using mean-squared error and an adaption of Fitt's law bit rate, a measure of throughput. In both animals, when comparing RN and NN, the intention-based retraining step substantially improved throughput by 42% in Monkey N ($P < 10^{-5}$) and 19% in Monkey W ($P < 10^{-5}$). Over 7 days of testing, NN and RN decoders achieved a 36% increase in throughput over RK across both Monkeys N and W. Offline analyses in Monkey N across 2 days show that NN predictions have significantly lower MSE than RR predictions ($P < 10^{-5}$). In the top and bottom 10^{th} percentiles of recorded speeds, NN produced 32% higher and 44% lower mean speeds than RR respectively. This may suggest that the NN improves MSE by better matching the wide range of speeds produced by able-bodied hand control. When tested online in the same monkey, the RN produced 46% higher and 60% lower mean speeds than RK in the high and low regimes respectively. Overall, these results suggest that the NN/RN decoder may be able to better map neural signals to velocities than linear methods like RR and RK. However, stochastic training methods may cause the NN/RN decoders to overfit to training data and converge inconsistently. Further investigation must be done to assess the consistency of trained NN/RN decoders and their ability to generalize to variations in the task.

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Poster

218. Neuroprosthetics: Decoding of Neural Activity and Neuromuscular Stimulation

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Topic: E.05. Brain-Machine Interface

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Title: A low-power communication scheme for wireless, 1000 channel brain-machine interfaces

Authors: *J. T. COSTELLO[1], S. R. NASON-TOMASZEWSKI[1], H. AN[1], J. LEE[1], M. J. MENDER[1], H. TEMMAR[1], D. M. WALLACE[1], J. LIM[1], M. S. WILLSEY[1], P. G. PATIL[1], T. JANG[2], H.-S. KIM[1], D. BLAAUW[1], C. A. CHESTEK[1];
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Abstract: Brain-machine interfaces (BMIs) have the potential to restore motor function but are currently limited by electrode count and long-term recording stability. These challenges may be solved by using free-floating "motes" which wirelessly transmit recorded neural signals. In particular, motes that are powered by and transmit data through infrared light can be scaled to sub-250 um dimensions for high-density recording. However, a key challenge with implanted wireless devices is minimizing power usage to stay within safe tissue irradiation limits.
Here, we evaluated a pulse-interval modulation (PIM) communication scheme for infrared (IR)-based motes that aims to reduce the wireless data rate and system power consumption. It has previously been shown that recording power can be reduced by sampling at only 2000 Hz and using the 300-1000 Hz spiking band power (SBP) as a neural feature. In the PIM scheme, data packets contain each mote's ID and encode SBP by the rate at which packets are transmitted. Then, a receiver can resolve packet collisions using temporal and spatial filters matched to each mote's ID. Due to asynchronous transmission times, PIM-based motes can use clock speeds less than 10 kHz, unlike synchronized schemes that require clock speed and power to scale with the number of motes.
In offline analyses, we found that PIM at 1 kb/s per channel maintained strong correlations with true neural firing rate. Then, to test PIM's ability to efficiently communicate SBP, we simulated the communication scheme in a real-time BMI. Two rhesus macaque monkeys, previously implanted with Utah microelectrode arrays in motor-related precentral gyrus, were trained to control a virtual hand using a BMI. In this system, Kalman filter decoders predicted finger velocity from SBP or simulated PIM-SBP. At a rate of 100 packets/s per channel (1 Kbps/ch), PIM-SBP decoders performed as well as standard SBP decoders (target acquisition times of 1.26 and 1.36 sec, respectively). This suggests that BMI performance can be maintained with significantly lower bit rates than transmitting the full 30 kHz signal (480 Kbps/ch) or SBP signal (32 Kbps/ch). Additional circuit and IR-light simulations suggest that, unlike other communication schemes, PIM motes can stay below IR power limits, and despite the complexity of an asynchronous scheme, a receiver could accurately decode data from 1000 motes using 3 mW. Finally, decoder accuracy can be improved using power-efficient, sparse, artificial neural networks.
These results suggest that PIM-based communication could significantly reduce power usage of wireless motes to enable the development of high-performance, high channel count, wireless BMIs.

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Poster

218. Neuroprosthetics: Decoding of Neural Activity and Neuromuscular Stimulation

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Program #/Poster #: 218.05

Topic: E.05. Brain-Machine Interface

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Title: Characterizing sensorimotor neural activity in Octopus Bimaculoides arms

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Abstract: Current prosthetic arms are not flexible and adaptable as they lack fine motor ability needed for specific movements, such as gripping. This work is designed to develop an adaptable and independent limb prosthesis based on the octopus' neural circuitry. The octopus offers a unique model for studying gripping behavior as it possesses eight arms that move with unlimited degrees of freedom. Each axial cord resembles the spinal cord seen in vertebrates and is capable of producing movement even when severed from the main body. Our approach utilizes electrophysiology (EP) recordings in Octopus Bimaculoides arms. A high-density carbon fiber 16 channel electrode array was inserted into isolated axial cords of two severed arms from separate animals. Arms were perfused with artificial saltwater originating from the octopus' tank. EP recordings were collected using Intan and Spike2 software. Sensorimotor stimuli, including tactile and electrical stimulation, were performed three times at each location along the arm. Tactile stimuli consisted of manual pinches using plastic forceps located at the isolated cord, proximal arm, and distal arm. Stimulation time and resulting movement of the arm was captured using a high-resolution camera that automatically synchronizes with the data. Multi-unit activity and single unit spike detection was performed using Spike2 with a band-pass second order filter and threshold of 4*Std to identify activity. We recorded unit amplitudes ranging 10-120 μV with an approximate spike duration of 1.05 ms. Preliminary results suggest that response to stimulation follows a Gaussian curve with cord pinches, representing efferent information, and distal pinches showing a highly left-skewed distribution. Time of maximum response after stimulation and the average number of events at that point, within 0.01 ms, for cord, proximal, and distal pinches was 250 ms (0.89 events), 700 ms (1.58 events), and 250 ms (2.15 events), respectively. When inserted transversely, the electrode channels show a spatial distribution of activity suggesting an anatomical component that could play a role in neural activity recorded. Both the number of neuron clusters and events were increased in channels that lie in the oral, aboral, and midline region of the axial cord compared to the channels between these regions. We are now identifying neural activity patterns that predict arm movement through MATLAB machine learning.These preliminary findings are an important step towards understanding the complex sensorimotor circuitry within the octopus' arm to improve future development of prosthetics.

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Poster

218. Neuroprosthetics: Decoding of Neural Activity and Neuromuscular Stimulation

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Topic: E.05. Brain-Machine Interface

Support: NIH NINDS R01NS119160

Title: Incorporating stiffness modulation into upper limb neuromuscular stimulation systems

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Abstract: Functional electrical stimulation (FES) of arm and hand muscles combined with the decoded motor intentions from intracortical recordings can restore reaching ability to individuals with spinal cord injury. Our goal is to improve FES-generated arm movements by incorporating real-time adjustments in limb stiffness in addition to limb kinematics. In our previous study, we have developed ways to automatically modulate limb stiffness based on the intended limb velocity command decoded from the brain. That study showed how decreasing stiffness during acceleration and increasing stiffness during deceleration allows one to have good control with a lower overall stiffness level to maintain movement performance while reducing energy usage and fatigue. However, that proof-of-concept simulation work used a simplified model of the arm with no redundant muscles or muscles that span multiple joints (biarticular muscles). Here we build on that method by now addressing two key practical issues: 1) balancing stimulation across multiple redundant muscles, and 2) optimizing stimulation of biarticular muscles. In developing solutions to address these two complexities, we also transitioned to a more accurate and robust definition of 'stiffness' that takes into account both active and passive torques around each joint. With these advances, we demonstrate, through simulation, how to generate an upper limb FES control algorithm that is clinically practical to implement and allows one to control limb stiffness in addition to kinematics. This improved FES control method can be used with the beneficial automated stiffness modulation algorithm we previously describe, as well as with volitional stiffness commands decoded directly from the brain. To better understand how limb stiffness can be decoded from neural populations, we have trained macaques with intracortical microelectrodes to perform an EMG-controlled cursor task in which independent activation of antagonist muscle pairs control one dimension and a separate dimension of movement is controlled by their degree of cocontraction. Preliminary results suggest some information about cocontraction can be extracted from the motor cortex. We anticipate getting similar results from paralyzed human study participants as they attempt to generate different limb stiffness levels. Results from these studies will help improve restoration of reaching after paralysis by demonstrating a clinically practical stimulation control method that includes stiffness modulation as well as identifying ways to decode reliable volitional stiffness commands from intracortically recorded neural signals.

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Poster

218. Neuroprosthetics: Decoding of Neural Activity and Neuromuscular Stimulation

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Title: Neural Population Dynamics of Simultaneous Finger Movements in Human Motor Cortex

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Abstract: Brain Machine Interfaces (BMIs), combined with dexterous movement neuroprostheses, can be used to restore functional reach and grasp movements to those with tetraplegia. However, current BMIs scale poorly to high degree-of-freedom (DoF) movements such as coordinated finger movements, potentially due to an incomplete understanding of how the brain generates motor commands for several joints simultaneously. Previous studies have shown neural population activity in motor cortex during single joint movements is well modeled as a dynamical system that evolves in time following intrinsic dynamical rules. This study aims to explore whether simultaneous finger movements also share consistent intrinsic dynamics in human motor cortex. A study participant in the BrainGate2 Pilot Clinical Trial, with chronic tetraplegia due to high cervical spinal cord injury (C4, AIS A), received two intracortical microelectrode arrays in the hand knob area of primary motor cortex (M1). The participant attempted a virtual reality multi-finger matching task requiring imagined flexion and extension movements of his index finger, thumb, and middle-ring-pinky finger group. Trials consisted of either a 1 DoF movement condition, where the goal was to move a single joint or finger group to a target position in a virtual space, or a 2 DoF condition, where the goal was to move pairs of fingers simultaneously. Recorded neural data was fit to low-dimensional linear dynamical systems using both regression to find the dynamics that optimally describe the data and jPCA

which imposes a constraint that the dynamics are rotational. When fit to 1 DoF movement conditions only, the optimal linear system had an R^2 of 0.57 while the rotational system had an R^2 of 0.43, demonstrating that rotations are a core component of the dynamical structure of the data. The top jPCA plane, which projects the most prominent rotations, captured 35% of the total variance of the neural data. Similar results were seen when the models were fit to 2 DoF conditions only (R^2 0.54 and 0.46, 33% variance explained by top jPCA plane) and to all conditions (R^2 0.48 and 0.34, 31% variance explained). Our analyses provide evidence that individual and simultaneous finger movements share a consistent dynamical structure, and the structure prominently features a rotational motif much like that seen in previous investigations of reaching and index finger movements. Furthermore, our study participant had a spinal cord injury that left him with no intact somatosensory feedback, yet rotational dynamics remained, suggesting the rotational structure is due to the recurrent connections in motor cortex and not sensory feedback.

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Poster

218. Neuroprosthetics: Decoding of Neural Activity and Neuromuscular Stimulation

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Program #/Poster #: 218.08

Topic: E.05. Brain-Machine Interface

Support: NIH NINDS R01NS119160

Title: Building fatigue resistance into upper limb neuromuscular stimulation control schemes

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Abstract: Functional electrical stimulation (FES) of arm and hand muscles can be used to reanimate paralyzed upper limbs. Our research focuses on improving current FES control methods by incorporating modulation of limb stiffness into current kinematic control methods. Previously, we demonstrated an automated method of modulating limb stiffness that improved reaching performance while reducing energy expenditure. However, that initial study utilized a simplified arm model with no redundant muscles or muscles that span more than one joint. Here our FES arm model was expanded to account for redundant and biarticular muscles and joint 'stiffness' is now more accurately defined as the degree of opposing net (active plus passive) flexion vs extension torques at each joint angle. In this improved method of calculating a person's unique FES control parameters, we first measure net passive torques and the relative active torque producing ability of each muscle at key points distributed throughout the workspace. At each point, these data are used to generate a system of equations for flexion vs

extension torques for each joint. The relative active flexion vs extension torque producing abilities of each muscle are on the right side of these equations, while the desired opposing flexion or extension torques minus any passive torques are on the left side. Redundant muscles are listed as separate terms in the same equation and biarticular muscles have their relative torque producing abilities included in multiple equations for the different affected joint angles. Solving this system of equations determines scale factors for each muscle that will produce the activation levels needed to exactly balance the desired degree of opposing flexion/extension torques (i.e. desired stiffness) while stabilizing the limb in the desired position. Although there are multiple ways to solve this system of equations, we use a right pseudoinverse function because it most efficiently distributes the work across redundant muscles to minimize fatigue. Additionally, we can further reduce the impact of fatigue by artificially manipulating the balance of relative muscle torques within the system of equations to account for differences in the fatigue rates of different muscles. Through simulations we show how this skewing process can produce efficient FES control systems that are more robust to fatigue over time. This work provides a novel, clinically-feasible process for optimizing FES parameters for simultaneous control of limb movement and stiffness for short and extended periods of time thereby bringing restoration of arm function after paralysis closer to clinical practice.

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Poster

218. Neuroprosthetics: Decoding of Neural Activity and Neuromuscular Stimulation

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Topic: E.05. Brain-Machine Interface

Support: DoD CDMRP Award SC180308
VAMR 5I01RX002654

Title: Motor recruitment properties of multi-contact composite flat interface nerve electrodes (C-FINEs) in the human upper extremity

Authors: *B. ALEXANDER[1], W. MEMBERG[1,3], R. ADAMS[4], J. MILLER[4,2,3], B. AJIBOYE[1,3,2], R. F. KIRSCH[1,3,2];
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Abstract: Functional electrical stimulation (FES) can activate paralyzed muscles in order to create functional movements. FES can be combined with neural, mechanical, or other methods of control in order to restore function. Composite flat interface nerve electrodes (C-FINEs) have been evaluated for the purpose of restoring functional control in the human lower extremity and in animal models. In this study, we used 16-contact C-FINEs implanted on 6 nerves innervating

the right upper extremity of a tetraplegic human participant to acquire pulse width (PW) modulated recruitment curves at multiple amplitudes. We used these curves to characterize the recruitment properties of the cuffs and aid in the creation of functional stimulation patterns. Muscle activation was quantified as the rectified and integrated, intramuscularly measured, electromyographic (EMG) response in the first 40ms after a twitch activation of given PW and pulse amplitude (PA). A novel sampling algorithm based on the assumption of a Gompertz curve fit of the form $M(PW)=a*e^{-b*e^{\wedge(-c*PW)}}$ was used to minimize the number of points required for each curve. We calculated maximum selectivity values for each muscle as the percent activation of that muscle compared to its measured maximum before any other muscle reached a 20% activation threshold. We found that it was possible to selectively activate 24/25 of the muscles recorded, though individual maximum selectivity values varied from 22.6% to 95.3%. We were able to successfully create functional stimulation patterns for arm and hand movements based on the acquired recruitment information. We also found cuff-wide trends in activation thresholds that indicate the spatial positioning of each nerve within its respective cuff. The marked variability in activation threshold values across the contacts of each cuff suggest that future characterization work should employ a sampling algorithm capable of intelligently varying PA and PW in order to ensure regions of low change on both axes are not overly sampled and areas of high change are sampled sufficiently.

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Poster

218. Neuroprosthetics: Decoding of Neural Activity and Neuromuscular Stimulation

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Title: Distinct neural modes are associated with static and dynamic grasping force

Authors: G. H. BLUMENTHAL[1,2,5], B. M. DEKLEVA[1,2,5], A. P. BATISTA[3,5], M. L. BONINGER[1,2,3], S. M. CHASE[6,5,7], R. A. GAUNT[1,2,5,3], J. A. GONZÁLEZ-MARTÍNEZ[4], B. M. YU[6,5,7,8], J. L. COLLINGER[1,2,5,3,7];
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Abstract: Humans can perform complex and diverse hand movements to manipulate objects that require a range of force application, from static forces such as when holding a suitcase, to dynamic forces such as when painting a picture. Understanding the role of the motor cortex (M1)

in the control of grasp force is particularly important to develop a brain-computer interface (BCI) that can restore dexterous hand function. However, many upper-limb BCIs rely on velocity information as opposed to force information, in part because cortical control of grasp force is not well understood, and complex hand actions are difficult to study in animal models. Two human participants with cervical spinal cord injury and intracortical microelectrode implants were instructed to attempt motor actions while observing a virtual hand performing grasps. M1 activity was recorded as participants observed trials that varied in target force and rate of force application. The participants attempted to match grasp forces that varied between forces required to hold a tomato and a can of soup, and rate of force application which varied between 1.5 and 6 seconds to reach the target force. Using dimensionality reduction techniques, we found low dimensional neural activity patterns that were either independent of or dependent on target force production. Specifically, independent modes appear to signal timing information about the task such as grasp onset, adjustment, and release. Separable, force-dependent neural modes were observed during periods of dynamic force adjustment and static holding, during which force conditions could be classified with a high degree of accuracy. Previous work has shown that some of these force-dependent components become less prominent when combined with more complex motor behaviors. We plan to evaluate the extent by which these components are impacted by task-relevant and -irrelevant contexts including motor actions and somatosensory feedback. These results demonstrate that there are temporally separable components that convey information about both motor output and task state even during attempted motor action, expanding our understanding of how M1 encodes grasp force. The findings from this study may inform future BCI applications which aim to improve dynamic force control during grasping, especially when combined with additional motor complexity such as arm translation.

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Poster

219. Rhythmic Motor Pattern Generation: Cellular Properties

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: CIHR 14392
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Title: Identifying the brainstem areas activated during mastication in mice

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Abstract: The core of the masticatory CPG lies between the rostral poles of the trigeminal (NVmt) and the facial (NVII) motor nuclei. This area includes some trigeminal primary afferents, premotor interneurons of the peritrigeminal area (PeriV), the trigeminal main sensory nucleus (NVsnpr) and the rostral pole of the spinal nucleus (NVspo). We have shown previously that lowering extracellular Ca^{2+} triggers rhythmic firing in neurons of the dorsal part of the NVsnpr by enhancing a persistent sodium current (I_{NaP}). Astrocytes actively participate by releasing the Ca^{2+}-binding protein S100β, which can be simulated by local applications of BAPTA (a calcium chelator). Induction of rhythmic activity in NVsnpr by local applications of BAPTA produces rhythmic activation of motor neurons (MNs) innervating the jaw muscles. However, we do not know if this activation is transmitted directly to trigeminal MNs or if it is relayed through PeriV neurons. Furthermore, we do not know whether other populations of neurons contribute to the genesis of the masticatory rhythm and project to different populations of MNs. To answer these questions, we performed Ca^{2+} imaging in an in vitro slice preparation maintaining all these areas to analyze the responses of neurons and astrocytes of PeriV to electrical stimulation of sensory afferents or activation of NVsnpr by local applications of BAPTA. As expected, electrical stimulation produced Ca^{2+}-responses in astrocytes or neurons (n=10 cells in 7 slices, latency = 4 ± 4s) of the NVsnpr. In PeriV, electric stimulation elicited, tonic (n= 10 cells in 4 slices) or rhythmic (n=10 cells in 4 slices) activation of cells of the parvocellular reticular formation (PCRt) (n=7 cells in 4 slices, latency = 9 ± 4s) as well as in the inter- (IntV, n=5 cells in 3 slices latency = 27 ± 4s) and supra-trigeminal (SupV, n=8 cells in 3 slices, latency = 43 ± 8s) regions. As for local applications of BAPTA in the NVsnpr, we first observed Ca^{2+}-responses in NVsnpr cells (n=8 cells in 9 slices, latency =7 ± 4s), followed by activation of PeriV cells in the following sequence: PCRt (n=19 cells in 9 slices, latency = 27 ± 5s) and IntV (n=4 cells in 8 slices, latency=28 ± 15s), SupV (n=28 cells in 14 slices, latency = 30 ± 6s) and Juxtatrigeminal region (n=4 cells in 4 slices, latency = 38 ± 14s). Finally, lowering extracellular Ca^{2+} induced rhythmic firing in only NVspo, outside of NVsnpr suggesting that it may also contribute to the rhythm generating circuitry. Together, these results suggest the involvement of the PeriV in rhythm transmission as well as a better understanding of the regions forming the CPG of mastication.

Disclosures: **O. Sanvi:** None. **D. Falardeau:** None. **D. Verdier:** None. **A. Kolta:** None.

Poster

219. Rhythmic Motor Pattern Generation: Cellular Properties

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 219.02

Topic: E.07. Rhythmic Motor Pattern Generation

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Title: Effects of leuprolide on the gait pattern in a tubulinopathy model: the taiep rat.

Authors: *J. AHUMADA JUÁREZ[1], V. PIAZZA[2], H. HERNÁNDEZ[3], J. R. EGUIBAR, Sr.[4], C. CORTES[5];
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Abstract: Tubulinopathies refer to a wide group of diseases affecting the central nervous system (CNS) with a mutation in the different alpha tubulins. Particularly, mutations in the tubulin β 4A (TUBB4A) gene cause in humans the leukodystrophy named hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC). Importantly, one of the most important signs of disability is an alteration on the locomotor pattern and ataxia. *Taiep* rats obtained at Benemérmita Universidad Autónoma de Puebla, and they had a motor syndrome whit tremor, ataxia, immobility episodes, epilepsy and paralysis. *Taiep* rat is the first model of tubulinopathy of human H-ABC whit a point mutation TUBB4A and similar MRI images. The aim of this study was to evaluate the effects of leuprolide acetate, an agonist of luteinizing hormone releasing hormone (LHRH), on gait parameters in adult *taiep* rats. We used twelve male *taiep* rats divided into three groups each with four subjects. Control group receive an intramuscular injection of physiological saline solution a second phase with the 1.2 µg/Kg or 2.4 µg/Kg doses of leuprolide acetate (Cryopharma, México). The stepping pattern analyzed using Catwalk™ system (Noldus Technologies, The Netherlands), and we measured the support base, the speed, the stride length, the duration of support, the swing and stand phase durations, as well the stepping sequence. The administration of leuprolide acetate with 1.2 µg/Kg improved the coordination among fore- and hind- limbs. ($P<0.05$) and the 2.4 µg/Kg dose significantly reduced the forelimb base of support ($P<0.05$). The other gait parameters did not change with the administration of this gonadotropin agonist. In conclusion, leuprolide acetate improved gait coordination and decreased the support phase in male *taiep* rats. These results are relevant because leuprolide will be a molecule to design future treatments for leukodystrophies and other central myelin alterations.Partially supported by PRONACES-CONACYT grant 194171 and by VIEP-BUAP 2021-2022 to CA in Neuroendocrinología (BUAP-CA-288). JCAJ student PhD on Physiological Sciences fellowship from CONACYT No. 885959

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Poster

219. Rhythmic Motor Pattern Generation: Cellular Properties

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Title: Spinal interneurons show progressive dysregulation in the SOD1^{G93A} ALS mouse model

Authors: *R. MONTAÑANA-ROSELL[1], R. SELVAN[1,2], P. HERNÁNDEZ-VARAS[3], D. B. AHLMARK[1], J. M. KAMINSKI[2], O. KIEHN[1,4], I. ALLODI[1];
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is characterized by the progressive degeneration of motor neurons leading to premature death. Due to their central role in the disease, research has typically focused on motor neuron cell autonomous events as a cause for degeneration. However, our previous findings showed early dysregulation and loss of connectivity of V1 inhibitory interneurons onto vulnerable motor neurons, indicating a potential role in disease initiation. Based on specific marker co-expression, V1 interneurons have been described as a highly heterogeneous population presenting up to 50 different clades. In the present study, we have conducted a comprehensive expression analysis of several interneuron markers in the SOD1^{G93A} mouse model utilizing different *in situ* multiplexing techniques. Our aim is to investigate the contribution of specific excitatory and inhibitory interneuron subpopulations to ALS progression. By *in situ* sequencing, we first validated expression of our chosen 24 interneuron markers, many of them typically described in the embryo and thought to be downregulated at later stages, in adult mouse tissue. We then analyzed transcript expression in our SOD1^{G93A} mouse model at three different timepoints: pre-symptomatic (postnatal day 30), at onset of locomotor phenotype (63), and upon motor neuron degeneration (112). For this purpose, we made use of RNAscope HiPlexUp, a new *in situ* hybridization quantitative technique which allows for co-detection of multiple transcripts on the same tissue. Our data confirm the early downregulation of the V1 specific Engrailed-1 transcription factor (from day 63) as well as the V1 cardinal clade-defining markers Foxp2, Pou6f2 and Sp8, with no preferential loss observed among these. Of note is the early and drastic dysregulation of V1 Calbindin 1 positive neurons in the ventral area, where Renshaw cells are located. The Calbinidin 2 marker, present in V1 Ia inhibitory interneurons among others, also shows early downregulation although to a lesser extent. Additionally, we report substantial downregulation of the Chx10 transcription factor, marker for V2a excitatory interneurons, at later stages of disease (from day 112). Interestingly, neurotransmitter expression is only affected at late stages and for specific inhibitory neurotransmitters, thus appearing after interneurons have already lost expression of their defining markers. All in all, our study denotes

the pronounced and progressive dysregulation of spinal interneurons throughout ALS pathology and points to several potential sources for non-autonomous motor neuron degeneration.

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Poster

219. Rhythmic Motor Pattern Generation: Cellular Properties

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: R35-NS097343
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Title: Robustness of reciprocally inhibitory circuits to perturbation and neuromodulation

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Abstract: An essential aspect of a healthy nervous system is its ability to produce reliable outputs while remaining plastic enough to respond to changes in the environment. Small reciprocally inhibitory circuits provide an excellent platform for studying the features that contribute to circuit robustness due to their abundance in the nervous system and a well-defined output. We used the dynamic clamp, a real-time neural-computer interface, to build reciprocally inhibitory circuits from the motor neurons of the crab stomatogastric ganglion by connecting them via artificial inhibitory synapses and adding a hyperpolarization-activated inward (H) current. We induced the two fundamental mechanisms of generation of antiphase oscillations in these circuits, “escape” and “release”. In the escape mode, the inhibited cell depolarizes above its synaptic threshold, terminating the firing of the active cell. In release, the presynaptic cell falls below its synaptic threshold, releasing the inhibited cell. We characterized the behavior and robustness of escape and release circuits to alterations in circuit parameters, such synaptic and H maximal conductances, temperature, and neuromodulation. We studied the effect of serotonin, proctolin, oxotremorine, and a simulated modulatory inward current (I_{MI}) on the circuit output. Neurotransmitters and hormones had little effect on escape circuits, but significantly changed the cycle frequency and oscillation amplitude of release circuits. We also showed that I_{MI} can restore oscillations in release circuits at high temperatures. Thus, the same perturbation can have dramatically different effects depending on the circuits’ mechanism of operation that may not be visible when looking at baseline circuit output. This study has implications for understanding individual variability in circuit responses to various stressors and modulators. To study how well the results obtained with the dynamic clamp experiments generalize to the circuits comprised of different neurons, we modeled large families of reciprocally inhibitory circuits with different underlying conductances and systematically applied perturbations. We then used nonlinear

embedding technique (t-SNE) to cluster circuit activity patterns to find parameter sets leading to a robust behavior in response to perturbations. We also explored how asymmetry in the intrinsic properties of the neurons comprising the circuits in escape and release affects their robustness to perturbations.

Disclosures: E. Morozova: None. **P. Newstein:** None. **E. Marder:** None.

Poster

219. Rhythmic Motor Pattern Generation: Cellular Properties

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Topic: E.07. Rhythmic Motor Pattern Generation

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Title: Activity and energy: The effect of K-ATP channel activity on network output of the pyloric circuit in the Cancer borealis stomatogastric ganglion

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Abstract: Prolonged changes in neuronal activity are triggers for homeostatic processes involved in maintaining activity levels around arbitrary 'set points'. ATP is intimately involved in maintaining a neuron's internal ionic environment and membrane hyperpolarization, especially in times of heightened activity. Fluctuations in levels of available ATP might serve as a readout of neuronal activity to balance its demand and supply. ATP-sensitive K^+ channels have been implicated in regulating neuronal activity in response to changing glucose levels and hypoxia. Increased levels of ADP cause channel opening and strong membrane hyperpolarization and high levels of ATP cause depolarizations. We explore the role of K-ATP channels in regulating activity of the pyloric circuit of the crab stomatogastric ganglion (STG). The continuous rhythmic output of the network is maintained *in vitro* and has been studied for its remarkable homeostatic and adaptive properties to changing environmental conditions but the molecular underpinnings of these adaptations remain uncovered. We used the sulfonylureas tolbutamide and glibenclamide to block channel K-ATP activity channels and diazoxide to activate the channels. We found that pharmacological activation of K-ATP channels can result in a complete cessation of rhythmic activity in the intact circuit. Channels blockers, on the other hand, caused minor increases in oscillation frequency in the intact network. Removal of neuromodulatory inputs to STG neurons (decentralization) results in a dramatic reduction of network activity. Blocking K-ATP channels restored oscillatory activity in decentralized networks. Hence, we report the presence of a potent pathway in STG neurons for the modulation of their activity based on their intracellular ATP levels.

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Poster

219. Rhythmic Motor Pattern Generation: Cellular Properties

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Topic: E.07. Rhythmic Motor Pattern Generation

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Title: Differential postnatal development of excitatory and inhibitory synaptic inputs to jaw-closing and jaw-opening motoneurons

Authors: *T. INOUE, T. NOGUCHI, R. KAJIWARA, K. NAKAYAMA, A. MOCHIZUKI, M. DANTSUJI, S. NAKAMURA;
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Abstract: Jaw-closing and jaw-opening motoneurons receive glutamatergic, GABAergic, and glycinergic synaptic inputs, which essential for execution of orofacial functions including mastication, suckling, and swallowing. The nature of synaptic inputs to jaw-closing and jaw-opening motoneurons undergo developmental changes during the early postnatal period, during which feeding behavior transition from suckling to chewing. However, details related to the postnatal developmental of synaptic inputs to these neurons are not clear. Thus, we examined the developmental changes of both excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) in jaw-closing (masseter) and jaw-opening (digastric) motoneurons from postnatal day (P) 2-17. We divided the postnatal period to three time windows; P2-5, 9-12, and 14-17, which correspond to the suckling period before and after tooth eruption, and immature chewing period, respectively. Among the three time windows, the properties of miniature EPSCs (mEPSCs) through non-NMDA receptors was not altered in jaw-closing motoneurons during postnatal development, whereas NMDA receptor-mediated mEPSCs became less frequent in jaw-closing motoneurons during P9-12 and P14-17 compared with P2-5. Furthermore, the proportion of NMDA/non-NMDA EPSCs evoked by stimulation of the supratrigeminal region in jaw-closing motoneurons decreased in in P9-12 and P14-17 compared with P2-5. In jaw-opening motoneurons, both non-NMDA and NMDA EPSCs remained unchanged during the examined postnatal period. In contrast to the excitatory synaptic inputs, amplitude and frequency of glycinergic mIPSCs in both jaw-closing and jaw-opening motoneurons increased during the postnatal period, although mIPSCs in jaw-closing motoneurons were much more frequent than in jaw-opening motoneurons. GABAergic mIPSCs became less frequent in jaw-closing motoneurons during P9-12 and P14-17 compared with P2-5, whereas mIPSCs frequency in jaw-opening motoneurons remained unchanged. These differential developmental natures of glutamatergic, glycinergic, and GABAergic synaptic inputs to jaw-closing and jaw-opening

motoneurons may underlie the transition of suckling to mastication and maturation of other jaw motor function.

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Poster

219. Rhythmic Motor Pattern Generation: Cellular Properties

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Topic: E.07. Rhythmic Motor Pattern Generation

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Title: Dynamics of a high spike-frequency bursting in a Central Pattern Generator

Authors: ***G. CYMBALYUK**[1], M. FOMENKO[2], Y. SHAMS[2], P. J. ELLINGSON[1], J. PARKER[2], R. J. ERAZO TOSCANO[2], R. L. CALABRESE[3];
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Abstract: Life-supporting rhythmic motor functions like heartbeating in invertebrates and breathing in vertebrates require an indefatigable generation of a robust rhythm by oscillatory circuits, Central Pattern Generators (CPGs). CPGs are adjusted by neuromodulation to meet environmental challenges. Leech heartbeating is controlled by a CPG based on two pairs of mutually inhibiting interneurons (HNs) forming half-center oscillators (HN-HCO). Neuromodulation navigates HN-HCOs between dysfunctional regimes by reducing Na^+/K^+ pump current, I_{pump}, and increasing h- current, I_h, in a coordinated fashion, while naïve manipulation of I_{pump} or I_h leads to either seizure-like or asymmetric HCO bursting. Here, we report that this comodulation leads the HCO model through a domain where two functional bursting patterns coexist; and HCO can be switched between them by a current pulse. While cycle periods of the patterns are roughly the same, one of them has two-times higher spike frequency. Continuous operation of CPGs requires intracellular Na^+ concentration ($[Na^+]_i$) to remain in a functional range by having checks and balances on the Na^+ fluxes on the cycle-to-cycle basis of bursting. We suggest that a high spike-frequency bursting regime requires dynamic interaction of I_{pump} and persistent Na^+ current, I_P. I_P is a low voltage-activated inward current that initiates and supports the bursting phase. It does not inactivate and is the major source of Na^+ influx. I_{pump} is an outward current activated by $[Na^+]_i$ and is the main source of Na^+ efflux. Both currents are active and counteract each other between and during bursts. We apply a combination of electrophysiology, computational modeling, and dynamic clamp to investigate the role of I_{pump} and I_P in HN neurons. By introducing with dynamic clamp additional I_{pump} and I_P into the dynamics of a living synaptically isolated HN neuron, we show that their joint increase produces transition into a new high spike frequency bursting regime with a larger amplitude of the membrane potential oscillations. Further increase of I_{pump} speeds up the HN rhythm by

shortening burst duration and interburst interval.
$[Na^+]_i$, which reflects the intensity of the spiking during bursts, endows the Na^+/K^+ pump current with a pivotal role in sculpting bursting patterns. We show that the dynamic interaction of I_{pump} and I_P offers a mechanism for the robust generation and flexible control of the functional bursting patterns. We suggest that this new regime provides effective control over rhythmic motor programs with faster output to the rest of the CPG network.

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Poster

219. Rhythmic Motor Pattern Generation: Cellular Properties

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSF, DBI-1707312

Title: Toward Development of Three Photon Imaging of Mouse Spinal Cord CPG Neurons During Locomotion

Authors: *K. LETT[1], R. ZIRKEL[2], M. ISAACSON[2], M. V. GUZMAN[4], C. B. SCHAFFER[3]; [1]Neurobio. and Behavior, Cornell Univerisity, Ithaca, NY; [3]Biomed. Engin., [2]Cornell Univ., Ithaca, NY; [4]Florida Intl. Univ., Miami, FL

Abstract: Much work has been done to dissect the function of spinal central pattern generator neuron subtypes. Electrophysiology and calcium imaging has enabled researchers to explore intrinsic properties of identified spinal neurons *in vitro* under fictive locomotor conditions. To determine the natural activity patterns of spinal CPG neurons during locomotor behavior in intact animals new optical and behavior tools will have to be developed. *In vivo* spinal cord imaging in mice has so far been limited to the first few laminae, which is populated primarily by sensory neurons. We refined our previously established system for awake spinal cord imaging to record voluntary locomotor behavior in addition to calcium transients in identified CPG neurons of awake mice fixed by a spinal chamber under the microscope and running on a treadmill. This includes the use of 3PEF (3-photon excited fluorescence microscopy) with adaptive optics for deeper, higher resolution imaging into the spinal cord than can be achieved with standard 2-photon imaging. A new custom-built circular treadmill facilitates continuous voluntary locomotion for awake imaging with the spinal cord fixed under the microscope objective. Similar treadmills placed in the homecage before training, enables animals to familiarize themselves with treadmill running increasing the amount of voluntary locomotion during imaging sessions. Additional hands on training time reduced to approximately seven days. This has resulted in more consistent spine fixed running bouts averaging 4Hz compared to 2Hz in free running mice. In addition, we modified our spinal cord chamber design to reduce motion artifact during awake

imaging. We identify good candidates for awake imaging experiments by assessing window clarity, limited motion artifact in the z-dimension, and satisfactory training performance. This is followed by synchronously recording hindlimb kinematics, locomotor speed, and calcium transients in genetically-defined neural sub-populations labeled through viral transfection or transgenic animals, and subsequently analyzing this data through automated pipelines. Our newly developed pipeline will be used to determine correlations between spinal CPG interneuron calcium transients and hindlimb kinematics.

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Poster

219. Rhythmic Motor Pattern Generation: Cellular Properties

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: by NSF grant 2128484 (David Schulz, PI)

Title: Motor neurons use cell-type specific feedback mechanism to constrain relationships among ion channel mRNAs in the stomatogastric ganglion (STG) of Cancer borealis (Cb)

Authors: *J. A. VITERI[1], D. J. SCHULZ[2];
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Abstract: Activity dependent feedback has been proposed as the mechanism coordinating most ion channel mRNA relationships in motor neurons. However, in the absence of any obvious activity dependent feedback, how can neurons that are usually silent, coordinate the ion channel mRNA profile necessary to recapitulate and maintain reliable outputs? To resolve this, we identified two neuron populations from the Stomatogastric ganglion (STG) of *Cancer borealis (Cb)*: the transiently-on lateral gastric neuron (LG) and the continuously bursting pyloric dilator neuron (PD). While PD continuously burst, we stimulated the gastric mill rhythm (GMR) of the STG *in vitro* and recapitulated LG's active state. We then quantified the mRNA abundances and pairwise relationships of 11 voltage gated ion channels for both neuron types. We report that the active state of LG induced up-regulation of 7/11 mRNAs and caused 25/55 mRNA relationships to become more positively correlated (all p-values < 0.05; all r values > 0.6). PD did not exhibit any changes in ion channel mRNA abundances and only 5/55 mRNA relationships became more positively correlated. This suggests that LG's mRNA profile may be more sensitive to the neuromodulatory release from GMR stimulation than PD is.We then proceeded to determine whether neuromodulation or activity was responsible for inducing the 25/55 positively correlated LG mRNA relationships we observed, by collecting 2 additional experimental LG groups which were either under the influence of physiological activity or neuromodulatory influence.The

slopes of 10/25 of these relationships were not significantly different between the LG stimulated and the LG activity-only groups (ANCOVA P>0.05) and were classified as activity dependent. The slopes of 7/55 relationships were not significantly different between the LG stimulated and the LG neuromodulation-only groups (ANCOVA P>0.05) and were classified as neuromodulatory dependent. Thus, mRNA relationships that were induced in the active state of LG are coordinated by a combination of feedback signal. Lastly, all the mRNA relationships we observed in LG were also present in PD but were constrained by different feedback mechanisms. This suggests that motor networks favor multiple modes of feedback to coordinate the mRNA relationships of distinct motor neuron populations.

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Poster

219. Rhythmic Motor Pattern Generation: Cellular Properties

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Title: Developmental Expression of Potential Rhythmogenic Ionic Currents and Channels in Spinal Shox2 Interneurons

Authors: *S. SINGH[1], K. J. DOUGHERTY[2];
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Abstract: Locomotion enables the movement of an organism from one place to another. Rhythmicity is a central feature of locomotion throughout life, despite developmental shifts in neural connectivity properties that occur within the maturing central nervous system. Rhythmic firing in the central nervous system is thought to be generated through a balance of network and intrinsic cellular properties. Within vertebrate locomotor circuitry, these properties allow rhythmogenic spinal interneurons (INs) to covert tonic supraspinal descending input into rhythmic output that determines the timing of motor activity. The extent to which the balance between network and intrinsic properties changes to maintain locomotor-related rhythmicity in the maturing spinal cord has not been explored. Prior work from the lab shows that gap junctional coupling between Shox2 INs strongly contributes to rhythmicity during fictive locomotion in neonatal mice but declines with age and is undetectable by adulthood. However, Shox2 INs in the adult spinal slice, where network properties are limited, can be rhythmically active. We therefore hypothesize that the strength of putatively rhythmogenic ionic currents and the expression of voltage-gated ion channels underlying them increase in rhythm generating INs across development. Here, whole cell patch clamp recordings, immunohistochemistry, and

RNAscope targeting lumbar Shox2 INs were performed to study the properties of putatively rhythmogenic ionic currents and the expression levels of channels mediating them across postnatal time points. We show that subsets of Shox2 INs possess currents which shape firing properties and may underlie bursting such as T-type calcium, H, and A-type potassium currents. Additionally, our data show that a vast majority of Shox2 INs possess persistent inward currents (PICs) and low threshold non-inactivating potassium currents, which are conductances that may set the timing of burst initiation and termination, respectively, during ongoing rhythmic motor behavior. While these currents were seen with high prevalence in all age groups examined, they increased in amplitude with age. Shox2 INs indeed possess RNA transcripts for $Na_V1.6$, a voltage-gated ion channel implicated in PICs. $K_V7.2$ & $K_V7.3$ voltage-gated ion channels, which mediate M-type potassium current, were expressed at high rates in Shox2 INs overall and showed a marked increase by adulthood. This suggests a developmental shift in rhythmogenic ionic currents and their respective ion channels. These findings will inform future studies to reveal changes in mechanisms of locomotor rhythmogenesis from birth through adulthood.

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Poster

219. Rhythmic Motor Pattern Generation: Cellular Properties

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Title: Synchronous multi-segmental activity controls the speed of axial locomotion by modulating the interval between peristaltic waves in Drosophila larvae

Authors: *Y. LIU[1,2], A. NOSE[1,2], M. ZWART[3], H. KOHSAKA[1,4];
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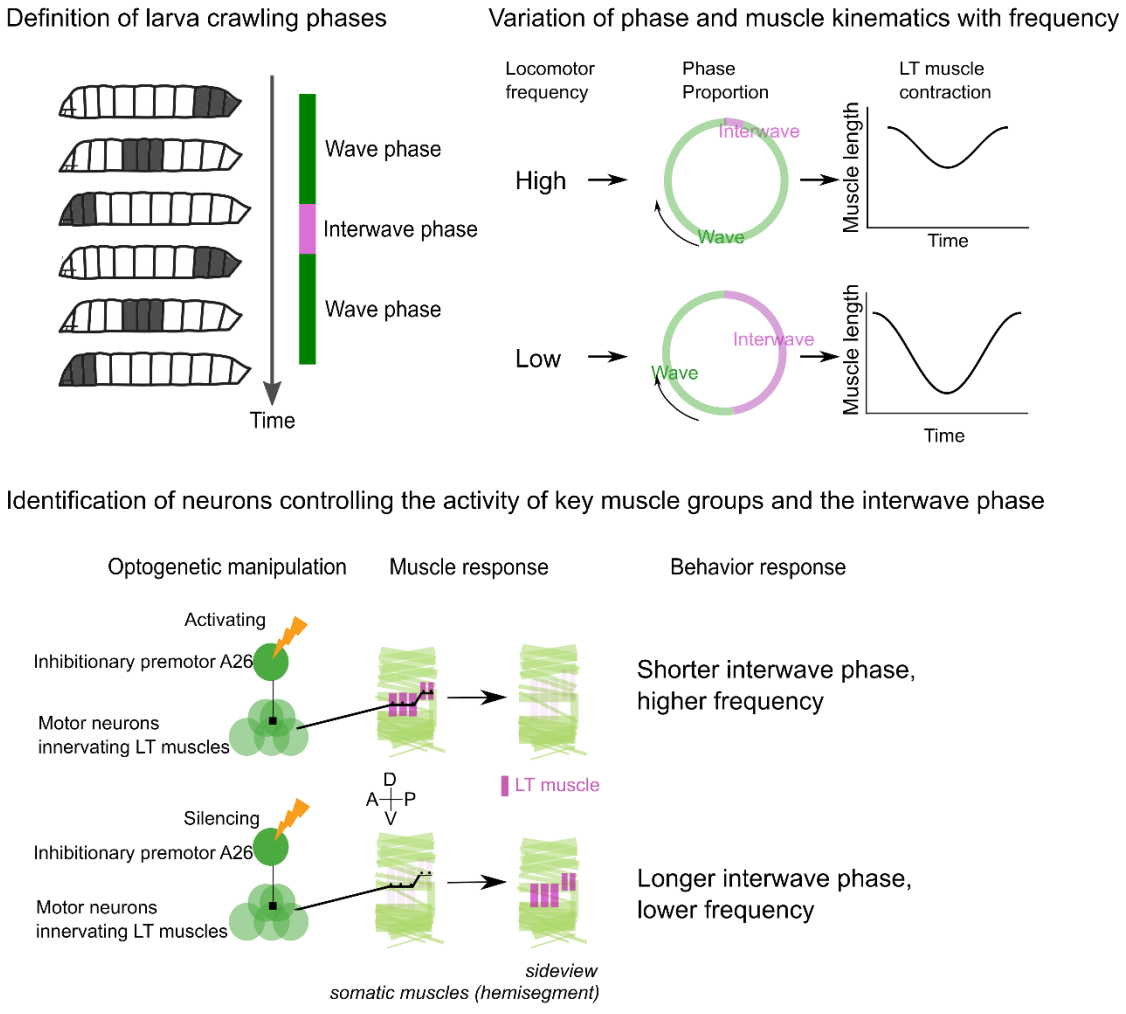
Abstract: The ability to adjust the speed of locomotion is essential for survival. In limbed animals, the frequency of locomotion is modulated primarily by changing the duration of the stance phase. The underlying neural mechanisms of this selective modulation are poorly understood. Here, we report a neural circuit controlling a similarly selective adjustment of

locomotion frequency in Drosophila larvae. Drosophila larvae crawl using peristaltic waves of muscle contractions. We find that larvae adjust the frequency of locomotion mostly by varying the time between consecutive contraction waves, reminiscent of limbed locomotion. A specific set of muscles, the lateral transverse (LT) muscles, co-contract in all segments during this phase, the duration of which sets the duration of the inter-wave phase. We identify two types of GABAergic interneurons in the LT neural network, premotor neuron A26f and its presynaptic partner A31c, which exhibit segmentally synchronized activity and control locomotor frequency by setting the amplitude and duration of LT muscle contractions. Altogether, our results reveal an inhibitory central circuit that sets the frequency of locomotion by controlling the duration of the period in between peristaltic waves. Further analysis of the descending inputs onto this circuit will help understand the higher control of this selective modulation.



Disclosures: **Y. Liu:** None. **A. Nose:** None. **M. Zwart:** None. **H. Kohsaka:** None.

Poster

219. Rhythmic Motor Pattern Generation: Cellular Properties

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 219.12

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant R01 NS047357

Title: The firing properties of Renshaw cells change with postnatal maturation of motor function.

Authors: *E. K. BICHLER, F. J. ALVAREZ;
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Abstract: Renshaw cells (RCs) are known to modulate motoneuron firing through recurrent inhibition. They were first identified *in vivo* because typical burst high frequency firing in response to motor axon inputs. Their unique firing properties were unexplored. Advances in RC genetics now permit visual targeting for whole-cell patch-clamp recording. We used the engrailed-1 (En1) and musculoaponeurotic fibrosarcoma oncogene homolog B (MafB) intersection and localization (ventral most spinal cord) to identify RCs for recording in L1-L5 spinal cord slices of *en1-cre::Ai9tdtomato::mafB-gfp* mice. Neurons were filled during recoding with biocytin to confirm post-hoc calbindin content and axon projections to motor pools. During recording each cell underwent a series of stimulation protocols: 400 ms square pulses to elicit action potential (AP) trains, 1-2 ms pulses to evoke single APs, triangular ramps to study slow activation and inactivating mechanisms and 1 sec long hyperpolarizing pulses to study Ih. Our goal was to describe RC firing properties and whether they undergo postnatal changes in parallel to maturation motor control milestones after birth. We divided RCs in four age groups P0-2, P4-5, P7-10, P13-14. Mature RC firing showed initial bursts of 2-4 spikes riding on a transient depolarization at the beginning of the pulse that manifests when RCs are held more negative than -70 mV. This property was associated with afterdepolarizations after single APs and was sensitive to T-type calcium current blockers. This property was found in 50% of P0-2 RCs but in almost all RCs after P5, thereafter it increased in size. Thus, initial bursting gradually rose with age from below 50 Hz to sometimes exceeding 300 Hz. Steady state (SS) firing also matured with age. APs became narrower due to rise times acceleration during the first postnatal week and advent of faster repolarizations and fast kinetic AHPs at P10. Thus, SS firing increased with age. High firing in mature RCs evoked long post-train AHPs that gave history-dependence to responses at intervals of 1 sec. In mature RCs, SS firing also showed high interspike-interval variability at middle depolarizations that transform into regular fast-spiking with no accommodation at high depolarization. Younger RCs were regular through different current pulse amplitudes and a few neonatal RCs fired single APs. Ih was examined by Sags in hyperpolarizing steps. Ih-Sags were faster kinetics and more frequent in P0-P2 RCs compared to P7-10 and became increasingly difficult to detect with age. In summary, RC firing changes significantly during postnatal development likely contributing to the maturation of spinal networks and motor function.

Disclosures: E.K. Bichler: None. **F.J. Alvarez:** None.

Poster

219. Rhythmic Motor Pattern Generation: Cellular Properties

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 219.13

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Swedish Research Council, Sweden (2017-02905)
Knut and Alice Wallenberg Foundation, Sweden (KAW 2018.0010)
Swedish Brain Foundation, Sweden (FO2021-0317)

Title: Single-cell RNAseq of motoneurons and V2a interneurons reveals molecular signatures of functional spinal circuit modules

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Abstract: Locomotion is an essential motor function and is driven by spinal circuits. Despite a growing understanding of the functional heterogeneity of neuronal populations within the locomotor circuit, a molecular characterization of this diversity is lacking. In mouse, recent studies have shown that the molecular diversity of motoneurons (MNs) reflects their muscle innervation pattern rather than electrophysiological subtypes, while no information is available at present regarding premotor interneurons (INs). In zebrafish, swimming is produced by axial muscles, which are spatially segregated into slow, intermediate and fast fibers each innervated by a separate MN pool with distinct electrophysiological properties. Similarly, the rhythm-generating V2a interneurons are organized into three subtypes that are selectively connected to the MN pools to form three speed circuit modules. These modules are sequentially recruited to increase the speed of swimming. However, it is still unclear if the functional diversity of MNs and V2a INs, and their modular circuit organization, are molecularly encoded. Here we use single-cell RNAseq to uncover the detailed molecular diversity of MNs and V2a INs in adult zebrafish. Cluster analysis revealed molecular subtypes within both the MN and V2a IN populations, which were validated using RNAscope, neuronal tracing and whole-cell patch-clamp recordings during swimming. Our results reveal clusters that correspond to the known functional subtypes of MN and V2a IN (slow, intermediate and fast). Specifically, the slow and fast MNs have distinct gene expression profiles while that of the intermediate MNs displays overlapping gene expression. The rhythm-generating V2a IN population clusters into three subtypes. One cluster of V2a INs expressing *esrrga* display pacemaker firing and are recruited during slow swimming. A second cluster expressing *shox2* comprises neurons displaying tonic firing that are mostly recruited at intermediate swim speeds. A third cluster includes neurons expressing *vachta* which display electrophysiological features of fast V2a INs. Furthermore, we show that the MN and V2a IN subtypes are defined by the expression of specific transcription factors. Finally, our analysis uncovers markers, including transcription factors, that are selectively expressed in MNs and V2a INs belonging to the same speed circuit modules, suggesting a shared molecular identity across CPG classes for neurons that are

electrophysiologically and functionally similar. Thus, our results reveal the molecular signatures of functional neuronal subtypes within and across different populations of the spinal locomotor circuit.

Disclosures: I. Pallucchi: None. **M. Bertuzzi:** None. **A. El Manira:** None.

Poster

219. Rhythmic Motor Pattern Generation: Cellular Properties

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 219.14

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant R01 NS5047357-15

Title: V1 interneurons expressing Foxp2 are a highly heterogeneous clade

Authors: *A. E. WORTHY[1], A. A. WANG[1], J. B. BIKOFF[2], F. J. ALVAREZ[1];
[1]Cell Biol., Emory Univ. Sch. of Med., Atlanta, GA; [2]Developmental Neurobio., St Jude Children's Res. Hosp., Memphis, TN

Abstract: Coordinated motor action depends upon spinal cord circuits that govern motoneuron output to skeletal muscles. The largest group of ventral, ipsilaterally-projecting inhibitory interneurons are V1 interneurons, defined by embryonic expression of the transcription factor (TF) Engrailed-1 (En1). Previously, V1s demonstrated extensive heterogeneity based on combinatorial TF expression, neurogenesis order, location, microcircuit assembly, calcium binding proteins, and firing properties. V1s were grouped into four clades each defined by a TF: MafA (Renshaw cells), Pou6f2, Foxp2, and Sp8. The largest V1 clade expresses Forkhead box protein P2 (Foxp2). Here, we use intersectional genetics and a dual-conditional GFP reporter mouse to lineage-label this group and investigate its diversity. We first used *en1-cre::foxp2-flpo* mice which revealed a larger number of Foxp2-V1s than detected previously with postnatal antibody staining of Foxp2. In the lower lumbar cord, we found 5 groups of Foxp2-V1s divisible by location (lateral or medial), neurogenesis, and postnatal day 5 (P5) expression of the TFs Foxp2, Foxp4, OTP, and Zinc finger homeobox 3 (Zfhx3). Next, we used *en1-flpo::foxp2-IRES-Cre* mice. These mice labeled similar Foxp2-V1s, but additionally revealed a dorsomedial cluster of cells that may transiently express Foxp2 during development. We suggest that differences in Cre- and FlpO-recombination efficiency combined with short-lived target gene expression best explains the slight differences between the models. The largest Foxp2-V1 subgroup (60%) expresses OTP at P5 and is clustered beside the lateral motor column, although OTP and Foxp2 co-expression was also found in some non-V1 interneurons located medially in the spinal cord. Recently, OTP and Foxp2 have been found in long projecting spinal interneurons marked by Zfhx3. We detected Zfhx3 expression in a subset of Foxp2-V1s (20%) and nearly all co-expressed OTP at P5. This suggests that the Foxp2 clade includes long- as well as locally-projecting interneurons. To further analyze OTP-V1s, we used *en1-cre::otp-flpo* mice. While

most OTP-V1s express Foxp2 at P5, we also detected V1s from the Pou6f2 and Renshaw cell clades in this lineage labeling model. This result suggests that OTP is transiently expressed by cells from multiple V1 clades during embryonic development, but expression is only maintained postnatally in lateral Foxp2-V1s. The diversity of Foxp2-V1s implies variations in connectivity and function which are currently under investigation.

Disclosures: **A.E. Worthy:** None. **A.A. Wang:** None. **J.B. Bikoff:** None. **F.J. Alvarez:** None.

Poster

219. Rhythmic Motor Pattern Generation: Cellular Properties

Location: SDCC Halls B-H

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Program #/Poster #: 219.15

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSERC Discovery Grant RGPIN-2022-03898
NSERC PGS-D 569969

Title: A role for a persistent subthreshold K^+ current in generating rhythm within larval zebrafish swimming circuits

Authors: *S. F. GAUDREAU, E. R. KACER, T. V. BUI;
Biol., Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Locomotion is the driving force of survival for most animals. In vertebrates, the ability to locomote is governed by circuits of neurons within the spinal cord. Spinal locomotor circuits have the intrinsic capability of producing the rhythmic activity needed to underlie repetitive locomotor movements. Rhythm generation for locomotion is shaped by connectivity patterns between spinal neurons as well as the specific ionic conductances that these neurons express. Since the emergence of the larval zebrafish as an attractive vertebrate model to the study of locomotion, appreciable progress has been made in uncovering locomotor circuit cell types as well as the connections they form with each other. The ionic basis, however, for locomotor rhythm generation in these circuits remains poorly investigated. A persistent subthreshold potassium current, I_M, is a known regulator of neuronal firing and recent evidence reveals its involvement in locomotor rhythm generation in rodents. We sought out to determine whether I_M is involved in shaping the locomotor rhythm in larval zebrafish which, to our knowledge, has remained uninvestigated. Our *in vivo* electrophysiological recordings of motor nerves during swimming activity of 4-5 days post fertilization larvae in the presence of ICA-069673 and XE-991 - agonist and antagonist, respectively, of the I_M-mediating Kv7.2/7.3 channels - reveal that I_M may limit the amplitude and frequency of tail beats produced during swimming. Furthermore, immunohistochemistry targeting Kv7.2 channels in zebrafish larvae supports motoneuron axon expression of these I_M-mediating ion channels. This suggests a potential last-order role for motoneurons in the regulation of the locomotor rhythm via I_M. Our findings further detail the mechanisms by which locomotor rhythm generation is governed in the larval zebrafish, all the

while highlighting that the ionic conductances at the basis of locomotor rhythm may be conserved across vertebrates.

Disclosures: S.F. Gaudreau: None. **E.R. Kacer:** None. **T.V. Bui:** None.

Poster

219. Rhythmic Motor Pattern Generation: Cellular Properties

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 219.16

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSERC CGS-M
NSERC Discovery Grant RGPIN-2022-03898

Title: Silifish: a software tool for computational modelling of spinal control of larval zebrafish (Danio rerio) and its use to study the spinal regulation of tail-beat frequency

Authors: *E. TOPCU[1], Y. ROUSSEL[2], T. V. BUI[1];
[1]Biol., Univ. of Ottawa, Ottawa, ON, Canada; [2]EPFL, Blue Brain Project, EPFL, Blue Brain Project, Geneva, Switzerland

Abstract: Locomotion is an important capability of animals that allows them to survive. Understanding the spinal control of locomotion is crucial to comprehend how this movement is generated, how impairments in locomotion arise due to injury or disease, and to identify targets for effecting recovery. Evolutionary conservation of many spinal circuits allows us to study simpler organisms to increase our understanding. Given its available transgenic lines and relative simplicity of its neuronal circuits, the zebrafish is an invaluable animal model for studying spinal control of motor movements. Recently our lab has developed a spinal cord computational model written in Python to model the activity patterns of spinal neurons involved in different types of swimming behaviour in larval zebrafish: single-coiling, double-coiling, and beat and glide (Roussel et al. 2021). To facilitate building upon our earlier model, we have developed SiliFish, an open-source software tool written in C# to model different swimming speeds of larval zebrafish (https://github.com/Bui-lab/SiliFish). This tool is based on the previous model generated within our lab with additional capabilities to facilitate modelling more complex behaviours. For example, SiliFish allows defining different spatial distributions for neurons or muscle cells, variable intrinsic properties for each cell or synapse, and probabilistic connections between cells. An easy-to-use graphical user interface allows making changes to a model, visualizing the created model in 3D, and testing the results promptly. The switch in programming language enables a 10-fold increase in performance, thus allowing the simulation of more realistic models with a higher number of cells if desired. We used SiliFish to explore how the spinal circuits of larval zebrafish can regulate various tail beat frequencies during beat-and-glide swimming. Modelling the generation of a wide range of tail beat frequencies required generating several modules composed of spinal interneurons dedicated to specific ranges of tail beat

frequencies. The tail-beat frequency modules were composed of cell populations with intrinsic properties, spatial distributions, and connectivity patterns based on existing literature. We tested how the control of these modules could generate particular patterns of tail beat frequencies. SiliFish will be a useful tool for the community not only to model the swimming speeds of larval zebrafish but also to test existing or to generate new hypotheses about locomotor control.

Disclosures: E. Topcu: None. **Y. Roussel:** None. **T.V. Bui:** None.

Poster

220. Hypothalamic-Pituitary-Gonadal Axis

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 220.01

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: T32 MH019524
U19 Oxytocin Brain Initiative Grant

Title: Oxytocin and lactationally-triggered embryonic diapause in mice

Authors: *J. L. MINDER[1], S. Y. KIM[2], M. V. CHAO[4], R. C. FROEMKE[3];
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Abstract: Embryonic diapause is a state in which the development of the pre-implantation embryo is paused under conditions of maternal duress, such as while nursing a previous litter. Embryonic development is halted at the blastocyst stage and implantation into the uterine endometrium is temporarily prevented, as the required estrogen surge for uterine receptivity is blocked due to an unknown mechanism. Development resumes as normal upon a return to maternal homeostasis (Renfree and Shaw, 2000). We hypothesize that oxytocin coordinates the embryo and uterus to carry out diapause in the context of lactationally-triggered facultative diapause in mice. To test if oxytocin could antagonize estrogen production, we implanted osmotic pumps and found the estrus cycle to be extended in animals with chronic oxytocin release vs. saline ($p=0.03$). This suggests that the development of the corpus luteum is slowed and perhaps less estrogen is released when oxytocin levels are raised. To model low estrogen levels and to test how this loss affects oxytocin neurons, we treated the hypothalamic cell line GT1-7 with the estrogen receptor antagonist 4-hydroxytamoxifen and saw that oxytocin mRNA levels were increased relative to saline treatment ($p=0.01$). This suggests that maternal oxytocin levels in oxytocin neurons are raised in the diapause state. We have documented oxytocin receptor expression in mouse blastocysts using reporter mice and tested the effect of oxytocin directly on mouse blastocysts ex vivo. We found that embryo implantation in the presence of oxytocin is delayed in comparison to saline using an in vitro model of implantation adapted from Bedzhov et al., 2014 (OT n=14, saline n=22). Furthermore, only ten percent of embryos knock-

out for the oxytocin receptor survive through diapause as compared to eighty percent of wild-type embryos when transferred into wild-type surrogate mothers followed by diapause induction. Our findings suggest that if pregnancy occurs coincident with nursing, maternal oxytocin levels rise and may initiate the pausing of embryonic development by preventing uterine receptivity for embryo implantation, while in parallel, also cuing the embryo to pause its development. This enables pregnancy progression between the mother and fetus to remain in sync during the phase of pausing. Understanding the role that the hypothalamic pituitary axis plays in altering the timing of embryo implantation and embryonic development has major implications for reproductive medicine.

Disclosures: **J.L. Minder:** None. **S.Y. Kim:** None. **M.V. Chao:** None. **R.C. Froemke:** None.

Poster

220. Hypothalamic-Pituitary-Gonadal Axis

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 220.02

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: This research was supported by a grant from the American Psychological Foundation's 2021 Division 1 Society for General Psychology Mary Whiton Calkins Grant.

Title: Periadolescent GnRH agonist exposure delays puberty and disrupts estrous cyclicity during treatment but recovers upon treatment termination

Authors: *M. A. KELLY[1], F. A. GUARRACI[1], L. AVENDANO[1], C. ESTOESTRA[1], B. SENCHEREY[1], H. S. VALDIVIA[1], A. GALE[1], L. YEPEZ[1], J. B. BELFIELD[1], K. M. CARTER[2], N. A. WILLIAMS[1], A. C. GORE[3];
[1]Psychology, Southwestern Univ., Georgetown, TX; [2]Biol., Francis Marion Univ., Florence, SC; [3]Univ. of Texas at Austin, Univ. of Texas at Austin, Austin, TX

Abstract: The present study examined the effects of periadolescent GnRH agonist exposure on pubertal onset and first estrous cycle during treatment, as well as sexual motivation within two weeks of treatment termination. We previously found that the GnRH agonist leuprolide acetate (50 µg/kg daily PD 25-50) significantly delayed puberty in female Long-Evans rats, but failed to have any long-term effects on estrous cyclicity or sexual motivation once treatment ended. Given that estrous cyclicity and sexual motivation were measured weeks after treatment ended in that study, we are still unsure if leuprolide administration directly affects estrous cyclicity or sexual behavior. Therefore, we investigated the effects of daily leuprolide treatment on pubertal onset (i.e., vaginal opening (VO)) and the first estrous cycle immediately after VO during the treatment period. In addition, we measured sexual motivation during their first day in behavioral estrus after treatment ended (starting on PD 51) using the partner-preference paradigm. Consistent with previous findings, we found that leuprolide acetate (50 µg/kg) significantly

delayed puberty. In addition, we found that the first estrous cycle was disrupted during the treatment period, as indicated by acyclicity and failure to cycle into estrus after VO. However, sexual motivation was not affected by periadolescent exposure to leuprolide when measured between PD 51-64. Furthermore, we found that leuprolide had no effect on fertility. Taken together with our previous findings, the present results indicate that not long after leuprolide treatment is discontinued, reproductive physiology and behavior return to normal.

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Poster

220. Hypothalamic-Pituitary-Gonadal Axis

Location: SDCC Halls B-H

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Program #/Poster #: 220.03

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant ASCEND

Title: A computational model for the excitability of hypothalamic neuronal circuitry during pubertal transition

Authors: ***P. LEE**[1], M. BANDORA[2], C. SÁENZ DE MIERA PATÍN[3], A. RUPP[3], C. F. ELIAS[4];
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Abstract: Energy sensing by the hypothalamus is crucial for sexual maturation. Excess energy is associated with early puberty, which, in turn, is correlated with a high risk of obesity, diabetes, and comorbidities later in life. The hypothalamic circuitry integrating metabolism and reproduction is poorly described. We have shown that the ventral premammillary nucleus (PMv)has a dense collection of neurons expressing the LepR and innervates sites associated with reproductive neuroendocrine and behavioral function. LepR PMv neurons have a fundamental role in pubertal development.
This neuronal population, however, is not homogeneous. A subset of LepRb PMv neuronsdepolarizes, whereas another hyperpolarizes in response to leptin. Specifically, we havecharacterized PMv neurons expressing dopamine transporters (DAT) suppress femalereproductive function, and this is inhibited by leptin. Through the physiological pubertalmaturation, we have identified that 1) DAT mRNA expression in the PMv is higher in prepubertalmice and DAT mRNA is responsive to factors other than estradiol during pubertal maturation, 2)PMv DAT neurons project densely to Kisspeptin neurons in the anteroventral periventricularnucleus (AVPV), and minimal projection to Kisspeptin neurons in the arcuate nucleus (ARC) orAgRP neurons of adult females. The DAT innervation of AVPV is very small

in prepubertal mice.
We hypothesize that 1) downregulation of DAT and innervation of Kiss1-AVPV in the PMv LepRb-inhibited subpopulation modulates the pubertal maturation and GnRH pulsatility, 2) the difference in DAT mRNA expression between prepubertal and adult mice is a direct signature that PMv-LepR neurons display developmental plasticity associated with the metabolic control of sexual maturation.
We have developed a computational model of conductance-based electrophysiology to supportthese hypotheses. Input signals to PMv and AgRP (agouti-related peptide) neurons by leptin,and the output response of bursting spikes and their relays to Kiss1 neurons in the AVPV andthe ARC, and ultimately to GnRH neurons is assessed. Among PMv neurons, the primary ionchannels are identified by single-cell RNA-seq data considering subpopulations of LepRneurons, i.e., DAT+ and DAT-. The computational neuronal circuit model predicts that the contribution of downregulated DAT and dense projection of PMv DAT neurons to Kiss1-AVPV is necessary for surging GnRH excitability and modulation of GnRH pulsatility, essential for pubertal maturation.

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Poster

220. Hypothalamic-Pituitary-Gonadal Axis

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 220.04

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: USDA NIFA 2018-06471
NIGMS Grant P20GM103434 to WV- INBRE
WVU Microscope Imaging Facility

Title: Evidence that proopiomelanocortin neurons may play a role in ovine puberty

Authors: *E. G. AERTS[1], M. J. GRIESGRABER[1], A. D. SEMAN[1], C. C. NESTOR[3], S. M. HILEMAN[1,2];
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Abstract: The timing of puberty onset is crucial as both delayed and precocious puberty can have negative health outcomes. Puberty relies on an increase in episodic gonadotropin-releasing hormone (GnRH) from the hypothalamus, resulting in a corresponding increase in luteinizing hormone (LH) secretion from the pituitary. Prior to puberty onset, ovarian estradiol (E_2) inhibits GnRH/LH release. This inhibition wanes at some point during development, allowing for the GnRH/LH surge and ovulation. However, how E_2 acts within the hypothalamus to do this is not clear as GnRH neurons do not have ER-α, the receptor for E_2. Neurons within the arcuate (ARC)

of the hypothalamus that contain kisspeptin, neurokinin B, and dynorphin (KNDy neurons) are critical for the generation of GnRH pulses. While our previous data suggests that direct actions of E_2 on KNDy neurons may not be instrumental for puberty onset, proopiomelanocortin (POMC) neurons in the ARC may regulate KNDy neurons as they have been shown to contain ER-α receptors. The goal of this study was to determine if POMC peptide expression, input to KNDY neurons, and neuronal activity change in a puberty-specific manner. Female sheep (n=5-6 per group) were ovariectomized and given subcutaneous E_2 implants at 5 months (prepubertal), 8 months (peripubertal) and 10 months (postpubertal) of age. Two weeks after surgery, blood samples were collected via jugular venipuncture every 12 minutes for 4 hours and assessed for LH via radioimmunoassay. The ewes were then sacrificed and hypothalamic tissue was perfused, collected, and assessed for POMC, kisspeptin, and cfos, a known marker of neuronal activity, using immunohistochemistry. Pulsatile LH secretion was lowest at 5 months and highest at 10 months, with pulse frequencies being intermediate at 8 months. Numbers of ARC POMC cells did not increase significantly over development ($p = 0.45$). However, the number of POMC close-contacts onto ARC kisspeptin neurons increased significantly from 5 months to 10 months of age ($p=0.02$) and the percentage of ARC POMC cells expressing cfos increased significantly from 5 months to 10 months ($p=0.003$) and from 8 months to 10 months ($p=0.034$) of age. These results indicate POMC neurons increasingly contact KNDy neurons with neuroendocrine puberty onset and become more active throughout development in association with increased LH secretion. Therefore, it is possible that puberty onset involves increased activation of ARC POMC neurons which act through KNDy neurons to stimulate GnRH/LH release.

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Poster

220. Hypothalamic-Pituitary-Gonadal Axis

Location: SDCC Halls B-H

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Program #/Poster #: 220.05

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSERC Grant

Title: Low testosterone activity predicts depressive affect in young men

Authors: *C. J. PURKIS[1], J. S. SANKAR[2], E. HAMPSON[2];
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Abstract: The relationship between testosterone (T) and depressive affect in men is poorly understood. This is particularly true in young men. Recent research suggests that inadequate T activity may be associated with increased depressive affect and that genetic variation in the androgen receptor (AR) may contribute to this relationship. This observational study investigated the association between bioavailable T concentration, the polyglutamine (CAG) repeat length

variant in exon 1 of AR, and depressive affect scores in 218 physically healthy young men ages 18-35 with diverse patterns of mood. Participants collected saliva specimens for endocrine monitoring, controlling for time of day, seasonal variation in T production, and medication use. Saliva was analyzed at two collection timepoints using high-sensitivity radioimmunoassays to quantify bioavailable T concentrations and basal cortisol levels. Genotyping using polymerase chain reaction and capillary electrophoresis was used to quantify AR CAG repeat length. All participants completed the Profile of Mood States (POMS), a standardized well-validated psychometric mood inventory with 6 empirically-derived subscales. A principal components analysis of the POMS data with oblique rotation confirmed 7 mood components, including a Negative Affect (NA) component. A median split on mean T level showed that in young men with low T, but not those with average or higher T levels, lower levels of bioavailable T and a longer AR CAG repeat length, indicative of a less effective AR, predicted greater NA scores. Bioavailable T concentrations and AR CAG repeat length failed to predict any other mood components. NA was unrelated to basal cortisol levels. These findings indicate a complex relationship in men between T activity and depressive affect, and suggest that the levels of T biologically available to the nervous system may modulate the severity of depressive affect experienced in young men.

Disclosures: **C.J. Purkis:** None. **J.S. Sankar:** None. **E. Hampson:** None.

Poster

220. Hypothalamic-Pituitary-Gonadal Axis

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 220.06

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: COST CA20121 BenBedPhar

Title: The Relationship of Neuroendocrine Signalling and Sex Differences among Expressed Genes in Mouse Hippocampal Gonadotropin-Releasing Hormone Neurons

Authors: *A. SALIHOGLU;
Dept. of Physiol., Karadeniz Tech. Univ. Fac. of Med., Trabzon, Turkey

Abstract: Gonadotropin-releasing hormone (GnRH) neurons play an important role in the hypothalamic-pituitary-gonadal axis modulation in a sex-dependent manner. However, the cellular mechanism of intersexual differences in GnRH neurons is not fully understood yet. The aim of this study was to detect the sexually dimorphic gene expression profile of GnRH neurons by using *in silico* tools. For testing the hypothesis, GSE66806 dataset downloaded from Gene Expression Omnibus database was re-examined for this research. In the dataset, the transcriptome of hippocampal GnRH neurons obtained from six intact, metestrous female and six male C57BL/6J mice was examined and gene expression profiles of their hippocampi were re-analyzed in the R program, based on Benjamini-Hochberg correction, adjusted p-values<0.05

were accepted as significant. Gene set enrichment analyses were performed in Gene Ontology and ENRICHR tools. Gene expression levels indicated that pro-opiomelanocortin-alpha (POMC), prodynorphin (PDYN), arginine vasopressin (AVP), gastrin (GAST), somatostatin receptors (SSTR1,R3), neuropeptide Y receptors (NPY1R,5R), pancreatic polypeptide (PPY), prolactin (PRL), calcitonin-related polypeptide genes (CALCA-B), natriuretic peptide type C (NPPC), gastrin-releasing peptide receptor (GRPR), endothelin genes (EDN1-3), glucagon (GCG), vasoactive intestinal polypeptide (VIP, VIPR1), growth hormone (GH), growth hormone-releasing hormone (GHRH), gastric inhibitory polypeptide (GIP), corticotropin-releasing hormone receptor-1 (CRHR1), urocortin (UCN), urotensin 2 receptor (UTS2R), tachykinin 1 (TAC1), neuromedin U receptor-1 (NMUR1), kininogen-1 (KNG1), ghrelin (GHRL), galanin receptor-1 (GALR1), gonadotropin-releasing hormone receptor (GNRHR), parathyroid hormone-like peptide (PTHLH), brain-derived neurotrophic factor (BDNF), prokineticin receptor-1 (PROKR1), prokineticin-2 (PROK2), persephin (PSPN), period circadian clock-2 (PER2), nuclear factor erythroid derived 2-like-2 (NRF2) genes were upregulated ($p<0.05$); and preproenkephalin (PENK), prepronociceptin (PNOC), neurotensin receptor-1 (NTSR1), diazepam binding inhibitor (DBI), chromogranins (CHGA-B), secretogranins (SCG2-3), cerebellin-2 (CBLN2), period circadian clock-1 (PER1), cryptochrome-2 (photolyase-like) (CRY2), KISS1 receptor (KISS1R) genes were downregulated ($p<0.05$) in the female group, compared with the male group. Results from this study indicate imbalances in expression levels (up- and down-regulation) of genes known to be involved in many neuroendocrine signaling caused by sexual dimorphism in hippocampal GnRH neurons.

Disclosures: A. Salihoglu: None.

Poster

220. Hypothalamic-Pituitary-Gonadal Axis

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 220.07

Topic: F.02. Neuroendrocrine Processes and Behavior

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CIHR 02003PJT-437197 to MDW/MMH
NSERC Alexander Graham Bell Scholarship to MFM
Ontario Graduate Scholarship to MFM

Title: Gonadal axis activation in a socially-suppressed mammal

Authors: *M. FAYKOO-MARTINEZ[1,2], D. SOKOLOWSKI[1,2], K. YUKI[2], H. HOU[2], C. CHAN[1,2], T. KETELA[3], M. PALMERT[2,1], T. PUGH[3,1], M. WILSON[2,1], M. M. HOLMES[4]; [1]Univ. of Toronto, Toronto, ON, Canada; [2]Hosp. for Sick Children, Toronto, ON, Canada;

[3]Princess Margaret Cancer Ctr., Toronto, ON, Canada; [4]Psychology, Univ. of Toronto Mississauga, Mississauga, ON, Canada

Abstract: Social stress inhibits puberty (i.e., sexual maturation) via suppression of the hypothalamic-pituitary-gonadal axis. Naked mole-rats (NMR) are long-lived rodents residing in large colonies and remain pre-pubertal regardless of age due to the presence of a single breeding pair. Puberty can begin within one-week when a mature NMR (at least 6 months of age) is removed from suppressive cues of the colony. However, the molecular pathways underlying socially-mediated pubertal suppression are unknown. Here, we compared the hypothalamic-pituitary-gonadal axis transcriptome of female and male NMRs within colony (breeders and subordinates) to animals that were removed from colony for 1 or 4 weeks. Gene expression differences pre-/post-colony removal revealed changes in the hypothalamic-pituitary-gonadal axis as soon as 1-week, with effects more pronounced in females. Notably, *TRH* (thyrotrophin-releasing hormone) was upregulated in the ventral hypothalamus. *TRH* is closely linked to the metabolic control of puberty by stimulating growth-related development. *ESR1* is a key marker of reproductive onset and was upregulated in ovaries. We then removed subordinate females from colony for 1, 4, or 7 days and to compare expression at single-cell resolution to within-colony subordinates and breeders. We identified two large neuronal clusters in the hypothalamus with high expression of genes including *KISS1, CRHBP* (corticotrophin-releasing hormone binding protein) and *TRH*: genes with clear connections to the stress/metabolic regulation of puberty. In the ovary, sex-differentiating genes (e.g., *STAR, AMH*) peaked in expression as early as 4 days post-removal across developing granulosa (cells supporting maturing oocytes). These data reveal puberty-related development in NMRs occurs within the first week of removal from colony, with ovarian transcriptional changes identified as early as 4 days. Endocrine changes in the hypothalamus provide evidence that release from social suppression initiates sex-specific processes for reproduction quite rapidly; these changes occur in the hypothalamus within four days post-removal in both sexes and in the gonads within the first four days in females. Thus, mechanisms suppressing pubertal onset in NMRs include an interaction of reproductive, stress and energy metabolism-related pathways.

Disclosures: M. Faykoo-Martinez: None. **D. Sokolowski:** None. **K. Yuki:** None. **H. Hou:** None. **C. Chan:** None. **T. Ketela:** None. **M. Palmert:** None. **T. Pugh:** None. **M. Wilson:** None. **M.M. Holmes:** None.

Poster

220. Hypothalamic-Pituitary-Gonadal Axis

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 220.08

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant 5R01HD087347

Title: Gonadotrope receptor activation modulates MMP9 and non-muscle myosin II function

Authors: *P. MOHAMMAD ZADEH, G. C. AMBERG;
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Abstract: Gonadotropes are a population of endocrine cells located in the anterior pituitary that are part of the HPG axis and play a key role in the regulation of reproductive function. Gonadotropes respond to the binding of GnRH to its cognate receptor (GnRHR) that stimulates transcriptional pathways which regulate the gonadotropins (LH and FSH) release. Evidence suggests that gonadotrope cells move towards the hypophyseal portal capillary endothelium, possibly to facilitate the release of gonadotropins into the bloodstream. However, mechanisms underlying the integration of GnRHR signaling mechanisms involved with gonadotrope plasticity at the molecular and cellular level are unclear. We used a combination of immunocytochemical (ICC) markers and protein analysis techniques in clonal αT3-1 gonadotropes to investigate signaling components associated with stress fiber formation and cell movement. Our data shows subcellular colocalization of the GnRHR, non-muscle myosin IIB (NMIIB) and matrix metalloprotease (MMP9) with the actin fibers at cell-cell junctions following application of GnRH. Our data also indicate that matrix metalloprotease 9 (MMP9) plays a role in facilitating αT3-1 cell movement. To examine the effects of GnRHR signaling on the expression and release of proMMP9, we examined the effects of GnRH and stress fiber function on pro-MMP9 using a quantitative ELISA kit. Specifically, we measured pro-MMP9 release following exposure of GnRH, cetrorelix acetate (GnRHR antagonist), blebbistatin (a NMII antagonist), and an inhibitor of the small GTPase Rac1. We found that GnRH, blebbistatin, and rac1 inhibition all increased the release of pro-MPP9 while GnRHR antagonism with cetrorelix acetate reduced release. Together, these data suggest a role for NMII, Rac1, and MMP9 in mediating gonadotrope responses to GnRHR activation.

Disclosures: P. Mohammad Zadeh: None. **G.C. Amberg:** None.

Poster

220. Hypothalamic-Pituitary-Gonadal Axis

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 220.09

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: USDA NIFA 2022-67015-36370

Title: Evidence that elevated AgRP signaling during undernutrition inhibits arcuate kisspeptin neurons in female sheep.

Authors: S. L. SHUPING, K. HARLOW, J. R. SOMMER, *C. C. NESTOR;
Animal Sci., North Carolina State Univ., Raleigh, NC

Abstract: Proper energy balance is important to ensure reproductive success. While it is known that nutrient restriction impairs hypothalamic-pituitary function, the central mechanism whereby undernutrition inhibits reproduction remains to be fully elucidated. Given recent evidence in

sheep and mice that the majority of arcuate kisspeptin neurons express melanocortin 3 receptors (MC3R), anorexigenic neurons which produce agoutirelated peptide (AgRP), an endogenous MC3R antagonist, may play an important role in relaying energy status to the reproductive axis. Since kisspeptin neurons are essential for reproduction and undernutrition increases AgRP expression, we hypothesized feed restriction would increase AgRP input to kisspeptin neurons and inhibit kisspeptin expression in MC3Rexpressing neurons in female sheep. We selected tissue containing the arcuate nucleus from ovariectomized ewes that were fed to maintain pre--study body weight (FM; n=4) or feed-restricted to lose 20% of pre-study body weight (FR; n=4). Using left brain hemisections (n=2/animal), we conducted duallabel immunofluorescence with primary antisera for kisspeptin and AgRP. Using right brain hemisections (n=2/animal), we conducted RNAscope for mRNA expression of kisspeptin, MC3R, and MC4R. Immunofluorescence revealed significantly more AgRP close-contacts onto ARC kisspeptin cells in FR ewes (4.94 $\pm$ 0.3 contacts/cell) than in FM ewes (1.82 $\pm$ 0.2 contacts/cell). RNAscope analysis revealed significantly fewer total kisspeptin cells in FR animals (38.8 $\pm$ 18 cells) compared to FM animals (91.4 $\pm$ 21 cells) and significantly fewer total MC3R cells in FM ewes (22.8 $\pm$ 5 cells) compared to FR ewes (86.8 $\pm$ 33 cells), while no difference was seen in total MC4R numbers between groups (FM, 24.5 $\pm$ 86 cells; FR, 28.7 $\pm$ 13 cells). Furthermore, we observed that the integrated density (mRNA per cell) for kisspeptin in kisspeptin/MC3R cells was significantly lower in FR ewes (470760 $\pm$ 144752 AU) compared to FM ewes (1298098 $\pm$ 378459 AU). Together with our previous data in male sheep, we suggest that AgRP neurons inhibit arcuate kisspeptin neurons and play a key role in relaying low energy status to reduce GnRH/LH secretion to ultimately impair reproduction in male and female sheep.

Disclosures: S.L. Shuping: None. **K. Harlow:** None. **J.R. Sommer:** None. **C.C. Nestor:** None.

Poster

220. Hypothalamic-Pituitary-Gonadal Axis

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 220.10

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: RO1ES0322163

Title: Adult phthalate mixture exposure alters the transcriptome in the mPOA of female mice

Authors: *J. MAXON, S. SORIANO, D. MELING, M. LAWS, J. FLAWS, M. MAHONEY; Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: Humans are exposed daily to mixtures of phthalates, which are plasticizers and stabilizers used in industrial products. Exposure occurs through food containers, cosmetics, medical tubing, children's toys, and flooring. Previous research suggests that phthalate exposure causes reproductive defects by disrupting the hypothalamic-pituitary-gonadal axis, which is

regulated in part by the medial preoptic area (mPOA). The sexually dimorphic mPOA regulates hormone secretion and reproductive behaviors and expresses sex steroid receptors. While phthalates' effects on reproductive tissues, behavior, and outcomes have been previously studied, gene expression changes in the hypothalamus due to phthalate exposure is underexplored. Thus we characterized gene-expression changes in the mPOA of female CD-1 mice after exposing them to an environmentally relevant mixture (mix) of phthalates during adulthood. Adult female CD-1 mice were fed control chow or chow dosed with a phthalate mixture for 34 days. The doses were either a medium dose of 1.5 ppm phthalate mix or a high dose of 1500 ppm phthalate mix. At the end of dosing, animals were euthanized, and 1 mm brain punches of the mPOA were collected for RNA-sequencing analysis. RNA-sequencing analysis identified 1938 differentially expressed genes (DEGs) in the medium and high dose groups (false discovery rate FDR < 0.1), respectively, compared to control. Gene Ontology Enrichment analysis depicted DEGs involved in localization, signaling, and response to chemical (medium dose) and translation and apoptotic processes (high dose). Pathway Enrichment analysis depicted DEGs involved in the neuroactive-ligand receptor, mTOR, and nicotine addiction pathways (medium dose). Pathway Enrichment analysis depicted DEGs involved in the glutamatergic, MAPK, calcium signaling, mTOR, and circadian entrainment pathways (high dose). This study identified candidate genes and pathways that are modulated as a consequence of phthalate exposure. The molecular targets of phthalate disruption defined here may point the way towards yet undiscovered behavioral and proteomic consequences of phthalate exposure.

Disclosures: J. Maxon: None. **S. Soriano:** None. **D. Meling:** None. **M. Laws:** None. **J. Flaws:** None. **M. Mahoney:** None.

Poster

221. Effects of Early Life Stress on the Brain and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 221.01

Topic: F.03. Stress and the Brain

Title: The advantages of using automated home-cage monitoring to characterize defensive and social deficits in adult mice exposed to complex trauma early in life.

Authors: *B. POLIS[1], R. ISLAM[1], T. D. PREVOT[2], A. KAFFMAN[1];
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Abstract: Children exposed to early life stress (ELS) usually experience a complex and unpredictable set of adversities, including maltreatment, neglect, poverty, neighborhood violence, and toxic pollution. ELS is a major risk factor for abnormal brain development and higher rates of multiple psychiatric and medical conditions later in life. Current rodent models of ELS rely on a single predictable adversity that is typically applied during the first two weeks of life, the equivalent of the third trimester to two years of age in human development. Such models

fail to reflect the complexity, unpredictability, and developmental time-span that characterize ELS in the clinical setting. Further, most behavioral tests used to assess anxiety-like outcomes in adult mice exposed to ELS (e.g., open field test, elevated plus maze, dark-light test) are notoriously difficult to replicate because they are brief, sensitive to environmental factors that are difficult to control, and usually conducted during the light phase when mice are less active. To address these concerns, we developed a mouse model of multiple and unpredictable adversities we named unpredictable postnatal isolation (UPI). Pups exposed to UPI are raised with limited bedding and nesting (stressor 1), are socially isolated for 1hr in small cylinders during the 2nd and 3rd weeks of life using unpredictable schedule (stressor 2), and experience nest disruption when return to their home cage (stressor 3). We tested the effects of UPI and sex on anxiety-like behavior using traditional tests of anxiety (open field test and elevated plus maze) and the Spotlight test and social behavior test using automated home cage monitoring (Noldus PhenoTyper). Home-cage monitoring was conducted for 12hrs during the dark phase, in a complex novel cage environment, and in the absence of human contact. In contrast to the inconsistent pattern of anxiety-like behavior observed in the open field test and elevated plus maze, we found a highly reproducible and statistically significant increase in defensive behavior and social abnormalities using the automated home cage monitoring. These findings highlight the limitations of canonical behavioral paradigms of anxiety and the advantages of using automated home-cage monitoring to improve the sensitivity, reliability, and translational utility of preclinical ELS studies in rodents.

Disclosures: B. Polis: None. **R. Islam:** None. **T.D. Prevot:** None. **A. Kaffman:** None.

Poster

221. Effects of Early Life Stress on the Brain and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 221.02

Topic: F.03. Stress and the Brain

Support: T32 MH096331
K08 MH122893
R01 MH068542
Hope for Depression Research Foundation
F31 MH127888
MH115914
MH1159-4-s1

Title: Effects of early life stress on pattern separation and hippocampal neurogenesis

Authors: *A. SANTIAGO[1,2], C. DEMAESTRI[1,2], K. TEGANG[1,2], C. STUCKE[3], K. G. BATH[1,2], R. HEN[1,2], W.-L. CHANG[1,2];
[1]Psychiatry, Columbia Univ., New York, NY; [2]Psychiatry, New York State Psychiatric Inst., New York, NY; [3]Bates Col., Lewiston, ME

Abstract: Background: Early life adversity (ELA) is a strong risk factor for mental illnesses, including depression and anxiety. One mouse model of ELA uses low bedding and nesting material with a wire mesh floor during the early postnatal period to induce fragmented maternal care. We have previously shown that this protocol accelerates both developmental decline in neurogenesis and developmental suppression of contextual fear response in juvenile males. Our group has also shown that adult hippocampal neurogenesis (AHN) affects performance in pattern separation. Here, we investigate the long-term effects of ELA on AHN and pattern separation, as measured with a fear discrimination task. Methods: Male and female c57BL6/J mice were exposed to limited bedding and nesting or control conditions during postnatal days 4-11. They were then all returned to normal housing conditions until adulthood. We then assessed context discrimination or collected tissue for histological analysis. First the contextual fear conditioning paradigm was administered with a foot shock delivered in Context A, followed by the same context 1 day later and testing in a novel and different context (Context C) 1 day after that. For fear context discriminations, animals explored Context A, which was paired with a footshock, and a similar context (B) which was never associated with shock over the course of >7 days. Freezing was measured in all contexts, with temporal order of context exposure varying across days. For histology experiments, doublecortin (DCX) staining was quantified in the dentate gyrus of adult mice after ELA. Quantification was performed blind to experimental condition using Image J, and the effects of both group and sex are reported. (n=8 mice per group for behavior, n=6 mice per sex and group for histology). Results: While control mice spent significantly less time freezing in the non-shock context B ($P<0.05$) compared to context A, ELA mice continued to freeze indiscriminately in both contexts, indicating generalization of threat response between the two contexts. DCX quantification will also be reported. Discussion: Together, our findings detail the long-term effects of ELA on hippocampal circuitry and functioning, particularly in the dentate gyrus, a region that is crucial for pattern separation. We assess the discrimination of emotionally salient contexts and investigate the impact on AHN after ELA.

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Poster

221. Effects of Early Life Stress on the Brain and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 221.03

Topic: F.03. Stress and the Brain

Support: German Federal Office for Education and Research grant 01GQ1404

Title: Optogenetic elevation of glucocorticoid levels during development alters behavior and brain transcriptome in adult zebrafish

Authors: *A. COOK[1], M.-K. CHOI[2], H. BECKMANN[2], S. RYU[2,1];
[1]Univ. Med. Ctr. Mainz, Mainz, Germany; [2]Living Systems Institute, Univ. of Exeter, Exeter, United Kingdom

Abstract: Stress exposure during early development influences physiological and behavioral responses of an animal in adulthood. Glucocorticoids (GCs) mediate wide-ranging effects of early life stress (ELS) exposure, yet underlying molecular mechanisms are not completely understood. We have developed a novel zebrafish ELS model by optogenetically targeting the interrenal gland (zebrafish homolog of the adrenal gland) and altering GC levels during development. In this transgenic zebrafish line, an optogenetic protein, beggiatoa photoactivated adenylyl cyclase (bPAC) is expressed specifically in the interrenal gland using a promoter fragment of the steroidogenic acute regulatory (StAR) gene. Blue light stimulation of the transgenic fish leads to activation of bPAC, leading to an increase in cAMP in interrenal cells and subsequent elevation of cortisol level in intact zebrafish. Raising the transgenic fish under light conditions containing blue light led to chronic elevation of cortisol during development. The adult transgenic fish raised under such conditions showed a range of behavioral alterations compared to wildtype, including social interaction, feeding, and fear processing. To gain insights into underlying molecular alterations, we then analyzed time-course transcriptional changes in the whole brain at the larval (6 and 13 days post-fertilization) and adult (120 days post-fertilization) stages. We found more than 3,000 differentially expressed genes (DEGs) between wildtype and transgenic fish. In adult transgenic fish, expression analysis revealed increased expression of RNA processing genes and decreased expression of genes related to nervous system development and cellular neuronal signaling. We also found in the larval brains of the transgenic fish downregulation of genes linked to neuron projection mechanism, as well as known social behavior associated genes. Lastly, we identified that adult ELS fish were more sensitive to acute stress. Remarkably, differentially expressed genes identified following acute stress in ELS fish overlapped considerably with gene sets linked to human neuropsychiatric disorders. In conclusion, our study established a novel ELS model in zebrafish by altering GC levels during development and identified ELS-induced molecular changes and behavioral alterations.

Disclosures: A. Cook: None. **M. Choi:** None. **H. Beckmann:** None. **S. Ryu:** None.

Poster

221. Effects of Early Life Stress on the Brain and Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 221.04

Topic: F.03. Stress and the Brain

Support: R01 MH127850-01

Title: The Impacts of Early Life Adversity on Ultrasonic Vocalization- and Light-Enhanced Acoustic Startle

Authors: *A. PARAKOYI, L. GRANATA, H. C. BRENHOUSE;
Psychology, Northeastern Univ., Boston, MA

Abstract: Early life experiences strongly influence neuronal maturation and affective processing. Early life adversity (ELA) has been shown to increase risk of anxiety, depression, and cognitive impairments in adolescence and adulthood. For example, ELA alters threat responsiveness, which is an expression of anxiety and its regulation by the prefrontal cortex (PFC). In this study, we used the acoustic startle response in the presence of potential threat as a measure of threat responsiveness. We measured threat-potentiated startle reflex responses in either maternally separated (ELA) or control reared (CON) rats on postnatal day 55 (emerging adulthood). Ultrasonic vocalizations (USV), specifically 22 kHz vocalizations, are naturalistic forms of communication among rats that can be used to indicate the presence of a threat stimulus and warn others of said threat. Additionally, rats are nocturnal and easily preyed upon in nature so aversion to light is a natural threat response. On day 1 rats were placed in the acoustic startle chamber and played randomized bursts of white noise over 20 minutes to get baseline startle measures. Then on day 2 rats were either exposed to playback of 22 kHz USVs prior to the startle paradigm or were exposed to light during the startle paradigm. Male rats showed a heightened baseline startle response than their female counterparts overall. On day 2 we found that in the presence of USVs, male but not female CON rats showed a heightened startle response compared to baseline. However, ELA blunted this enhancement, as males showed a similar startle response to baseline. Preliminary findings suggest that in the presence of light CON rats show heightened startles response, whilst ELA rats show blunted startle responses like USV data. Ultimately, this study can aid in our understanding of how ELA impacts threat responsivity as measured by the startle reflex.

Disclosures: **A. Parakoyi:** None. **L. Granata:** None. **H.C. Brenhouse:** None.

Poster

221. Effects of Early Life Stress on the Brain and Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 221.05

Topic: F.03. Stress and the Brain

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Academic Investment Research Program-Individual Principal Investigator Award to YMY

Title: Targeting cerebello-thalamo circuit to rescue affective and social deficits induced by social isolation

Authors: *S. S. PATHAK[1], O. Y. CHAO[1], E. MARRON FERNANDEZ DE VELASCO[2], M. SLATTERY[1], Y.-M. YANG[1,3];
[1]Dept. of Biomed. Sci., Univ. of Minnesota Med. Sch., Duluth, MN; [2]Dept. of Pharmacol., [3]Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Early-life stress, such as social isolation (SI), increases the likelihood of developing neuropsychiatric disorders including anxiety, depression, autism, schizophrenia, and substance abuse. The high comorbidities of these disorders suggest common pathogenic mechanisms related to structural and functional alterations in the brain. Although many brain areas can be affected by SI stress, the cerebellum is increasingly recognized as a pivotal mediator of stress-induced behavioral changes. In the cerebellar cortex, Purkinje cells carry the sole output to deep cerebellar nuclei (DCN), which further gate outgoing information to the neocortex primarily via the thalamus. Through the cerebello-thalamo-cortical connections, the cerebellum is viewed as an estimator of the internal state in response to stress. To investigate the neural basis, we isolated mouse pups for 4 weeks after weaning. These animals showed long-lasting behavioral changes such as high anxiety and social recognition deficit when compared to group-housed controls. *Ex vivo* patch-clamp recording from Purkinje cells revealed that SI significantly reduced the activity of these output neurons, raising the hypothesis that altered downstream cerebello-cortical connectivity may underlie the behavioral deficits. Using an adeno-associated virus (AAV)-mediated optogenetic approach, we infused AAV vectors containing ChR2-YFP or eNpHR-YFP in the fastigial nucleus of DCN, and implanted optic fibers in the paraventricular thalamus (PVT) to respectively excite or inhibit the nerve terminals of DCN neurons in the PVT with photostimulation. We found that excitation of the DCN-PVT circuit alleviated the social recognition deficit in SI mice while inhibition of this pathway had no effect on the social deficit. Moreover, excitation of the DCN-PVT circuit rescued the anxiety phenotype in SI mice whereas inhibition of this pathway exacerbated the anxiety phenotype. Taken together, we have identified a key circuit underlying the cerebellum-mediated response to SI. Precise control of the cerebello-thalamo circuit activity may provide an effective avenue for rescue of affective and social impairments associated with SI stress.

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Poster

221. Effects of Early Life Stress on the Brain and Behavior

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

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Topic: F.03. Stress and the Brain

Support: NIH DA052909
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Emory National Primate Research Center (ENPRC) Base Grant OD P51OD011132

Title: Long-term effects of early life stress and adolescence psychostimulant use on adult emotional regulation and underlying neurocircuitry: a nonhuman primate study

Authors: B. A. BEESLEY[1], R. LEBOVIC[1], V. REDDY[1], E. R. SIEBERT[1], Z. A. KOVACS-BALINT[1], M. A. STYNER[2], M. A. NADER[3], L. HOWELL[1], A. M. KAZAMA[1], ***M. SANCHEZ**[1];
[1]Emory Univ., Atlanta, GA; [2]Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; [3]Wake Forest Univ. Sch. of Med., Winston Salem, NC

Abstract: Early life stress (ELS), including child maltreatment (MALT), has been linked with deficits in emotional and stress regulation and an increased risk of developing anxiety and drug use disorders. However, due to limitations of prospective, longitudinal designs in humans, the developmental trajectories, emergence, and underlying biological mechanisms of these psychopathologies have been difficult to understand. Using a translational macaque model of infant MALT, we studied the long-term effects of ELS in 22 adults (13 MALT 7M, 6F; 9 Control 5M, 4F). Previous data from these animals during adolescence showed higher baseline acoustic startle amplitude (higher anxiety) in the ELS than Control group, as well as faster acquisition, and higher intake of a psychostimulant drug (cocaine) during an IV self-administration study, with interesting sex differences. The present study examined: (1) whether the elevated anxiety persists in MALT animals into adulthood, (2) the neurobiological mechanisms involved and (3) the potential synergistic effect of psychostimulant exposure during adolescence as well as ELS to increase anxiety and neural alterations. To study this, the adult macaques were administered an acoustic startle paradigm where we measured baseline startle amplitude as an established and translational biomarker for anxiety and emotional reactivity in human and animal models. Median acoustic startle amplitudes from an auditory stimulus of varying intensities (95dB, 100dB, 110dB, 115dB and 120dB) were collected on two testing days for each animal. We also examined long-term structural and functional effects in neurocircuits important for emotional/stress regulation and reward, including prefrontal cortex, amygdala, hippocampus, and nucleus accumbens using T1-/T2-weighted and resting state functional connectivity (FC) MRI scans. In the baseline acoustic startle paradigm, we found a significant interaction between group and dB levels (dB*group: $F_{4,72}$ =2.812, p=0.032) with higher startle in MALT than Control animals at the lowest dB levels, and no sex or total adolescence cocaine effects as covariates. These findings suggest persistently elevated anxiety/emotional reactivity in ELS animals into adulthood. Preliminary structural MRI data in a subset of animals show volumetric ELS by hemisphere effects in orbitofrontal cortex ($F_{1,8}$ =7.080, p=0.029) and in ICV-corrected ventrolateral prefrontal cortex (t_8=-5.206, p<0.001). We are currently investigating the long-term impact on the functional connectivity (FC) of these neurocircuits as well as whether the neurobiological alterations predict the persistent elevated anxiety in MALT animals.

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Poster

221. Effects of Early Life Stress on the Brain and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 221.07

Topic: F.03. Stress and the Brain

Support: Carver Foundation Start-up Funds

Title: Early life stress and the dynamic maternal brain: Intersecting impacts on stress vulnerability and maternal care

Authors: *S. MITCHELL, M. EBERLE, M. JOHNSON, A. JIMENEZ, M. MATKOVICH, R. VELAMURI, B. HING, H. STEVENS, R. HULTMAN;
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Abstract: The maternal brain arises from rapid adjustments during normal development critical for meeting the demands of motherhood. Adverse early life experiences can impact components of the brain later responsible for maternal care and maternal care behaviors themselves. Early life stress (ELS) also alters neural pathways relevant to depression and anxiety, some of which overlap with those relevant to maternal care. In this study, we aim to elucidate the intersecting impacts of ELS on vulnerabilities relevant to depression and maternal care at multiple levels of investigation. We used a combination of maternal separation with early weaning (MSEW) in addition to limited nesting material as a model of ELS in CD1 mice, P2-P17 (n = 8-9 litters/group). Following weaning, juvenile mice were assessed for behavior on tasks relevant to negative and positive affect and anxiety (n = 12-15/group and sex) and for brain morphology and immunohistochemistry (n = 5-6/group and sex). At six weeks of age, prior to breeding, female mice were surgically implanted with muti-site in-vivo electrodes for monitoring brain-wide electrical activity. Electrical recordings were performed in the home cage and in response to negative affect stimuli at multiple stages, capturing the course of maternal brain changes from pre-gestation to pregnancy, early motherhood, and post-weaning (n = 6-7/group). Electrode design targeted brain regions linked to stress vulnerability and pup-directed social interactions - prelimbic and infralimbic cortices, nucleus accumbens, basolateral, medial, and central amygdala, ventral hippocampus, ventral tegmental area. Maternal behaviors were recorded following parturition for evaluation alongside electrical signatures. Forced interaction tests were also performed twice - prior to gestation and following weaning - to determine if the adult stressor of motherhood altered the animals' vulnerability signatures. Multiple regions of adult brain were additionally assessed for gene expression (n=6-8/group). Findings show ELS animals are initially smaller but normalize weight by adulthood. ELS heightened levels of freezing during fear conditioning training and showed a deficit in cued fear learning. Results thus far suggest that changes occur in animals exposed to ELS involving regions at the intersection of depression vulnerability and maternal care networks. Additional electrical signatures, behavior, and brain regional morphology, protein, and gene expression data currently being collected will be presented.

Disclosures: **S. Mitchell:** None. **M. Eberle:** None. **M. Johnson:** None. **A. Jimenez:** None. **M. Matkovich:** None. **R. Velamuri:** None. **B. Hing:** None. **H. Stevens:** None. **R. Hultman:** None.

Poster

221. Effects of Early Life Stress on the Brain and Behavior

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

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Topic: F.03. Stress and the Brain

Support: 5R01MH117004-03

Title: Early life adversity reprograms the methylome and transcriptome of ventral dentate gyrus granule cells to alter neuronal function and anxiety in adulthood

Authors: *A. NABILA[1], E. BRINDLEY[3], L. J. ZALLAR[2], S. ODELL[1], M. J. SKELLY[4], J. G. TOTH[3], A. ALONSO[5], K. E. PLEIL[3], M. TOTH[3];
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Abstract: Developmental exposure to environmental challenges like early life adversity (ELA) can lead to maladaptive behaviors and an increased susceptibility to disease later in life. Such ELA-associated phenotypes have previously been linked to DNA methylation and histone modifications in both human and rodent studies. However, the molecular mechanisms by which the epigenome regulates neuronal function and behavior following ELA are not well understood. We hypothesize that ELA permanently alters the epigenome and in turn, the transcriptome, at behaviorally relevant brain regions to manifest maladaptive behaviors and disease in adulthood. To test this hypothesis, we utilized two models of ELA: MA, in which serotonin 1A receptor deficiency in the mother ($5HT1AR^{+/-}$) causes a maternal autoimmune-like condition and anxiety, and ML, in which late gestational LPS administration induces maternal inflammation. In both models, adult male offspring exhibit innate anxiety-like behaviors on the elevated plus maze. Since the ventral dentate gyrus has been implicated in anxiety, we performed reduced representative bisulfite sequencing of ventral dentate granule cells (vDGCs) in ELA and control offspring. Both ELA models show differential CpG methylation at thousands of short genomic regions with mostly partial methylation in control animals. Overlapping differentially methylated regions (ELA-DMRs) are enriched in exons, 3' UTRs and CpG island shelves and shores, suggesting that ELA-DMRs may regulate transcription via multiple mechanisms. Single nucleus RNA sequencing of vDGCs of MA and control mice identified 6 subclusters of vDGCs and 70 total differentially expressed (DE) genes, 48 of which were also differentially methylated (DM). Gene ontology of DExDM genes showed enrichment in categories related to synaptic organization and plasticity. Notably, 33/48 DExDM genes were downregulated, indicating synaptic dysfunction in agreement with electrophysiological data that showed altered excitability of MA vDGCs. To assess whether these changes were subtype specific, we also separately

analyzed differential expression in the vDGC subclusters. However, we found that differentially expressed genes were broadly similar in MA vDGCs across all clusters. Overall, our data suggest that MA vDGCs are epigenomically, transcriptionally and functionally different from control vDGCs, resulting in anxiety-like behaviors.

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Poster

221. Effects of Early Life Stress on the Brain and Behavior

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 221.09

Topic: F.03. Stress and the Brain

Support: CAPES 001

Title: Exercise during prenatal zika virus exposure inhibits behavioral disturbances in newborns and teenagers mice

Authors: *R. LEONI DE SOUSA, M. F. DIAS-PEIXOTO, P. M. LOPEZ, S. F. F. OLIVEIRA, G. H. B. OLIVEIRA, K. H. SAMPAIO, P. P. CRUZ, C. O. D. MAGALHAES, R. C. CASSILHAS;
Univ. Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, Brazil

Abstract: Zika virus (ZIKV) infection during pregnancy is related to microcephaly and neurobehavioral disorders at birth, while prenatal exercise is supposed to provide neuroprotection in newborns pups. We investigated the potential neuroprotective effect of prenatal swimming exercise during ZIKV exposed pregnancy in mice pups. Twelve weeks old female and male Swiss mice were mated in our facilities in a 2:1 ratio respectively for 24 hours. Dams were separated from the males after this period. ZIKV (10^6 PFU) or an equal volume of 100uL of saline (Control) was injected intraperitoneally in the dams at embryonic day 10.5. The exercise protocol for the dams consisted of 1 adaptation week and 4 weeks of swimming training. Swimming training sessions consisted of 60 minutes, 5 following days per week during 4 weeks and started 1 week before mating. Female mice were randomly assigned into three groups: Control group, intraperitoneally injected with saline (Control); untrained group, intraperitoneally injected with ZIKV (ZIKV); and trained group, intraperitoneally injected with ZIKV (ZIKV/swim). Maternal behavior was evaluated to guarantee that neurobehavioral changes seen in the offspring were due to the infection. Body weight of the dams was measured during gestation. Pup's body mass and brain weight were measured at P1. Behavioral tests were performed at postnatal days P2-P8 and P30-P35 in female and male pups. Maternal behavior and body weight of the dams did not present significant changes between the groups. Exercise during prenatal ZIKV exposure prevented in female and male mice pups brain atrophy at P1, disturbances in negative geotaxis at P2, development of anxious, depressive and anti-social

behaviors at P-35. Our findings reveal beneficial effects of exercise during pregnancy exposure to ZIKV in newborns and teenagers mice, independently of sex.

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Poster

221. Effects of Early Life Stress on the Brain and Behavior

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Topic: F.03. Stress and the Brain

Support: NRF Grant NRF-2019R1C1C1011390
KBRI Grant 22-BR-02-03
KIST Grant 2E31524

Title: The effect of many different types of stress on mice behavior: Acute stress (PTSD+SPS), chronic unpredictable stress, and social isolation stress

Authors: *D. KIM, M. CHOI, Y.-J. YOUN, Y. JEONG;
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Abstract: Modern people are exposed to various stressful situations. Stress is a crucial factor for depression and is related to the imbalance of physiological and psychological homeostasis, including brain function and structure. Stress induces the endocrine alterations characterized by the activation of hypothalamic-pituitary-adrenal axis and sympathetic adrenal-medullary system. There are many causes of stress and each affects us differently. The goal of this study is to determine the effects of different types of stress on mice behavior.In modern society, excessive food intake and calories due to stress and reduced physical activity can lead to obesity. The association between stress and obesity has been known to be interrelated, and there are individual differences in these intricate relationships. However, the difference of individual stress responses such as stress susceptibility or resilience has not been studied well in obesity. Interestingly, we found the stress resilience only in an obese group in our long-term (chronic)/short-term (acute) stress-induced depression mice model. These individual differences in stress responses in obesity can play a crucial role in stress maintaining and recovering mechanisms against long-term/short-term stress. The finding of this study can lead to the development of effective and customized stress treatment strategies upon individuality.Since the outbreak of COVID-19, lockdown, social isolation and social distancing change our daily lifestyle thoroughly. Therefore, we focused on the effect of long-term social isolation stress on mice behavior in life cycle model by age. Interestingly, we observed different behavioral phenotype in childhood stage compared with adolescence and manhood stage. Therefore, we further study the effect of social isolation on the development of sociability through age by

transcriptome analysis. Through this study, we can find critical factor involved in the sociability that might be utilized to the diagnosis indicators or therapeutic agents for stress-related diseases.

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Poster

221. Effects of Early Life Stress on the Brain and Behavior

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Program #/Poster #: 221.11

Topic: F.03. Stress and the Brain

Support: CIHR
NSERC

Title: Role of thioredoxin-interacting protein in corticosterone-impaired neuronal differentiation and neurodegeneration

Authors: *M. A. LLANES CUESTA[1], H. TAN[2], S. ACHARYA[1], J.-F. WANG[1];
[1]Pharmacol. and Therapeut., Univ. of Manitoba, Winnipeg, MB, Canada; [2]Kleysen Inst. for Advanced Med. (KIAM), Winnipeg, MB, Canada

Abstract: During stress, the hypothalamic-pituitary-adrenal axis releases glucocorticoids into the bloodstream and targets various organs including the brain. Evidence indicates that chronic stress reduces the length and branching of neuronal dendritic arbors, and the number of astrocytes in the hippocampus and prefrontal cortex. These deleterious effects on the brain by chronic stress have been associated with the development of psychiatric disorders such as depression. Thioredoxin-interacting protein (Txnip) is an inhibitor of thioredoxin (Trx), a protein that plays an essential role in regulating redox balance. Txnip binds to Trx and prevents Trx from binding and inhibiting the apoptosis-signaling kinase 1 (ASK1), thus promoting apoptosis. Previously, we found that Txnip is increased in primary mouse cortical neurons during neurodegeneration from 22-32 days in vitro (DIV). Txnip is also upregulated in primary neurons exposed to chronic corticosterone (CORT) treatment. This data suggests that CORT-induced upregulation of Txnip might lead to neuronal death and impairment of neuronal function. The aim of this work is to understand the role of Txnip in chronic CORT treatment-impaired neuronal differentiation and neurodegeneration. First, we determined whether chronic CORT treatment impairs neuronal differentiation and promotes neurodegeneration in primary cortical neurons. Neuronal cells were treated with 1 µM CORT at 2 and 7 DIV for 5 days. Immunocytochemistry analysis showed that CORT treatment impairs dendritic outgrowth and branching and induces cell death in the MTT assay at early and late stages of differentiation. No significant changes were observed in the expression of synaptic proteins. Second, because chronic stress causes profound atrophy of astrocyte branches and volume, we treated mouse primary astrocytes with CORT concentrations ranging from 0.125 to16 µM at different time points from 1 to 7 DIV. Our western blot analysis showed that all CORT concentrations and treatment length significantly increased Txnip protein

levels but no Trx levels. Finally, we evaluated the expression of Txnip in post-mortem brain tissue using immunohistochemistry. We observed that Txnip protein levels were increased in the anterior cingulate cortex of patients with Major Depressive Disorder when compared to healthy controls. Altogether this data suggests that chronic CORT-mediated upregulation of Txnip might lead to inhibition of Trx and thus impairs neuronal differentiation and promotes neurodegeneration. Consequently, contributing to the development of depression.

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Poster

221. Effects of Early Life Stress on the Brain and Behavior

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Program #/Poster #: 221.12

Topic: F.03. Stress and the Brain

Support: NIMH R01MH117459-01
NSF GRFP 2021318039

Title: Early life adversity affects social recognition memory and hippocampal plasticity: differential effects of sex and specific experience

Authors: *R. C. WATERS[1,2], H. WORTH[1], S. POWLEY[1], E. GOULD[1,2];
[1]Princeton Neurosci. Inst., Princeton, NJ; [2]Dept. of Psychology, Princeton, NJ

Abstract: Early life adversity (ELA) has long been recognized as a predisposing factor to the emergence of several neuropsychiatric diseases, many of which involve social dysfunction. Importantly, specific types of ELA have been shown to increase the likelihood of different neuropsychiatric outcomes in humans. Furthermore, adult humans who experienced ELA have altered social memory, with differential effects associated with exposure to either neglect or abuse. We used two rodent models of ELA to understand the alteration of social memory: maternal separation and early weaning (MSEW), a model of neglect, and limited bedding and nesting (LBN), a model of scarcity and abuse, to investigate effects on social recognition. We found that MSEW and LBN male mice showed social memory impairments that differed, with MSEW males showing a social recognition deficit and LBN males showing reduced social novelty preference. By contrast, both MSEW and LBN female mice showed enhanced social novelty preference. We next investigated whether ELA-induced social memory impairments in males are associated with altered plasticity mechanisms in the hippocampus. Studies have shown that adult-born granule cells (abGCs) in the dentate gyrus are important for social memory (Cope et al., 2020). These neurons project to the hippocampal CA2 region, an area considered to be a "sociocognitive hub", which integrates inputs from multiple afferents to support social recognition memory (Hitti and Siegelbaum, 2014). Pyramidal neurons and interneurons in the CA2 are surrounded by perineuronal nets (PNNs), specialized extracellular matrix structures that play a role in social memory (Cope et al., 2021). Thus, we investigated both abGCs in the

dentate gyrus and PNNs in the CA2 region in MSEW and LBN male mice. First, we found that MSEW, but not LBN, mice had diminished abGC numbers as well as a lower density of abGC axons in the CA2 compared to controls. Second, we found that LBN, but not MSEW, mice had elevated PNNs in the CA2 region compared to controls. These findings suggest that different types of ELA produce deficits in social memory function through different mechanisms. Next steps will involve determining whether ELA-induced changes in abGCs and PNNs are responsible for alterations in social recognition in males and how MSEW and LBN affects hippocampal plasticity in females.

Disclosures: R.C. Waters: None. **H. Worth:** None. **S. Powley:** None. **E. Gould:** None.

Poster

221. Effects of Early Life Stress on the Brain and Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 221.13

Topic: F.03. Stress and the Brain

Support: Emmanuel College

Title: Increased susceptibility of male CD-1 mice to developmental stress in a two-hit paradigm: long-term effects on anxiety and depression-related behaviors

Authors: *S. T. ZDON[1], A. P. GEMOS[1], V. N. APPIAH-DANQUAH[1], C. E. DONAHOE[1], S. G. DANIEL[1], A. C. DRAKE[1], M. P. LEUSSIS[2];
[1]Psychology and Neurosci., [2]Emmanuel Col., Emmanuel Col., Boston, MA

Abstract: Evidence demonstrates that early adversity and stress are linked to an increased likelihood of developing mental illness later in life. Maternal separation in rodents has been used to model early adversity and stressful events in childhood, while social isolation has been used as a stressor in adolescence. This study focused on the long-term effects of multiple stressors during development, essentially a two-hit model with impacts during both childhood and adolescence and evaluated behaviors related to anxiety and depression in adulthood. Male and female CD-1 mice received either maternal separation (MS; PND 2-12), adolescent social isolation (ASI; PND 35-56), or a combination of both (MS-ASI). The control group (CON) received no maternal separation or adolescent social isolation. Our results showed that across multiple behavioral tests, males were more susceptible to the developmental stress manipulations than females. For example, we found higher anxiety in all three developmentally stressed male groups compared to controls in both the elevated plus maze (EPM total time in open arms, $p=0.003$) and novelty suppressed feeding paradigm (latency to eat, $p = 0.08$). While females did not exhibit consistently higher anxiety in these two tests, MS-exposed and ASI-exposed females showed somewhat reduced time in the open arms of the EPM. In the social preference test, once again males were more affected, as all developmentally stressed male groups showed higher preference for social contact in a three-chamber test compared to controls, while in the females there was no

effect of treatment condition (group x sex interaction p = 0.004). There was no major effect of treatment in either the forced swim test or the sucrose preference paradigm. Similarly, locomotor behavior in an open field was not affected by developmental stress exposure in any group. Behavioral data analysis is ongoing as sample sizes in each group range from 4-8 currently and larger group sizes will help validate the reported findings. Future analyses will seek to assess potential changes in glucocorticoid receptor levels in different brain regions following the different developmental stress treatments to assess whether similar sex differences can be detected at this level.

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Poster

221. Effects of Early Life Stress on the Brain and Behavior

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Program #/Poster #: 221.14

Topic: F.03. Stress and the Brain

Support: Brain & Behavior Research Foundation
Roy J. Carver Charitable Trust

Title: Sex-specific brain network signature and differential gene expression involved in vulnerability to early life stress

Authors: *M. EBERLE, B. HING, M. JOHNSON, M. MATKOVICH, Y. FILALI, S. MITCHELL, W. WYCHE, R. HULTMAN;
Univ. of Iowa, Iowa City, IA

Abstract: Negative stressors such as early life stress (ELS) can increase vulnerability to subsequent stress exposures increasing susceptibility to disease. While some individuals can be vulnerable to the adverse effects of stress, the mechanism underlying this vulnerability to stress has not been fully elucidated. Although the prefrontal cortex was previously observed to be involved in generating an electrical brain network signature of stress vulnerability (EF1) in C57BL/6J mice, the sex-specific difference of this stress-associated vulnerability signature has not been investigated. To study this, we first validated EF1 detection in C57BL/6J mice. Using a machine learning approach, we analyzed local field potential across seven brain regions to identify EF1. Although the vulnerability scores were similar between males (N = 6) and females (N = 6) under resting conditions, they were significantly elevated in females when exposed to an intruder CD1 mouse (P &lt 0.05) suggesting increased vulnerability to stress in female mice. To evaluate gene expression between the sexes, we performed single cell RNA-Seq using the prefrontal cortex and identified differentially expressed genes in glutamatergic and GABAergic neurons in female mice. These genes were significantly over-represented (FDR &lt 0.05) for neuro-related pathways including axon guidance, neurotrophin signaling pathway, dopaminergic

synapse and GABAergic synapse. To begin to explore the universality of sex-specific vulnerability to ELS, we also exposed nine litters of CD1 mice aged P2 - P17 to maternal separation with early weaning, along with limited nesting material. Unstressed CD1 controls received standard weaning and nesting material. Results from the elevated plus maze of these mice in adulthood (N = 23 males, N = 23 females) showed that male mice from the stress (N = 11) and control (N = 12) groups did not show significant difference in time spent in the open arms ($p = 0.2$). In contrast, female mice from the stress group (N = 12) showed a trend in spending less time in the open arms ($p = 0.05$) compared to the control group (N = 11) suggesting increased anxiety which is consistent with increased EF1 score in females. Overall, these data provide insights into mechanisms spanning levels of analysis related to stress vulnerability differences between males and females. Further analyses exploring the relationship between behavior, neurophysiology, and gene transcription with regard to ELS are underway. We hope that this research will contribute to a greater appreciation for the divergent nature of stress at the individual level.

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Poster

221. Effects of Early Life Stress on the Brain and Behavior

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Program #/Poster #: 221.15

Topic: F.03. Stress and the Brain

Support: Maine INBRE - NIGMS Grant P20GM103423

Title: Early adversity alters affective processing in response to 22 kHz ultrasonic vocalization playback probe with sex-specific responses to therapeutic ketamine

Authors: *S. M. BONAUTO, S. N. ELLIS, E. NOEL, K. N. PATEL, L. M. O'SULLIVAN, A. M. HOCKING, J. A. HONEYCUTT;
Bowdoin Col., Brunswick, ME

Abstract: Investigating the effects of early life adversity (ELA) through maternal separation in rats provides a translational window to study behavior and mood disorders in humans with a history of adversity. In rats, ultrasonic vocalizations (USVs) are thought to communicate affectively valenced social information. These USVs are within-species cues that may serve as analogues to human facial expressions under certain circumstances. Specifically, 22 kHz USVs are characterized as aversive and negatively valenced, while 55 kHz appetitive calls are thought to be positively valenced. By exposing ELA and control rats to aversive 22 kHz USVs as a probe for hypervigilance and/or alterations in affective processing, we can examine how ELA impacts behavioral responses to emotionally valenced stimuli in a translational manner. Here, male and female rats were evaluated in an open field test (OFT) during USV playback at postnatal day

(PD) 25 or 45 to assess whether responses to playback may be influenced by an interaction between development and ELA history. Behavioral results show clear sex differences across development and ELA experience where females with a history of ELA were most influenced by USV playback. In experiment 2, male and female adult rats were exposed to silence, 22 or 55 kHz USVs. Findings from this experiment suggest that there are distinct behavioral responses to varying frequencies of USV playback over time in the OFT. Differences in behavior as a function of sex and/or ELA exposure suggest the likelihood of different behavioral approaches to affective stimuli, as well as the possibility of different neural recruitment dependent on these factors. Activity of brain regions associated with affective processing (e.g., amygdala, prefrontal cortex, bed nucleus of the stria terminalis) - quantified via density of cFos+ cells - indicate condition-dependent neural recruitment during USV playback that is specific to playback type and may be sex-dependent. In experiment 3, male and female adult rats (with or without history of ELA) were treated with acute ketamine (KET; 15mg/kg) and assessed days later in the OFT with playback of 22 kHz USVs. This generally revealed an anxiolytic effect of KET in males, while showing a deleterious effect on performance in ELA females. Taken together, this model provides an opportunity to characterize patterns of activity in the brain that correspond with emotionally valenced social signals and behavioral output while furthering our understanding of potential treatment options for at-risk populations.

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Poster

221. Effects of Early Life Stress on the Brain and Behavior

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Program #/Poster #: 221.16

Topic: F.03. Stress and the Brain

Support: Maine INBRE - NIGMS Grant P20GM103423

Title: Exposure to early life adversity produces developmental and sex-specific impacts on anxiety-like behavior and DNA methylation in a rat model

Authors: E. NOEL, A. CHEN, S. BONAUTO, S. ELLIS, Y. PEÑA, *J. A. HONEYCUTT; Bowdoin Col., Brunswick, ME

Abstract: Early life adversity (ELA), such as abuse or neglect in childhood, can result in adverse behavioral and neurological outcomes in brain regions connected to emotional processing. Individuals with a history of ELA exhibit higher rates of mental illness later in life (i.e., anxiety, depression), with females showing a higher prevalence; however, the mechanisms underlying these outcomes remain poorly understood. DNA methylation, an epigenetic modification of DNA readout, may contribute to ELA-induced vulnerability by altering neural development through epigenomic pathways, particularly in certain neural subtypes. GABAergic parvalbumin

(PV) containing cells are sensitive to ELA and show sex-related outcomes that are linked to affective processing, and therefore these neurons may be uniquely vulnerable to methylation outcomes. To investigate the degree to which changes in DNA methylation - particularly in PV cells - may underlie ELA-related sex disparities, both male and female Sprague-Dawley rats experienced ELA in the form of pre-weaning maternal separation. Rats were evaluated at two developmental timepoints (postnatal day (P)25 or P45) for anxiety-like behavior and DNA methylation both globally and in PV cells. Behavioral results show a developmental decrease in anxiety-like behavior from P25 to P45 in the open field test and elevated zero maze. Thus, older animals exhibited less anxiety-like behavior than younger animals regardless of sex and pre-weaning condition. Neural results were gathered using both immunohistochemistry and quantitative ELISA to discern patterns of DNA methylation in brain regions associated with anxiety, with the goal of understanding how DNA methylation may connect to ELA and/or anxiety-like behavior. The epigenetic results suggest an age and sex-dependent increase in 5-methylcytosine (5-mc) in PV cells in prefrontal cortex. ELA males exhibited a significant increase in 5-mC intensity in PV cells over development compared to control males. Females displayed a relationship between increased 5-mC intensity and decreased anxiety-like behavior, suggesting a biochemical linkage between heightened PV methylation and anxiolytic effects. These results suggest developmental differences in 5-mC intensity following ELA for both males and females, which may also elucidate a possible role of 5-mC changes in female resiliency. Overall, by characterizing regional and cell-specific differences in DNA methylation with respect to sex and age, critical windows for epigenetic alterations, symptom onset, and even treatment intervention may be discerned.

Disclosures: E. Noel: None. **A. Chen:** None. **S. Bonauto:** None. **S. Ellis:** None. **Y. Peña:** None. **J.A. Honeycutt:** None.

Poster

221. Effects of Early Life Stress on the Brain and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 221.17

Topic: F.03. Stress and the Brain

Title: Role of the serotonergic 5HT1A receptor in the periaqueductal gray matter on the response to stress and alcohol consumption in the rat

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Abstract: Alcohol use disorders are complex, and stress plays a fundamental role. The periaqueductal gray matter (PAG) is a target structure of the acute effects of alcohol for

regulating, among other responses, anxiety. Alcohol consumption from the stage juvenile modifies the expression of serotonin (5-HT) in the PAG and this contributes to excessive alcohol consumption during adulthood. In the present study, juvenile male Wistar rats were unexposed (alcohol-naïve group; A-naïve) or exposed to intermittent access to 20% alcohol for 12 sessions (alcohol-exposed group; A-exposed) at the end of the alcoholization period the open field test was performed. Posteriorly, all the animals were subjected to an unpredictable chronic stress protocol, at the end the open field test was performed for the second time. Subsequently, animals received intra dorsal-PAG (D-PAG) injections of 0.5µL of vehicle (isotopic saline solution, 0.09%), a selective 5-HT1A agonist (8-OH DPAT 1nmol / 0.5 µL) and 5-HT1A antagonist (WAY 100635 0.6 nmol / 0.5 µL). Finally, the open field test, elevated zero maze, and a choice paradigm of 4 bottles were performed. Chronic unpredictable stress causes decreased time in the center, the number of crossings, and increased grooming time, while intra-D-PAG microinjection of 5-HT1A receptor antagonist significantly decreased alcohol consumption. Chronic unpredictable stress causes behaviors related to anxiety and excessive alcohol consumption, and the 5-HT1A receptor in the D-PAG is one of the main players.

Disclosures: P. León: None. **A. Villanueva:** None. **H. Sanchez-Castillo:** None.

Poster

221. Effects of Early Life Stress on the Brain and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 221.18

Topic: F.03. Stress and the Brain

Support: DGAPA-PAPIIT IN 208722

Title: Separation of the motivational and hedonic reaction after stress exposure.

Authors: *Y. B. VIDAL-DE LA O[1], P. TORRES-CARRILLO[2], K. B. VALENCIA-FLORES[3], D. B. PAZ-TREJO[4], H. SANCHEZ-CASTILLO[5];
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Abstract: Stress is considered main predisposing factor for development of stress related disorders (depression, anxiety, trauma, etc). Particularly the subjects with Major Depressive Disorder (MDS) shows lack of motivation and anhedonia as major symptoms. However, there is few reports where motivation and hedonic valence are conjoint evaluated. Besides, the stressor type is not considered in many studies, nevertheless, distinct kind of stressors have differential effects that where observed when they are compared. For all this, the main goal of this research was analyzing the dissociation of the hedonic reaction from the motivation in an animal model of

chronic mild stress and social isolation in male Wistar rats. We used a chronic unpredictable stress battery (CUSB) and social isolation model (SIM). We evaluated the motivation with a progressive-ratio paradigm (PR), the milk preference test (MPT) and the saccharine preference test (SPT) for the hedonic value. We found that both stressors induced anhedonia, this was observed trough the decrease in saccharine and evaporated milk consumption in SPT and MPT tests. On the other hand, only CUSB group showed a minor breaking point, increase in the latency to retrieve reward and more consumption of water in PR test. Those results demonstrated a motivational impairment. In conclusion, the dissociation of hedonic reaction and motivation are stressor type dependent. This results demonstrate how important is to consider the stressor type in depression models in rodents.

Disclosures: Y.B. Vidal-de La O: None. **P. Torres-Carrillo:** None. **K.B. Valencia-Flores:** None. **D.B. Paz-Trejo:** None. **H. Sanchez-Castillo:** None.

Poster

221. Effects of Early Life Stress on the Brain and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 221.19

Topic: F.03. Stress and the Brain

Support: DGAPA IN208722
CONACYT CVU-925597.

Title: An approach to screen the susceptibility in rats and the efficacy of an antidepressive treatment after chronic stress.

Authors: *K. VALENCIA[1], Y. VIDAL-DELAO[1], H. SÁNCHEZ-CASTILLO[1,2,3];
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Abstract: Chronic stress during life has been considered a risk factor for development psychiatric illness in humans. Chronic unpredictable stress battery (CUSB) is an animal model to study depression through stress exposition in rodents. CUSB can induce a depressive and anxiety behavioral markers that could be modulated with an antidepressant treatment (fluoxetine). However, it is well known that not all subjects develop depression-like behaviors or do not respond to antidepressant treatments. The way to screen these individual differences in susceptibility to stress or efficacy of antidepressant therapy in depression models are not well studied. Here we show that saccharine consume, immobility and time spend in center of the area could be useful as behavioral markers for screening susceptibility to stress and depression, also we show that fluoxetine treatment had different efficacy at individual level depending on dose and age of exposure to stress. First, we found that after CUSB (during adolescence, adulthood, or double hit) rodents show depression and anxiety profiles. Second, we found that fluoxetine (10

mg/kg) could recover depression but no anxiety profile in groups of stress during adolescence and adulthood. Third, fluoxetine (20 mg/kg) could recover stressed subjects on the double hit group. Finally, we use machine learning clustering method (k-means), to classify the subjects in all groups based on the individual effects modulated by stress exposure and antidepressant treatment to find the ratio of stress effects and pharmacological efficacy. Using altered behaviors as classifiers, we found three possible clusters, (1) Without alterations, (2) Moderated anxiety and depression profile (3). High anxiety and depression profile, suggesting two groups of susceptible animals with different intensities of altered behavior. Also, fluoxetine could change the ratio of rats affected by chronic stress depending on the dose, showing beneficial effects of antidepressant treatment at 10 mg/kg but no at 20 mg/kg. Our results demonstrate that CUSB has different consequences even in subjects that experienced the same stress protocol. Also, fluoxetine has different efficacy recovering behavior associated to the age of exposure to stress. Finally, we suggest k-means as an easy and useful method to classify susceptible rodents in studies of depression and to study the individual efficacy of antidepressant treatments.

Disclosures: K. Valencia: None. **Y. Vidal-delaO:** None. **H. Sánchez-Castillo:** None.

Poster

221. Effects of Early Life Stress on the Brain and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 221.20

Topic: F.03. Stress and the Brain

Support: F31-MH127888 (CD)
RO1-MH115914 (KGB)
RO1-MH115049 (KGB)

Title: Early life adversity in mice leads to sex-differences in threat reactivity and activity of corticotropin-releasing hormone neurons in the central amygdala

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Abstract: Experiencing early life adversity (ELA), such as resource scarcity, is associated with an increased risk for anxiety-related disorders. Females are twice as likely to develop anxiety-related disorders compared to males. A core feature of these disorders is enhanced reactivity to real and perceived threat. Sex biases in risk and symptom presentation may be related to changes in corticotropin-releasing hormone (Crh), a sexually-dimorphic neuropeptide that is released in response to stress and is important for driving hyperarousal associated with threat reactivity. In males, ELA has been associated with changes in Crh levels in the amygdala, however, the effects on females and the impact on threat reactivity is still largely unexplored. We used the limited

bedding and nesting (LBN) model of ELA in mice to test its effects on fear-potentiated startle, a translationally relevant behavioral phenotype used to assess threat reactivity. We also measured the activity of Crh+ neurons using two methods. First, we quantified the number of active Crh+ neurons post-testing in the central amygdala using cFos as a marker of neuronal activity. We also measured Ca^{2+} of Crh+ neurons in the central amygdala during fear-potentiated startle using fiber photometry. We found that LBN enhanced acoustic startle in females, but not males, both when the threat was unpredictable and predictable. Female mice reared in LBN had increased co-labeling of cFos+ and Crh+ in the central amygdala, and sustained Ca^{2+} activity of Crh+ neurons during the tone previously associated with a threat (foot shock). The current work identifies a potential sex-specific mechanism for enhanced threat responding as a result of ELA. Ongoing studies will lay the groundwork for understanding sex differences in pathology and will contribute to the improvement of treatments and interventions that are individualized to the prior experience and sex of the individual.

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Poster

221. Effects of Early Life Stress on the Brain and Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 221.21

Topic: F.03. Stress and the Brain

Support: RO1-MH115914
RO1-MH115049

Title: Effects of early life adversity and secondary stress exposure on the VTA CRF system and alcohol intake

Authors: *J. BRETON[1,2], M. CRITZ[2], Z. CORT[3], I. MUSTAFA[4], K. G. BATH[1,2];
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Abstract: Experiencing early life adversity (ELA) increases the risk for substance use, including alcohol consumption. The VTA corticotropin releasing factor (CRF) system has been functionally linked with ethanol consumption and stress-induced reinstatement of drug seeking. Emerging evidence also suggests that ELA alters reward and stress circuits, including in the ventral tegmental area (VTA), providing a potential mechanism underlying vulnerability to addiction. Animal models of ELA, such as the limited bedding and nesting (LBN) paradigm, may provide insights into the neurobiological factors that contribute to risk for drug seeking. Here, we assessed the consequences of LBN on the expression of CRF-related genes in the VTA of both male and female adult mice to test the effects of sex as well as rearing condition on the development of a key brain center supporting ethanol consumption and stress responding. LBN

was associated with a decrease in both CRF cell numbers and CRF gene expression levels in the VTA of both sexes. LBN reared mice also had decreased CRF binding protein (CRF-BP) expression levels, though CRF-receptor expression remained unchanged. These changes were validated and localized across the VTA using fluorescent in situ hybridization in a separate cohort of animals. In another cohort of mice, exposure to a secondary stressor increased CRF and CRF-BP expression levels in LBN animals relative to controls. This stress-associated shift in CRF and CRF-BP expression may provide a mechanism through which a secondary stressors may interact with ELA to increase risk for drug seeking. Based on the observed effects, we are testing the impact of secondary stressors on ethanol consumption in control and LBN reared mice. Continued work will assess whether the observed changes in the VTA CRF system are functionally related to stress-induced changes in ethanol seeking. Overall, these studies provide critical insight into the effects of ELA on stress-related behaviors, their circuits and the overlap between stress and the risk for addiction.

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Poster

221. Effects of Early Life Stress on the Brain and Behavior

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Program #/Poster #: 221.22

Topic: F.03. Stress and the Brain

Support: NIH Grant F31DA053088-01
NIH Grant RO1-MH115914
NIH Grant RO1-MH115049

Title: Early life adversity slows mouse reinforcement learning and undermines reward expectation

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Abstract: Early life adversity (ELA) confers risk for reward-related psychopathologies, with likely effects on the development of the neural substrates supporting reward learning and decision making. Specifically, a stressful and highly unpredictable early environment may undermine expectations about richness and stability. In turn, animals may adopt strategies to optimize reward pursuit according to diminished expectations. To test this hypothesis, we use a

mouse model of ELA to manipulate the reliability and quality of early life care and subsequently test adult outcomes on reward learning and decision making with a two-arm bandit task. Exposure to ELA led to poorer choice discrimination, slowed learning, decreased the impact of richness on choice, and slowed reaction times as a function of reward. We formalized group differences in bandit task performance with different assumptions in a biologically informed reinforcement learning model. Our modeling data lead us to posit that group differences are accounted for with changes in learning rates, choice stochasticity, and a parameter indexing sensitivity to environmental richness. Each of these parameters have been independently linked with aspects of striatal dopamine signaling, supporting the hypothesis that ELA alters the midbrain dopamine system. Ongoing modeling work is aimed at resolving the respective contributions of these parameters by leveraging behavior, striatal dopamine receptor expression (via rtPCR and RNAScope), and the dynamics of dopamine signaling (via dLight fiber photometry) in the nucleus accumbens core.

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Poster

221. Effects of Early Life Stress on the Brain and Behavior

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 221.23

Topic: F.03. Stress and the Brain

Support: MH115914
MH115914-s1
MH115049

Title: Sex differences in stress-induced feeding behavior as a consequence of early life adversity in adult mice

Authors: *M. CRITZ, K. G. BATH;
Developmental Neurosci., Columbia Univ. - New York State Psychiatric Inst., New York, NY

Abstract: Early life adversity (ELA) increases the risk for stress-related disorders later in life, including eating disorders. Binge eating is a core symptom of many eating disorders, and is commonly triggered by stress or negative affect. ELA during sensitive developmental windows can alter the development and functioning of systems associated with stress and feeding, which may underlie an increased risk of developing disordered feeding patterns later in life. Further, ELA has the potential to alter sensitivity to hedonic food signals, which may further interact with stress to promote altered food choice and impact caloric intake. Here, we tested the effects of ELA on stress-induced feeding using the limited bedding and nesting paradigm, a resource-based model of ELA. Given that the effects of ELA on development are sex-specific, and that disordered eating is most commonly diagnosed in young women, we tested both male and female

mice in order to further investigate behavioral and neural sex differences in the feeding response to acute stress. ELA rearing resulted in hyperphagic “binge-like” feeding behavior on highly-palatable food in adult males following an acute stress. In contrast, female ELA mice required a second exposure to an acute stressor to elicit a hyperphagic ‘binge-like’ response. To test for possible mechanisms underlying sex-differences in the effects of ELA on stress-induced binge-like feeding, we carried out studies assessing changes in gene expression and neuronal activity using immediate early gene cFos in stress, reward, and feeding-specific regions, including the ventromedial hypothalamus, amygdala, and ventral tegmental area (VTA). Ongoing studies seek to elucidate the effects of ELA on feeding behavior as well as on the development of neural circuits underlying stress and feeding. This work will contribute to the development of more refined treatments and interventions for stress-induced disordered eating.

Disclosures: M. Critz: None. **K.G. Bath:** None.

Poster

221. Effects of Early Life Stress on the Brain and Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 221.24

Topic: F.03. Stress and the Brain

Support: UMEA
CRC 1280, project number 316803389

Title: Increased fear responses to safety cues in young adults born very preterm

Authors: *B. ALBAYRAK[1], L. JABLONSKI[1], U. FELDERHOFF-MUESER[1], B. M. HUENING[1], T. M. ERNST[2], D. TIMMANN[2], G. BATSIKADZE[2];
[1]Dept. of Pediatrics I and C-TNBS, Essen Univ. Hosp., [2]Dept. of Neurol. and C-TNBS, Essen Univ. Hosp., Univ. of Duisburg Essen, Essen, Germany

Abstract: Very preterm birth (VP) is associated with an increased risk for attention deficit, autism spectrum and anxiety disorders, denoted as "preterm behavioral phenotype“. Disturbed learning processes are thought to play an important role in the development of emotional disorders. Abnormal brain development in VP infants may result in disordered learning processes, which are exacerbated by environmental risk factors and persist in adulthood. Initial studies in rodent models of prematurity have reported altered fear conditioning. We tested the hypotheses that VP young adults displayed higher levels of fear conditioning, less differentiation between the CS+ and the CS- (that is, safety) signals and stronger resistance to extinction relative to healthy controls. A group of 37 VP (23 to < 33 weeks) young adults (mean age 20.03 ± 2.80 years) and 31 age- and sex-matched term-born (> 36 weeks) controls (22.23 ± 2.27 years) performed a differential fear conditioning paradigm on two consecutive days, which has been introduced by Milad et al. (Biol Psychiatry 2007;62:446-54). Acquisition and extinction training were performed on day 1. Recall and reinstatement were tested on day 2. Visual stimuli served as

conditioned stimuli (CS), and an aversive electric shock as unconditioned stimulus. A higher percentage of VP (43.2%) showed mild to moderate levels of fear compared to controls (12.9%) based on the Depression, Anxiety and Stress Scale (DASS-21). There was a tendency of VP to show higher SCR levels than controls throughout the experiment. Both VP and controls showed significantly higher skin conductance responses (SCRs) towards the CS+ compared to the CS- in the acquisition phase, which vanished during the extinction phase. Acquisition and extinction learning was not significantly different between VP and controls (non-parametric ANOVA). In early recall, controls showed significantly higher SCRs towards a CS+, that had been extinguished (CS+E) and towards a CS+, that had not been extinguished (CS+U) compared to the CS-. In VP, SCRs were significantly higher related to the CS+U compared to the CS-, but not related the CS+E (least squares means test). In early reinstatement, controls showed significantly higher SCRs towards CS+U, but not CS+E compared to the CS-. In VP, SCRs related to CS+U, CS+E and CS- were not significantly different. Reduced differentiation between safety and fear cues in recall and reinstatement may contribute to increased anxiety in very preterm born adults. Findings suggest that development of neural substrates involved in processing of safety cues are perturbed in prematurity.

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Poster

221. Effects of Early Life Stress on the Brain and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

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Topic: F.03. Stress and the Brain

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Märta Lundqvist foundation and Swedish Society of Medicine
Swedish Research Council (VR: 2015-00495)

Title: Exploring the relationship between early life adversity, telomere length and peripartum depression

Authors: *M. VRETTOU[1], S. TOFFOLETTO[2], S. LAGER[1], A. SKALKIDOU[1], E. COMASCO[1];
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Abstract: Over the last decade, depression and anxiety disorders have been increasing in young women. Yet, there is a lack of knowledge about psychobiological factors influencing sensitivity

to hormonal fluctuations as those occurring during pregnancy and postpartum. Preliminary evidence suggests a link between telomere length (TL), as biological marker for cellular senescence, and peripartum depression (PPD), but the directionality of this association remains unclear. Furthermore, adversity experienced early in life has been linked with PPD, as well as deviant TL. The aim of the present study was to examine the association between early life traumatic events, TL and PPD. A total of 298 women including 99 with PPD and 199 healthy controls were included in the study. Blood samples were collected from the participants during childbirth. Early-life adversity was assessed using self-reports, likewise depressive and anxiety symptoms at pregnancy week 17, pregnancy week 32, and six-month postpartum. TL was measured by quantitative real-time PCR. Between-group comparisons were assessed using the Mann-Whitney test. Correlations were assessed using the Spearman test. Multiple regression analyses were performed to identify predictors of depressive scores throughout pregnancy. TL (at delivery) was positively associated with depressive symptoms at pregnancy week 17, but not week 32 or six-month postpartum. Early life adversity (interpersonal events), age and parity significantly predicted depressive symptoms at pregnancy week 17. At week 32, interpersonal traumatic events and age were significant predictors of depressive symptoms. The PPD group had significantly longer telomeres at delivery compared with controls. The positive relationship between TL, early-life adversity and PPD is in line with few previous studies reporting longer TL in individuals with clinically significant depressive symptoms or exposure to traumatic events. Aberrant inflammatory reactions during pregnancy and peripartum may cause impaired telomere regulation. Elucidating the determinants of telomere length, given its potential as a biomarker of mental illness affected by early life stress could enable an early identification of women with an increased risk of PPD, and early intervention within the clinical practice.

Disclosures: M. Vrettou: None. **S. Toffoletto:** None. **S. Lager:** None. **A. Skalkidou:** None. **E. Comasco:** None.

Poster

221. Effects of Early Life Stress on the Brain and Behavior

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Program #/Poster #: 221.26

Topic: F.03. Stress and the Brain

Support: NRF-2021R1C1C1009128

Title: Identification of exercise-induced antidepressant response utilizing a multifaceted depressive and anxiety behavior analysis: evidenced by transcriptomic analysis

Authors: D.-J. HWANG, Y.-J. HAN, *T.-K. KIM;
Korea Natl. Sport Univ., Seoul, Korea, Republic of

Abstract: Despite extensive effort, the available evidence is insufficient to support the use of pharmaceutical agents to prevent depressive symptoms due to its genetic and behavioral

complexity and heterogeneity. Therefore, it's necessary to find a therapeutic alternative that reflects the understanding of differences in individual response to stress, and exercise may be a possible option for the treatment of depression. The purpose of this study was to elucidate the underlying mechanism of depressive disorder by developing a stress response analysis method and applying it to the antidepressant effect of exercise. Male 9-week-old C57BL6/J mice were subjected to restraint stress (RST) for 2 h daily for 14 days, and RST mice treated with voluntary exercise (VE) were housed in home-cage with a wheel running. Mice were measured for depressive and anxiety behavioral tests, and a two-dimensional (2D) behavioral matrix was introduced to segregate mice into susceptible and resilient subpopulations. In addition, comparative transcriptomic and bioinformatics analysis based on mRNA sequencing data were performed in the hippocampus (vHPC) to identify differentially expressed genes (DEGs) responsible to the susceptibility and resilience of exercise-induced antidepressant effect. After being exposed to RST, approx. 75% (53/70) of RST mice were susceptible to stress, and despite a similar average daily running distance (RST-Sus: 3.9 ± 1.1 km/d; RST-Res: 4.5 ± 1.4 km/d), RST mice treated with VE (RST-Ex) exhibited an antidepressant [susceptibility to exercise intervention, approx. 65% (21/32)] and anxiolytic effect [resilience to exercise intervention, approx. 78% (25/32)] in a mixed anxiety and depression state analysis, respectively. We have also found evidence of the involved biological processes derived from gene ontology (GO) functional enrichment analysis of the DEGs arising from susceptibility and resilience to stress and exercise intervention. These findings provide a better characterization of exercise-induced antidepressant effect through a multifaceted depressive behavior analysis method, providing a reliable and reasonable framework for revealing the underlying mechanisms of exercise-induced antidepressant effects.

Disclosures: D. Hwang: None. **Y. Han:** None. **T. Kim:** None.

Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.01

Topic: F.06. Autonomic Regulation

Support: BECA DOCTORADO NACIONAL #21181378
FONDECYT 1190729

Title: Chemical modification of the antidepressant fluoxetine to prevent its absorption through the intestinal wall

Authors: *F. ROJAS-HIDALGO[1], J. SOTO[2], J. A. BRAVO[3];
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Abstract: Depression is a neuropsychiatric disease, that has large burden on the patient and society. To treat this condition, several drugs are available, including fluoxetine (FLX), a specific serotonin reuptake inhibitor (SSRI). Recently, it has been shown that FLX also has effects beyond the brain, including the gut microbiota. Gut bacteria are sensitive to FLX, and there are descriptions of bacterial proteins that bind FLX. In addition, gut symbionts affect brain function, in what has been called the microbiota-gut-brain axis. Therefore, is it possible that FLX affects the gut microbiota, and thus contribute to its effect on the brain? To test this, we propose to chemically modify FLX, in order to make it less permeable trough the intestinal wall, and thus making it more available to gut microbes. To achieve this, we propose the addition of methyl groups to the secondary amine of FLX, in an attempt produce a charged quaternary amine group. For this, firstly FLX was left to react with methyl iodide, in dimethylformamide for 48h. Then the tertiary amine was left to react again with methyl iodide, but in tetrahydrofuran with tributylamine. The reaction product of each step was evaluated through ^{1}H-NMR and ^{13}C-NMR. This spectrometric analysis reveals that this procedure allows the transformation of the secondary amine of FLX into the quaternary form, a product named FLX^{+}. To date the synthesis and scaling up of this reaction has been optimized, improving reaction yields (it increased from 4.3% to 70.6%). Additionally, solubility curves have been made to estimate the K_{ow} (octanol/water) Furthermore, this synthesis procedure has also been carried out with sertraline (SRT) and paroxetine (PAX), as these compounds have similar structures to FLX, yielding the corresponding quaternary amines SRT^{+} and PAX^{+}. Future studies will require characterization of binding properties of FLX^{+}, SRT^{+} and PAX^{+} to the serotonin transporter, while at the same time testing the inability of these novel compounds to traverse the intestinal barrier. Finally, this chemical modification of SSRI's should provide the pharmacological tools needed to test the hypothesis that drugs affecting the brain, also impact on gut microbes, and how the effect on gut symbionts contributes to the antidepressant effects of SSRI's.

Disclosures: F. Rojas-Hidalgo: None. **J. Soto:** None. **J.A. Bravo:** None.

Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.02

Topic: F.06. Autonomic Regulation

Support: DI PUCV 039.316/2022

Title: Colonic neurons in the young rat: morphological changes happen shortly after weaning

Authors: V. VILLARROEL-GUZMÁN, A. LÓPEZ-AGUILERA, J. ESCOBAR-LUNA, J. EYZAGUIRRE-VELASQUEZ, J. A. BRAVO, ***M. JULIO-PIEPER**;
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Abstract: The enteric nervous system (ENS) is remarkably adaptable to environmental stimuli. Plastic changes in the ENS, including variations in neuron count, size and chemical coding, are in turn related to functional adaptations in muscle contractility and epithelial absorption/secretion. We have previously shown that juvenile rats subjected to protein deficiency, display a reduced neuronal body size in various submucosal neuron types, in comparison to age-matched controls. On the other hand, we found that high-fat diet induced a decrease in the number of colon submucosal ganglia as well as the total number of neurons per area in juvenile rats.
Weaning is a naturally-occurring, major environmental challenge which includes changes in diet, mucosal barrier function and microbiota composition. However, the adapting features of ENS throughout this biological milestone are largely unknown. In the present work we evaluated the effects of weaning and age, on the morphology of rat colon submucosal neurons.
Colon samples were obtained from rats at postnatal day (PND) 21 (pre-weaned), PND 35 and PND 45. Submucosal wholemount preparations were subjected to immunofluorescence against the pan-neuronal marker PGP9.5 and the sensory neuron marker N200K, and microphotographs were captured to perform neuron morphometry.
At PND 35 and PND 45, the soma of submucosal neurons had a significantly greater area in comparison to that observed in PND21 pre-weaned rats. The percentage of neurons that had a sensory phenotype was also increased after weaning. On the other hand, there was a significant reduction in the number of neurons and ganglia in PND 35 and PND 45 rats, in comparison to PND 21 pre-weaned rats.
Future studies should focus on characterizing the chemical code of these neurons and evaluate the functional adaptations they undergo early after weaning. These data will increase our understanding of the relationship between nutrition and infantile-juvenile digestive physiology. In addition, being the ENS a key component to the gut-brain axis, these data will provide a better picture of the potential implications of dietary changes at a young age on the ability of enteric submucosal neurons to convey local information to the central nervous system.

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Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.03

Topic: F.06. Autonomic Regulation

Support: NIH Common Fund SPARC Program (Grant OT2 OD030534)

Title: Biophysically detailed cellular and network models of right atrial ganglionic plexus principal neurons identified from transcriptomics data

Authors: *S. GUPTA[1], A. J. H. NEWTON[1], A. MOSS[2], J. S. SCHWABER[2], R. VADIGEPALLI[2], W. W. LYTTON[1,3];
[1]Physiol. and Pharmacol., SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY; [2]DBI Functional Genomics/Computational Biol., Thomas Jefferson Univ., Philadelphia, PA; [3]Dept. of Neurol., Kings County Hosp., Brooklyn, NY

Abstract: The Right Atrial Ganglionic Plexus (RAGP), a component of the Intrinsic Cardiac Nervous System (ICNS), mediates pacemaker response of the sinoatrial node to vagal stimulation. We developed single-compartment models of RAGP principal neurons and local networks based on the transcriptomics (HT-qPCR -- High Throughput quantitative Polymerase Chain Reaction) of 321 principal neurons of Yucatan minipig RAGP. 14 of 241 genes in the transcriptomics map encoded ion channel proteins: voltage-gated sodium [*Scn1a*, Nav 1.1], voltage-gated potassium [*Kcna1*, Kv 1.1; *Kcnc1*, Kv 3.1], voltage-gated calcium [*Cacna1a*, Cav 2.1 (P/Q); *Cacna1b*, Cav 2.2 (N); *Cacna1c*, Cav 1.2 (L); *Cacna1d*, Cav 1.3 (L); *Cacna1g*, Cav 3.1 (T); *Cacna1i*, Cav 3.3 (T)], hyperpolarization activated [*HCN1*, *HCN2*, *HCN3*, *HCN4*] and inward rectifying potassium channels [*Kcnj3*, Kir 3.1]. Associated Hodgkin-Huxley-based ion channel models were selected from ModelDB and Channelpedia, and simulations were run on NetPyNE/NEURON software. Physiological models for each ion channel were selected in order to produce spiking behavior in all 104 cell types, each with distinct ion channel combinations. These types produced 3 firing patterns under current clamp: 65% produced single-spike phasic response; 2% elicited 2-3 spikes; 21% produced tonic firing (of which 33% continued firing post-stimulus). A double-exponential synapse model was developed which matched the power-law dependence for paired-pulse vagal stimulation. This synapse model was used for both vagal inputs and for intrinsic connections. The number and size of neural clusters were chosen to match reported values, with variable numbers of neurons, 10-100. We developed models of neuromodulation by neuropeptide-Y (NPY) and acetylcholine (Ach). NPY lowered Ca^{2+} channel HCN current permeabilities. Ach also lowered calcium currents while increasing inwardly rectifying potassium channels. We used NetPyNE to explore the unconstrained parameter space of connectivity strength.

Disclosures: S. Gupta: None. **A.J.H. Newton:** None. **A. Moss:** None. **J.S. Schwaber:** None. **R. Vadigepalli:** None. **W.W. Lytton:** None.

Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.04

Topic: F.06. Autonomic Regulation

Support: Supported by the Depatment of Psychology, Illinois State University

Title: Effects of the antidepressant vortioxetine on heart rate and respiration in anesthetized rats

Authors: P. B. ROGERS[1], S. J. CAHUE[1], ***B. A. HEIDENREICH**[1,2];
[1]Psychology, [2]Biol. Sci., Illinois State Univ., Normal, IL

Abstract: Heart rate and breathing are controlled by the CNS through brainstem areas that receive input of the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT). Consequently, drugs that affect the 5-HT system, including antidepressants, often change the frequency of heart contractions and respiration. For example, the selective serotonin reuptake inhibitor (SSRI) citalopram (0.1 - 6.4 mg/kg i.v.) decreases heart and breathing rates in the majority (67% and 81%, respectively) of urethane-anesthetized rats examined (Morrison & Heidenreich, Program 382.15, 2008 Neuroscience Meeting Planner, Society for Neuroscience, Online). A recently developed antidepressant drug, vortioxetine, acts as an SSRI but is also a 5-HT_{1A} receptor agonist, a 5-HT_{1B} partial agonist, and a 5-HT_3 and 5-HT_7 antagonist, and may produce clinical improvement more rapidly than other SSRI medications. Because multiple 5-HT receptor subtypes influence cardiac activity and breathing, we investigated the effects of vortioxetine on these parameters. Adult male Sprague-Dawley rats were anesthetized with urethane (1.5 g/kg i.p.) and prepared for recording as previously. Following collection of baseline heart and breathing rate data, vortioxetine HBr (0.25 - 8 mg/kg) was administered i.v. every 2 minutes in doses that doubled to a cumulative dose of 16 mg/kg. Eight of 10 rats showed a change of 3% or more in cardiac activity to vortioxetine; in one rat, heart rate transiently increased but returned to basal level. Seven rats decreased heart activity by the final dose of vortioxetine, including 4 that showed a transient increase in cardiac contractions. The E_{max} for altering heart rate was 82.3 ± 3.0% of baseline (mean ± S.E.M.) with an ED_{50} of 7.6 ± 1.7 mg/kg cumulatively. Eight of 10 rats showed a change of 3% or more in breathing rate to vortioxetine. Three rats increased respiration, with an E_{max} of 125.4 ± 5.8% of baseline and an ED_{50} of 1.4 ± 0.4 mg/kg cumulatively. Five rats decreased breathing by the final dose (E_{max} = 74.4 ± 4.5% of baseline; ED_{50} = 4.9 ± 2.2 mg/kg), including 1 that transiently increased breathing rate. Together, these results indicate that vortioxetine i.v. produces relatively small effects in physiological parameters in anesthetized rats, consistent with a report that 17 mg vortioxetine i.v. had no significant effects on heart activity or vital signs in depressed patients (Vieta et al. (2019), Int. Clin. Psychopharm., 34:153-160). Interestingly, vortioxetine produced less uniform effects in anesthetized rats than citalopram, suggesting that the 5-HT agonist/antagonist actions of vortioxetine are relevant to mechanisms of 5-HT regulation of heart and breathing rates.

Disclosures: P.B. Rogers: None. **S.J. Cahue:** None. **B.A. Heidenreich:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); H. Lundbeck A/S.

Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.05

Topic: F.06. Autonomic Regulation

Support: NHLBI R15HL145645-01
NHLBI R15HL147286-01

Title: Differential activation of viscerally-sensitive glutamatergic neurons within the rostroventrolateral medulla in cardiogenic sympathetic afferent reflex.

Authors: *M. R. ZAHNER[1], E. BEAUMONT[2];
[1]Hlth. Sci., [2]Biomed. Sci., East Tennessee State Univ., Johnson City, TN

Abstract: The Cardiogenic Sympathetic Afferent Reflex (CSAR) is a cardiovascular reflex characterized by increased sympathetic activity and blood pressure during myocardial ischemia. Metabolites such as bradykinin (BK) are released from the ischemic myocardium to stimulate cardiac sensory afferents causing an increase in sympathetic activity. The rostroventrolateral medulla (RVLM) is a key sympathetic output center for cardiovascular-related sympathetic activity and plays an important role in generating the reflex activity in the CSAR. However, the specific characteristics of sympathetic activation of RVLM in the CSAR are not fully understood. The goal of this study was to characterize the differential activation of barosensitive and ionotropic glutamatergic neurons within RVLM in the CSAR. To do this renal sympathetic nerve activity (RSNA), arterial pressure, and heart rate, were recorded in α-chloralose (100 mg/kg I.V.) anesthetized rats during epicardial application of BK (10 μg/ml). During control recordings, epicardial BK increased RSNA 180 ± 17% from baseline and induced a 21 ± 3 bpm tachycardia. Inhibition of barosensitive RVLM activity, by increasing arterial pressure to 165 ± 3 mmHg with I.V. phenylephrine, decreased basal RSNA to 29 ± 4% of baseline, but only partially attenuated the RSNA response (96 ± 18% increase from baseline, $P < 0.05$, N=15) and had no effect on the magnitude of BK-induced tachycardia (16 ± 3 bpm, $P > 0.05$). Bilateral microinjection of the $GABA_A$ agonist muscimol (1.0 nmol in 100 nl) into RVLM decreased basal RSNA to 31 ± 3% of baseline and abolished the RSNA response to epicardial BK (3% ± 3% from baseline, $P < 0.05$, N = 8). However, muscimol inhibition of RVLM had no significant effect ($P > 0.05$) on the magnitude of the BK-induced tachycardia (28 ± bpm) compared with vehicle (21 ± 6 bpm). To determine the glutamate receptor subtypes of RVLM neurons we tested the response to epicardial BK before and after bilateral microinjection of either AMPA/Kainate antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 0.7 nmol) or 2-amino-5-phosphonopentanoic acid (AP5, 1.25 nmol), an NMDA receptor antagonist into RVLM. Preliminary data suggest that microinjection of both CNQX (N=6) and AP5 (N=3) attenuates both the arterial pressure and RSNA response, but not the reflex tachycardia elicited by epicardial BK. These data suggest that glutamatergic viscerally-sensitive neurons within the RVLM, which are non-barosensitive are also involved in the CSAR. While muscimol inhibition of the RVLM abolished the arterial pressure and RSNA response to epicardial BK it did not affect the reflex tachycardia suggesting an alternative cardiac chronotropic pathway.

Disclosures: M.R. Zahner: None. **E. Beaumont:** None.

Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.06

Topic: F.06. Autonomic Regulation

Title: The effect of Tension-type headache on Heart rate variability

Authors: *E. TUMURBAATAR[1,3], A. BADARCH[2], O. ZAMBAL[4], A. GANGAA[5], O. JARGALSAIKHAN[6], R. ERKHEMBAYAR[7], B. LKHAGVASUREN[8];
[1]Dept. of clinical neuroscience, [2]Brain and Mind Res. Institute, Mongolian Acad. of Sci., Ulaanbaatar, Mongolia; [3]Hlth. Res. Dept., Mongolian Natl. Univ. of Med. Sci., Ulaanbaatar, Mongolia; [5]Dept. of clinical neuroscience, [4]Brain and Mind Res. Inst., Ulaanbaatar, Mongolia; [6]Dept. of psychology, Univ. of the Humanities, Ulaanbaatar, Mongolia; [7]Intl. cyber education department, Mongolian national university of medical sciences, Ulaanbaatar, Mongolia; [8]Dept. of Psychosomatic Med., Intl. Univ. of Hlth. and Welfare Narita Hosp., Narita, Japan

Abstract: Background: Tension-type headache (TTH) is characterized by recurrent episodes of bilateral pressing or tightening pain on the head that is not accompanied by nausea. Although TTH is a common neurological disorder among the general population, its impact on autonomic control remains poorly understood in Mongolia. The aims of this study were to investigate whether participants with TTH differ from population without TTH in heart rate variability (HRV) measures of autonomic nervous system activity and whether BMI has a moderating effect on this association. **Methods:** This population-based, cross-sectional study among the Ulaanbaatar population (n=217, mean age = 41.8±11.5 years), was carried out between July and September 2020, in 38 sampling centers in the capital city. Trained researchers applied 1) a 17-item structured interview to diagnose TTH based on the International Classification of Headache Disorders (ICHD) III; 2) Heart rate variability (HRV) record to detect autonomic activity. Demographics, physical examination, and psychological factors were measured using Hospital Anxiety Depression Scale, and WHOQOL-BREF. Binary logistic regression analysis was used to examine the association of TTH with other variables. **Results:** In this study, 117 (53.9%) participants met ICHD-III criteria for TTH. HRV decreased in male obese participants with TTH. Alongside increased in female obese participants with TTH compared to groups without TTH. HRV differences associated with gender. Partial sample binary logistic regression analyses revealed, TTH was associated with gender $\beta = 1.16$, $p = 0.002$; younger ages $\beta = 2.181$, $p = 0.003$; depression probable cases calculated by HADS $p = 0.042$. **Conclusions:** These results suggest that TTH highly prevalent among urban population in Mongolia. TTH associated between HRV indices and psychological factors vary between obese men and women.

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Poster

222. Neurovisceral Physiology II

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Topic: F.06. Autonomic Regulation

Support: NIH HEAL/SPARC U01 NS113867-01
NIH R15HL137143-01A1

Title: Stellate ganglion sympathetic postganglionic efferent innervation in the flat-mount of the whole heart (atria and the ventricles) of rats: anterograde tracing

Authors: *J. CHEN[1], K. T. BENDOWSKI[1], J. MADAS[1], Y. ZHANG[1], D. B. HOOVER[2], T. L. POWLEY[3], Z. CHENG[1];
[1]Univ. of Central Florida, Orlando, FL; [2]Dept. of Biomed. Sci., East Tennessee State Univ., Johnson City, TN; [3]Perdue Univ., W Lafayette, IN

Abstract: The autonomic nervous system (sympathetic and parasympathetic) plays an essential role in controlling cardiac functions. Previously, we used anterograde tracing to examine the vagal afferent and efferent (preganglionic) innervations of the normal heart, their anatomical remodeling and their functional changes in disease models of rats and mice. In addition, we used vesicular acetylcholine transporter (VAChT, a parasympathetic marker) and tyrosine hydroxylase (TH, a sympathetic marker) to determine the parasympathetic and sympathetic postganglionic innervation of the whole heart (atria and ventricles). Immunohistochemical labeling with TH is useful for determining the broad distribution of sympathetic efferent axons in the heart. However, it is difficult to distinguish its extrinsic and intrinsic origins using TH labeling. Furthermore, the topographical organization and morphological terminal structures of cardiac sympathetic postganglionic efferent axons from the stellate ganglia (SG) remains unknown. To address this we anterogradely labeled individual sympathetic postganglionic axons projecting to the heart, using tracer Dextran-Biotin microinjections into the right SG of Sprague-Dawley rats (male, n=6, 3-6 months). Fourteen days after the injection, animals were euthanized, and their hearts were dissected into the left/right atria (LA and RA) and ventricles (LV and RV) and prepared as flat-mounts. The tracer-labeled axons were digitized and analyzed using The Neurolucida 3D tracing system and imaged using a Zeiss M2 Imager. We found that stellate sympathetic neurons had projections to the LA, RA, LV, and RV. Individual sympathetic axons formed complex arbors of varicose neurites in the areas of the sinoatrial node (SAN) and atrioventricular node (AVN) as well as the other areas of the atria and throughout the ventricles. Sympathetic axons also innervated blood vessels. Using the Neurolucida Digitization and Tracer system, we determined the regional density and characterized different types of axons. Some axons formed relatively simple terminal structures with only a few branches, whereas other axons produced very complex terminals with extensive branching. For the first time, we anterogradely labeled the sympathetic postganglionic axons and characterized their terminal architecture in the whole heart. These results will provide an anatomical foundation for future study of cardiac sympathetic control and its changes in pathological conditions such as aging, diabetes, sleep apnea and heart failure. This study was supported by NIH HEAL/SPARC U01 NS113867-01 and NIH R15HL137143-01A1.

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Poster

222. Neurovisceral Physiology II

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Program #/Poster #: 222.08

Topic: F.06. Autonomic Regulation

Support: NIH HEAL/SPARC U01 NS113867-01
NIH R15HL137143-01A1

Title: Topographical Distribution and Morphology of Parasympathetic Postganglionic (Cholinergic) Axons in The Flat-Mounts of Whole Atria and Ventricles of Mice Hearts

Authors: *Y. ZHANG[1], A. BIZANTI[1], J. CHEN[1], T. L. POWLEY[2], D. B. HOOVER[3], D. GOZAL[4], Z. CHENG[1];
[1]Univ. of Central Florida, Orlando, FL; [2]Perdue Univ., W Lafayette, IN; [3]Dept. of Biomed. Sci., East Tennessee State Univ., Johnson City, TN; [4]The Univ. of Missouri Sch. of Med., Columbia, MO

Abstract: The autonomic nervous system plays an essential role in controlling cardiac functions. Parasympathetic activation may have important therapeutic implications in cardiovascular diseases (CVD). Parasympathetic control of the heart is also mediated by the intrinsic cardiac nervous system, which is comprised mainly of cholinergic neurons. Previously, we used anterograde tracing to examine the vagal afferent and efferent (preganglionic) innervations of the normal heart and their anatomical remodeling and functional changes in rodent disease models. We further used tyrosine hydroxylase (TH) and anterograde tracer microinjection into the stellate ganglia to examine the sympathetic postganglionic innervation of the whole heart (atria and ventricles). However, the cardiac parasympathetic postganglionic innervation remains poorly documented. Here, we aimed to determine the topographical distribution and morphology of the parasympathetic postganglionic innervation of the atria and ventricles of mice (male, 3-6 months, n=6). The flat-mounts of whole left and right atria (LA and RA) and ventricles of mice were immunostained for vesicular acetylcholine transporter (VAChT) and scanned using a confocal microscope coupled with a Zeiss M2 Imager to generate montages of the whole LA or RA. To calculate the regional density of VAChT-IR axons and terminals, we used an automated software algorithm “Axon Tracer” to trace and digitize labeled axons. We found 1) **Atria**: VAChT labeled the principal neurons (PNs) in all intrinsic cardiac ganglia (ICG) on the dorsal surface of LA and RA. ICGs were located at the junctions of pulmonary veins to the LA as well as the junctions of the superior vena cava (SVC), left pre-caval veins (LP-CV), and inferior vena cava (IVC) with the RA. VAChT-IR axons and terminals innervated PNs densely and covered the entire LA and RA. Furthermore, the density of VAChT-IR varicose terminals in the cardiac muscles within different locations was in the following order: sinoatrial node (SA) > atrioventricular node (AV) > left atrium-pulmonary veins junction > auricles. VAChT-IR terminals also innervated blood vessels and adipose tissues. 2) **Ventricles**: VAChT-IR axons densely innervated the cardiac muscles and the blood vessels of the left and right ventricles (LV and RV). Topographical mapping of cardiac cholinergic axon innervation provides an anatomical foundation for functional analysis of parasympathetic control of the heart and of its remodeling in pathological

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Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.09

Topic: F.06. Autonomic Regulation

Support: Office of the Assistant Secretary of Defense for Health Affairs through the Autism Research Program under Award No. W81XWH-16-1-0321

Title: Psychophysiological Predictors of the Treatment Response to Propranolol on a Standardized Assessment of Anxiety in Individuals with Autism Spectrum Disorder

Authors: *N. NURAINI[1], C. APPLING[1], D. BEVERSDORF[2], M. PRENDERGAST[1], A. KALATHIL[3], B. FERGUSON[4];
[1]Interdisciplinary Neurosci. Program, [2]Departments of Radiology, Neurology, & Psychological Sci., [3]Psychology and Biol. Sci., [4]Hlth. Psychology and Radiology, Univ. of Missouri, Columbia, MO

Abstract: Autism spectrum disorder (ASD) is characterized by repetitive behaviors and impaired social communication. Many individuals with ASD have co-occurring anxiety disorders which have been shown to be associated with markers of autonomic arousal. For the treatment of anxiety, it is plausible that those individuals with greater adrenergic tone at rest may receive the most benefit from propranolol, which is a nonselective, beta-adrenergic antagonist, which is under study for the treatment of anxiety in ASD. One of the psychophysiological assessment tools that have been shown to be sensitive to anxiety is skin conductance level (SCL), which is an indicator of sympathetic nervous systems activity. Many studies have shown the sympathetic branch of the autonomic nervous system to be heightened in many individuals with ASD, but there is a high degree of variability. We utilized baseline SCL to determine whether adrenergic tone, would predict the response to propranolol. Subjects diagnosed with ASD aged 7-24 took propranolol for 12 weeks in an open-label manner. Baseline (i.e., resting and prior to taking propranolol) skin conductance level (SCL) was obtained through over 5-minute recordings. Anxiety outcomes were assessed using the Spence Children's Anxiety Scale. We hypothesized that SCL during baseline would predict the anxiety response to propranolol in this sample of individuals with ASD. Further exploration will be needed with larger trials targeting anxiety in patients with ASD to predict changes in anxiety response to propranolol- an inexpensive and more accessible drug, which could target anxiety utilizing precision medicine principles in ASD.

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Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.10

Topic: F.06. Autonomic Regulation

Title: Contribution of the anatomically embedded sympathetic trunk towards the physiological effects mediated by vagus nerve stimulation (VNS) in rats

Authors: *A. DESHMUKH, R. CHEN, B. KNUDSEN, J. TREVETHAN, K. LUDWIG; Univ. of Wisconsin, Madison, Madison, WI

Abstract: Title: Contribution of the anatomically embedded sympathetic trunk towards the physiological effects mediated by vagus nerve stimulation (VNS) in rats*Authors: Ashlesha Deshmukh, Rex Chen, Bruce Knudsen, James Trevethan, Kip LudwigRationale:* Vagus nerve stimulation (VNS) in rat is an established model to study various applications with VNS caused bradycardia used as a threshold biomarker for successful target engagement. Sympathetic trunk (ST) stimulation is known to cause tachycardia and there have been recent studies showing in pigs that the ST can “hitchhike” with the vagus nerve (VN), yet there is no clear anatomical documentation in a rat model if ST is anatomically embedded with the VN in the carotid sheath and how its activation affects the thresholds of bradycardia known to be mediated by VNS. *Methods:* In male Long Evans rats we traced the ST from the sympathetic cervical ganglion (SCG) caudally till the sternum to find its location in the carotid sheath with reference to the VN at the common location for VNS electrode cuff. We delivered bipolar charge balanced electrical stimulation of 300 usecs pulses at 6.25Hz to the VN and ST to build dose response curves, individually and combined, to measure the physiological changes caused in heart rate and breathing. *Results:* In rats, the ST frequently crosses over from the medial to lateral side in the carotid sheath when traced caudally from the SCG to end up in very close proximity – and sometimes conjoined with the cervical vagus and would likely be cuffed with the vagus in most preparations. VNS with the vagus well-isolated from the ST repeatedly caused bradycardia in rats with a maximum drop of up to 100 beats per minute at very low levels of stimulation, while isolated ST stim caused tachycardia with a maximum increase of 15 beats per minute. Stimulating the ST and VN in conjunction – as we believe is common in most experimental preparations unless carefully dissected required a two fold or more increase in currents applied to cause bradycardia. *Conclusion:* It is important to isolate both VN and ST in the carotid sheath and functionally verify by electrically stimulating both to cause bradycardia and tachycardia

respectively before chronic long-term studies. This will enable researchers to confirm target engagement by VNS while isolating the ST to avoid harmful side effects by using stimulation current values higher than necessary and activating two competing autonomic systems. Incidental stimulation of the pre-ganglionic sympathetic fibers within the frequently hitchhiking ST may provide a partial explanation for highly variable animal responses within studies, and inconsistent results of VNS across rat studies.

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Poster

222. Neurovisceral Physiology II

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Topic: F.06. Autonomic Regulation

Support: T32 DA007261
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R01-DA042499

Title: Evaluation of Brainstem-Vagal Circuits as a Mediator of Opioid-Induced Respiratory Depression

Authors: *B. RUYLE, R. KESARAJU, S. MASUD, N. MASSALY, J. MORON-CONCEPCION;
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Abstract: Evaluation of Brainstem-Vagal Circuits as a Mediator of Opioid-Induced Respiratory Depression
Intravenous fentanyl administration induces severe respiratory depression (OIRD) which can be fatal if not reversed in a timely manner. Previous data from our lab demonstrates that peripheral opioid receptors play a greater role in the onset and maintenance of OIRD than previously thought. The vagus nerve is involved in cardiorespiratory function and contains high expression of mu opioid receptors (MORs). Therefore, we examined effects of fentanyl on vagal-brainstem trafficking and its relevance to OIRD. Conscious rats received an intravenous fentanyl administration (20 ug/kg; Pre-Test), and cardiorespiratory parameters were recorded via a collar oximeter. Rats underwent a unilateral vagotomy caudal to the nodose ganglion or a sham surgery in which the nerve was exposed but not cut. 7-10 days later, rats received subsequent fentanyl administration fentanyl (20 ug/kg; Post-Test), and cardiorespiratory parameters measured. Brains were collected for immunohistochemical analyses. Vagotomized rats displayed a significant reduction in CHAT immunoreactive (IR) neurons in the ipsilateral dorsal motor nucleus of the vagus. In addition, vagotomy significantly reduced MOR-IR in the ipsilateral nTS, including a marked reduction in the solitary tract. Fentanyl induced robust Fos-IR throughout the nTS, with a

significant increase in Fos-IR observed in the contralateral nTS compared to ipsilateral nTS of vagotomized rats. Fentanyl induced rapid decreases in oxygen saturation, heart rate and respiratory rate in both groups, effects which lasted up to 15 minutes after injection. These responses were not significantly altered following vagotomy (Pre-Test vs Post-Test). Our data demonstrate that unilateral vagotomy reduces brainstem motor neurons and MOR-expressing vagal afferent fibers in the nTS. However, these changes do not appear to alter OIRD. These unaltered physiological responses may be due to compensation by contralateral vagal-brainstem connections.

Disclosures: B. Ruyle: None. **R. Kesaraju:** None. **S. Masud:** None. **N. Massaly:** None. **J. Moron-Concepcion:** None.

Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.12

Topic: F.06. Autonomic Regulation

Support: FCT fellowship

Title: An interoceptive role for glycinergic periaqueductal grey circuits during defensive states

Authors: *S. LOURENÇO DOS REIS, J. SIGNORET-GENEST, P. TOVOTE;
Inst. for Clin. Neurobiology, Würzburg, Inst. for Clin. Neurobiology, Würzburg, Würzburg, Germany

Abstract: Fear and anxiety are evolved defensive states as response to a threat. Inappropriate selection and inability to rapidly switch between defensive state are hallmarks of fear- and anxiety-like disorders. Evidences demonstrate that these states consist in a multitude of coordinated and integrated responses, such as behavioral and autonomic, in which the afferent signals reporting the body's physiological state, i.e. interoception, are crucial to regulate these emotional states. The midbrain periaqueductal grey (PAG) critically contributes to defensive states however, it remains poorly understood how its neuronal subtrates encode and integrate interoceptive signals as part of a defense reaction. Focusing on PAG glycinergic neurons we observed, using deep brain calcium imaging with a miniaturized microscope, activation during the switch between a freezing/bradycardia state to a no-freezing/increase heart rate state, in which heart rate precedes neuronal activity, suggesting that these neurons receive cardiac interoceptive information. Furthermore, optogenetic manipulation confirmed an involvement of PAG glycinergic neurons in maintaining the macrostate dynamics in physiological levels, with optoactivation increasing heart rate variability, and optoinhibion decreasing. Moreover, virally-mediated trans-synaptic retrograde tracing demonstrated monosynaptic connection to the nucleus of solitary tract (NTS) and rostral ventral medulla (RVML), cardiac regulatory areas within the medulla. Anterograde tracing demonstrated that the PAG glycinergic neurons project almost

exclusively to forebrain regions, such as the hypothalamus, suggesting that these neurons might be crucial to report the cardiac state to higher order brain regions. Overall, our data suggest that glycinergic neurons in the vlPAG are involved in defensive states potentially via regulating cardiac interoception.

Disclosures: S. Lourenço dos Reis: None. **J. Signoret-Genest:** None. **P. Tovote:** None.

Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.13

Topic: F.06. Autonomic Regulation

Support: NIH Grant UH3AT009145

Title: Effects of the Mindfulness-Based Blood Pressure Reduction (MB-BP) program on depression and functional neural connectivity

Authors: *B. NEPHEW[1], R. CALI[3], J. J. POLCARI[2], L.-B. LINNELL[2], F. SAADEH[4], E. LOUCKS[4], J. KING[2];
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Abstract: Hypertension is a leading cause of death and disability in the US, with a prevalence of 46% where approximately only 50% of patients have it adequately controlled. Due to the significant challenges in the long-term efficacy and adverse effects of pharmacological interventions, there is a critical need for complimentary interventions for hypertension. While the 8-week Mindfulness-Based Blood Pressure Reduction program (MB-BP) induces enduring decreases in blood pressure, the neural correlates of this effect are unknown. The objectives of this study were to identify functional neural connectivity correlates of MB-BP using resting state functional MRI in a subset of participants (14 MB-BP, 22 active controls) from the stage II MB-BP RCT and assess potential associations with key clinical outcomes. MB-BP participants exhibited increased interoception and decreased depressive symptoms compared to controls. Analyses of MRI data revealed significant effects of MB-BP in multiple functional neural networks: default mode, cerebellar, executive function, sensorimotor, frontoparietal, auditory, and visual. Changes in neural functional connectivity were associated with measures of interoception and depression. Limitations include small sample size (leading to insufficient power in the analysis of blood pressure), the study duration (3 months), and the inclusion of only 2 time points. It is concluded that MB-BP induces alterations in functional connectivity of the brain which could mediate beneficial changes in depression, interoceptive awareness, and blood pressure in individuals with hypertension.

Disclosures: B. Nephew: None. **R. Cali:** None. **J.J. Polcari:** None. **L. Linnell:** None. **F. Saadeh:** None. **E. Loucks:** None. **J. King:** None.

Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.14

Topic: F.06. Autonomic Regulation

Title: Heart Rate Variability and Physical & Mental Exhaustion in Postmenopausal State

Authors: E. TUMURBAATAR[1], ***B. CHOIJAMTS**[3], T. JADAMBA[2], B. LKHAGVASUREN[4];
[1]Dept. of clinical neuroscience, [2]Brain and Mind Res. Institute, Mongolian Acad. of Sci., Ulaanbaatar, Mongolia; [3]MNUMS, Ulaanbaatar,, Mongolia; [4]Dept. of Psychosomatic Med., Intl. Univ. of Hlth. and Welfare Narita Hosp., Narita, Japan

Abstract: Background: There is a little evidence regarding the extent to which age-related changes in Heart Rate Variability (HRV) depend on simultaneous changes in levels of estrogen and body composition as it occurs from premenopausal state to postmenopausal state. The aim of this study was to evaluate HRV comparing menopausal symptoms in pre and postmenopausal groups. **Methods:** This cross-sectional study was conducted between July and October 2020, as a part of MonTime-Line study. Autonomic function was assessed among perimenopausal (n=87) and postmenopausal (n=99) women (n = 186) aged between 40 and 65 (51.1±7.1). We used Menopause rating Scale (MRS) to measure menopausal symptoms. HRV 5 minutes short time recording was performed based on the guidelines of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. **Results:** "Q7: Physical & mental exhaustion" and "Q8: Sexual problems" level in postmenopausal women had significantly lower HF (p = 0.041; 0.003) and higher LF (p=0.042; 0.003) when expressed in normalized units. The ratio of LF/HF, the index of sympathovagal balance was significantly higher (p = 0.03; 0.042) among postmenopausal women. No significant differences of HRV index by the severity of menopausal symptoms were observed in women premenopausal state. **Conclusion:** Our result suggest that postmenopausal symptoms are associated with altered sympathovagal control. In particular, Physical & mental exhaustion and Sexual problems in moderate or severe degree are related to increase of sympathetic nerve activity.

Disclosures: E. Tumurbaatar: None. **B. Choijamts:** None. **T. Jadamba:** None. **B. Lkhagvasuren:** None.

Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.15

Topic: F.06. Autonomic Regulation

Support: FAPESP 2015/20500-3
CAPES
CNPq

Title: Changes in arterial pressure induced by taste stimuli in normotensive and spontaneously hypertensive rats

Authors: E. D. PEREIRA, Jr, L. A. DE LUCA, Jr, *J. MENANI, C. A. F. ANDRADE; Physiol. and Pathology, UNESP, Araraquara, Brazil

Abstract: The spontaneously hypertensive rats (SHRs), an animal model of primary hypertension, have a typical increased sodium preference compared to normotensive strains. Our previous results suggest that NaCl palatability is increased and independent from body fluid balance in SHRs. However, it still unknown if taste stimuli applied to the oral cavity would also change autonomic responses. Here, we investigated whether salt, sweet, and bitter taste stimuli would change mean arterial pressure (MAP) and heart rate (HR). Male adult SHRs and normotensive Holtzman rats with an intraoral (IO) cannula and a femoral artery catheter had MAP and HR recorded during IO infusion (1 ml/min) of 0.3 M NaCl, 2% sucrose, 1.4 mM quinine sulphate solutions. All tastes stimuli increased MAP in normotensive rats (NaCl: Δ 9 ± 1; sucrose: Δ 8 ± 1; quinine: Δ 12 ± 1 mmHg) and even more in SHRs (NaCl: Δ 26 ± 3; sucrose: Δ 17 ± 2; quinine: Δ 32 ± 3 mmHg). Alpha1-adrenergic receptor blockade with prazosin (1 mg/kg of body weight intravenously) decreased baseline MAP in SHRs (control: 173 ± 2, vs. prazosin: 93 ± 7 mmHg) and normotensive rats (control: 103 ± 2, vs. prazosin: 80 ± 2 mmHg) and increased HR in SHRs (control: 361 ± 10, vs. prazosin: 405 ± 18 bpm) and normotensive rats (control: 431 ± 17, vs. prazosin: 518 ± 14 bpm). Taste stimuli-induced changes in MAP were abolished by prazosin in SHRs (NaCl: Δ -1 ± 1; sucrose: Δ 4 ± 2; quinine: Δ 5 ± 3 mmHg) and in normotensive rats (NaCl: Δ -2 ± 2; sucrose: Δ 3 ± 1; quinine: Δ 3 ± 2 mmHg). The present results suggest that the affective component of taste induces cardiovascular responses mediated by the sympathetic nervous system. In addition, these responses were enhanced in SHRs compared to normotensive rats.

Disclosures: E.D. Pereira: None. **L.A. De Luca:** None. **J. Menani:** None. **C.A.F. Andrade:** None.

Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.16

Topic: F.06. Autonomic Regulation

Title: Cardiac Signals Affect Cortical Motor Excitability andMuscle Activity

Authors: *E. AL[1,2], T. STEPHANI[3], M. ENGELHARDT[4], S. HAEGENS[5], A. VILLRINGER[3], V. V. NIKULIN[2];
[1]Columbia Univ., Columbia Univ., New York, NY; [2]Neurol., Max Planck Inst., Leipzig, Germany; [3]Max Planck Inst. for Human Cognitive and Brain Sci., Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany; [4]Charite Universitatmedizin, Berlin, Germany; [5]Dept. of Psychiatry, Div. of Systems Neurosci., Donders Inst. For Brain, Cognition & Behaviour, New York, NY

Abstract: Internal bodily signals such as heartbeats can influence human perception and action. For instance, in a series of behavioral and EEG experiments, we have shown that somatosensory perception is impaired both during the systolic phase of the cardiac cycle and when heartbeats evoke strong cortical responses. Here, we examine whether these cardiac effects originate from general changes in excitability. For this purpose, cortical and corticospinal excitability was assessed using electroencephalographic and electromyographic responses to transcranial magnetic stimulation (TMS) while monitoring cardiac activity using electrocardiography in thirty-six participants. At the end of the TMS experiment, subjects also performed a motor pinch task. Our results demonstrated that cortical and corticospinal excitability was maximal during systole as compared to diastole. In line with this finding, in the motor task, muscle activity and desynchronization of sensorimotor oscillations (8-25 Hz) were observed to be stronger following muscle contractions during systole. Complementing these results, we also observed that TMS led to heart-rate decreases specifically in systole but not in diastole. In addition to the cardiac cycle effects, increases in cortical responses to heartbeats, as measured by heartbeat-evoked potentials, predicted stronger corticospinal excitability. These findings show that systolic cardiac signals are associated with a facilitatory effect on motor excitability. This is in contrast to the cardiac-related sensory attenuation previously reported for somatosensory perception. Altogether these findings thus suggest that action and perception have distinct windows in the cardiac cycle for optimal information processing.

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Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.17

Topic: F.06. Autonomic Regulation

Support: NIH/SPARC OT2OD026585
Dr. Miriam and Sheldon G Adelson Medical Research Foundation

Title: Ultrastructural Features of the Right Atrial Ganglionic Plexus (RAGP) in Rhesus Macaques Provide Insights into Autonomic Modulation of Cardiac Function

Authors: *G. CHIAROTTO[1], N. P. BISCOLA[1], J. D. TOMPKINS[3], L. A. HAVTON[2,4]; [1]Neurol., [2]Neurol. and Neuroscience,, Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Cardiac Arrhythmia Center, Dept. of Med., David Geffen Sch. of Medicine, UCLA, Los Angeles, CA; [4]James J Peters Veterans Affairs Med. Center, Bronx, New York, NY

Abstract: The right atrial ganglionic plexus (RAGP) is a component of the intrinsic nervous system of the heart. The mammalian RAGP ganglia contain cholinergic neurons and receives cholinergic input, and RAGP influence is essential for normal sinoatrial node function. The RAGP is an emerging target of interest for therapeutic interventions, including electrical stimulation strategies for neuromodulation, in cardiac conditions. However, the synaptic and neural organization of the RAGP varies between species, and there is limited information on the detailed and fine structure of the RAGP in primates, including humans, to guide development of refined neuromodulation strategies. We aimed to characterize the neural organization of RAGP ganglia in adult rhesus macaques using light microscopy (LM) and transmission electron microscopy (TEM) after plastic resin-embedding of tissues. Immunohistochemistry for PGP9.5 and LM analysis show the RAGP to be composed of multiple ganglia, which are interconnected by several nerve fiber bridges. Toluidine blue-stained LM sections of individual ganglia show clusters of ellipse-shaped neuronal somata with prominent nuclei, surrounded by a loose neuropil which includes small myelinated axons. TEM studies of RAGP ganglia show neurons with large nucleus and an organelle-rich cytoplasm. The RAGP neurons are surrounded by satellite cells with prominent nuclei and their processes in close apposition with the neuronal cell membrane. The neuropil includes dendritic profiles and axo-dendritic synaptic contacts, symmetric synaptic specializations and presence of clear spheroid vesicles and some dense-core vesicles in the presynaptic boutons. TEM studies of inter-ganglionic fiber bundles show small myelinated and predominantly unmyelinated fibers in multiple peripheral nerve bundles connecting individual ganglia. Each nerve fiber bundle is surrounded by a thick perineurium. We conclude that the RAGP in primates show a complex synaptic organization with axo-dendritic contacts and ganglionic innervation by both myelinated and unmyelinated fibers. The RAGP model system in rhesus macaques may be used for future studies of cardiac physiology and cardiovascular dysregulation in e.g. aging, neuropathy, and neurological trauma to the spinal cord.

Disclosures: G. Chiarotto: None. **N.P. Biscola:** None. **J.D. Tompkins:** None. **L.A. Havton:** None.

Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.18

Topic: F.06. Autonomic Regulation

Support: NIH SPARC Grant OT2OD023848

Title: Comparative neurobiology of intrinsic cardiac neurons from mice, pigs and humans

Authors: *J. D. TOMPKINS[1], J. PATEL[1], D. B. HOOVER[2], E. H. SMITH[2], L. A. HAVTON[3], N. BISCOLA[3], K. SHIVKUMAR[1], J. L. ARDELL[1];
[1]UCLA Cardiac Arrhythmia Ctr., UCLA, Los Angeles, CA; [2]East Tennessee State Univ., East Tennessee State Univ., Johnson City, TN; [3]Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Intrinsic cardiac neurons (ICNs) are integral to neural control of the heart. Mammalian and nonmammalian species have been used to study these cells, yet a paucity of data from human cells exists. As these neurons are targeted in the treatment of cardiac disease, a more developed understanding of human ICNs is necessary. We used a multidisciplinary approach to complete a detailed comparison of the structure and function of ICNs from humans, mice and pigs. Immunohistochemistry of whole and sectioned ganglia, transmission electron microscopy, intracellular microelectrode recording and dye filling for quantitative morphometry were used to define the neurophysiology, neurochemistry and ultrastructure of these cells. Human ICNs are distinct from those in pig and mouse in regards to multiple parameters. The densely packed, smaller ICNs of the mouse lack dendrites, form axosomatic connections, and have high synaptic efficacy evident of obligatory synapses. In pig and human, a convergence of discrete cholinergic inputs synapse onto extensive dendritic arbors supporting a greater integrative role. Neuropeptide staining shows unique patterns of chemical coding in ganglia across species. While basic membrane physiology is conserved, potassium conductances regulating membrane excitability diverge, with small-conductance potassium channels contributing more greatly to human ICN repolarization and excitability. We have created a publicly accessible, multimodal atlas of ICNs from mice, pigs, and humans identifying similarities and differences in the evolution of these epicardial neurons. A scaling of cellular complexity in relation to heart size, lifespan and phylogenetic classification is identified.

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Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.19

Topic: F.06. Autonomic Regulation

Support: NRF-2021M3E5D2A01022391

Title: Investigation of cardiovascular response to trigeminal nerve electrical stimulation

Authors: *C. KIM[1], H. KIM[2], S. LIM[2], Y. PARK[3], D. KIM[3], I. KIM[2], D. JANG[2];
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Abstract: Recently, many studies reported that non-invasive electrical stimulation related to the autonomic nervous system modulation is applied to various fields such as rehabilitation and treatment. The trigeminal nerve is a cranial nerve connected to the vagus nerve through the brainstem and controls the activity of the parasympathetic nerve stimulation of the central nervous system. However, few studies have previously shown cardiovascular response to trigeminal nerve stimulation. In this study, the trigeminal nerve stimulation experiment was performed with four groups (Sham, 2Hz, 20Hz, 200Hz stimulation frequency group). The experiment protocol total time was 21 minutes, repeating the stimulation on/off section for 2 minutes 3 times after the initial rest section of 7 minutes. This protocol monitored electrocardiography (ECG) and photoplethysmography (PPG) real-time. In addition, heart rate (HR) and pulse arrival time (PAT) were calculated from these signals. As a result, HR was significantly decreased in the 2Hz, 20Hz, and 200Hz groups except for the sham group after the first stimulation. The decrease in HR indicated that the activity of the parasympathetic nervous system was modulated through the trigeminal nerve. Unlike HR, which showed a recovery response after 30 seconds of stimulation, PAT recovered after 10 seconds and showed a sharper response. These results indicate that the recovery time from changes may differ from physiological measurements. In conclusion, the changes in HR and PAT after stimulation indicated that non-invasive trigeminal nerve stimulation could modulate parasympathetic nervous system activity.

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Poster

222. Neurovisceral Physiology II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 222.20

Topic: F.06. Autonomic Regulation

Support: HINRI LABS

Title: Can restoration of sexual behavior facilitate synergistic recovery of other organ systems after nine years of complete paralysis?

Authors: *I. MONTOYA[1], H. R. TORRES SOLANO[1], H. ZHONG[1], P. GAD[2], V. EDGERTON[1];
[1]RANCHO RESEARCH INSTITUTE, RANCHO LOS AMIGOS NATIONAL REHABILITATION CENTER, DOWNEY, CA; [2]SPINEX INC., NORTHRIDGE, CA

Abstract: All sensory modalities directly or indirectly project to the motor system via the interneuronal network in the spinal cord. Following severe spinal cord injury (SCI), all organ systems are markedly impaired. Post SCI, engaging sensory modalities may facilitate positive activity-dependent network reorganization of neural networks in the brain and spine. We hypothesized that the extensive multimodal sensations triggered during sexual behaviors may facilitate the functional reorganization of neural networks responsible for the control of sensory-motor and autonomic systems. To explore this hypothesis we have studied the changes in sexual function in a male subject, living with complete SCI at T4 for >9 years. Non-invasive spinal neuromodulation and activity-based neurorehab therapy (ABNT) were used to reactivate and retrain the spinal neural networks. Within weeks of therapy, the subject reported a response in erectile function to a range of tactile stimulations during foreplay to levels of 7-9 (self-reported rigidity scale 0-I0). Visual, olfactory, and taste stimulations facilitated erection typically to levels of 8, 6, and, 6 respectively, lasting 10-15 minutes with rigidity sufficient for vaginal penetration. To sustain the erection for vaginal ejaculation (30-45 min) a 1.0ml alprostadil penile injection was usually needed. During ejaculation, rigorous rhythmic lower body movements occur. Heightened pleasurable penile sensations occurred during insertion analogous to a climax. Climax and ejaculation were accompanied by rapid flexions of the lower limbs and novel sensations extending from the bottom of the feet to the testicles and anus. Anorectal sensations were similar to that of a bowel movement. Post sexual activity, significant changes in cardiovascular responses were observed including reddening of the face and increased heart rate from about 65 to 120 bpm. Changes in other autonomic functions include an increase in bladder capacity from 50 to 250ml and a reduction in emptying frequency of the clamped suprapubic tube-connected drainage bag by 50%.The time between detecting bladder fullness to a response in the urethral sphincter to prevent a leak increased.The time required for bowel emptying was reduced from about 60 to 30 mins, with a sensation of defecation and awareness of complete emptying.The ability to perspire as needed demonstrated improved thermoregulation. It is critical to note that all of these changes were observed in the absence of active spinal neuromodulation. These data demonstrate the integrative nature of sensorimotor-autonomic systems to ABNT and neuromodulation, and how they could be facilitated by sexual behaviors.

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Poster

223. Sleep Regulation and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 223.01

Topic: F.07. Biological Rhythms and Sleep

Support: NC3Rs PhD Studentship NC/S001689/1
NC3Rs Early Careers Engagement Award
MRC grant MR/S01134X/1

Title: Investigating sleep homeostasis and dynamics in the presence of fasting-induced torpor in laboratory mice

Authors: *S. L. WILCOX[1,2], V. MUNDAY[1,2], J. MENGUAL[1,2], E. MEIJER[1,2], L. E. MCKILLOP[1,2], V. VAN DER VINNE[5,1,2], S. N. PEIRSON[3,2], D. M. BANNERMAN[4], V. V. VYAZOVSKIY[1,2];
[1]Physiology, Anat. and Genet., [2]Sleep and Circadian Neurosci. Inst., [3]Nuffield Dept. of Clin. Neurosciences, [4]Exptl. Psychology, Univ. of Oxford, Oxford, United Kingdom; [5]Biol., Williams Col., Williamstown, MA

Abstract: Torpor is a profoundly altered state of physiology, characterised by hypometabolism and hypothermia, that is readily induced in mice by common food restriction (FR) paradigms. An interaction between torpor and sleep homeostasis has been described in other rodent species (Vyazovskiy et al 2017), however, this interaction has not been well characterised in mice. We aimed to address the question of how sleep regulation and torpor interact by performing chronic electrophysiological (EEG and EMG) and peripheral body temperature recordings in male C57BL/6J mice (n=9). This approach allowed us to simultaneously assess sleep and torpor during ad libitum feeding and FR. A subset of mice underwent auditory stimulation across vigilance states, including torpor. We found that FR resulted in a regular occurrence of torpor in all mice on most days. As a result, sleep-wake architecture was significantly altered, with mice spending more time awake in the light phase and overall, more time in EEG-defined sleep (primarily NREM, 56% vs 38%, $p<0.05$), as compared to ad libitum feeding ($p<0.05$). To investigate sleep homeostasis, mice were sleep deprived for 4 hours during ad libitum and FR. All mice were provided with food immediately after sleep deprivation to minimise acute effects of hunger on subsequent sleep. The rebound of EEG slow wave activity (0.5-4Hz, SWA), a measure of sleep intensity, following these manipulations was compared to sleep following an undisturbed torpor bout of comparable length. All conditions resulted in an increase in SWA above baseline, indicating an increase in sleep pressure. However, the peak SWA was significantly lower during FR, and following torpor, compared to ad libitum condition (Ad lib peak: 153 ± 1.3%; FR peak: 118 ± 2.1%; Torpor peak: 98 ± 4.6%; $p=0.027$). Auditory stimulation did not disrupt sleep in food restricted or ad libitum conditions, nor did it prevent torpor induction or induce arousal from torpor during FR. Evoked response potentials in the EEG were observed in all vigilance states. To our knowledge, this is the first time that sleep homeostasis in response to torpor induction has been investigated in laboratory mice. These results demonstrate that torpor and food restriction drastically alter sleep and activity patterns. Moreover, our result suggest that fasting-induced torpor has a sleep depriving effect, consistent with the effects of spontaneous torpor in other species.

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Poster

223. Sleep Regulation and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 223.02

Topic: F.07. Biological Rhythms and Sleep

Support: R37 HD081168

Title: Rhythmic bursting activity in the parafacial zone is associated with the developmental emergence of cortical delta waves during the second postnatal week in rats

Authors: M. AHMAD, *G. SOKOLOFF, M. S. BLUMBERG;
Psychological and Brain Sci., Univ. of Iowa, Iowa City, IA

Abstract: In developing rats, the cortical delta activity (1-4 Hz) characteristic of slow-wave sleep emerges suddenly between postnatal day (P) 9 and P11. The developmental emergence of delta activity could reflect anatomical changes in cerebral cortex and/or changes in subcortical structures that modulate cortical activity. Regarding the latter possibility, the parafacial zone (PZ), which is located lateral and dorsal to the facial nerve, has been identified in adult mice as being particularly important for the expression of slow-wave sleep: activation of the GABAergic/glycinergic projections from the PZ to the wake-promoting parabrachial nucleus triggers slow-wave sleep and cortical delta activity (Anaclet et al., *Nature Neuroscience*, 2014). Here, we hypothesized that the PZ would exhibit both state-dependent and delta-activity-dependent changes in neural activity in P12 rats, when delta activity is present, but not in P10 when delta activity is absent. To test this hypothesis, we recorded extracellular neural activity from the PZ and cortical EEG in P10 and P12 rats as they cycled freely between sleep and wakefulness. At P12, but not P10, PZ units exhibit state-dependent activity characterized by rhythmic bursts that are synchronized with cortical delta waves. Importantly, a recent study found that respiratory rhythms modulate delta activity during sleep (Karalis & Sirota, *Nature Communications*, 2022). As the PZ receives projections from brainstem regions involved in respiration, we further hypothesized that neural activity in PZ is synchronized with the respiratory rhythm only during slow-wave sleep; indeed, that is what we find. These results suggest that PZ activity contributes to the developmental emergence of delta activity and mediates the coupling between respiratory and delta oscillations during slow-wave sleep.

Disclosures: M. Ahmad: None. **G. Sokoloff:** None. **M.S. Blumberg:** None.

Poster

223. Sleep Regulation and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 223.03

Topic: F.07. Biological Rhythms and Sleep

Support: O'Sullivan Family Graduate Scholarship

Title: A reduction in sleep EEG spindle activity in 'layer 6b silenced' mice

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Abstract: In this study, we investigate the role of cortical layer 6b (L6b) in sleep-wake regulation. L6b is derived from the early generated subplate, which plays an essential role during cortical development. When this function is completed, a large proportion of subplate neurons undergo preferential cell death, but a proportion of subplate cells survive into adulthood to form L6b in rodents or interstitial white matter cells in primates. Yet, the function of L6b beyond cortical development is unknown. Anatomical input to L6b is predominated by long-range intracortical projections, allowing integration of ongoing activity. Moreover, it can be activated by orexin/hypocretin, a neuromodulator involved in arousal and energy homeostasis. Thalamic projections of L6b selectively target higher order thalamic nuclei, which are involved in cortico-thalamo-cortical networks and brain state control. All these characteristics suggests that layer 6b may be involved in regulation of sleep and waking. We investigated the role of L6b in a mouse model with a Cre-dependent truncation in the gene for Synaptosomal Associated Protein of 25 kDa (SNAP25) in a subset of L6b neurons (Drd1a-Cre::Snap25fl/fl)(Hoerder-Suabedissen et al., 2019). This makes the subset of L6b synaptically silenced from the time of birth. Continuous electro-encephalography/electro-myography (EEG/EMG) recording was undertaken over 24-hours in freely behaving animals, and vigilance states were manually annotated in 4-s epochs for subsequent analysis. We find that L6b silenced animals and Cre negative control littermates spent comparable time in each of the vigilance states (layer 6b silenced (n=9) vs controls (n=7), Wake 41.5±1.83% vs 42.50±1.32% (mean ±SEM, unpaired t-test, t(14)=0.52, p=0.61), NREM 46.65±1.57% vs 44.92±0.81% (t(14)=0.90,p=0.38), REM 7.41±0.41% vs 7.99±0.72% (t(14)=0.74, p=0.47)). EEG spectral analysis revealed a significant reduction in power density in the 13-22.75 Hz range in the frontal EEG during NREM sleep in L6b silenced animals (two-way ANOVA over 0.5-30Hz with genotype x frequency, Genotype, F(1,14)=10.51, p=0.0059, post-hoc tests with Bonferroni correction), which includes the sigma frequency range (10-15 Hz), an expression of sleep spindle activity. This is the first study to investigate the effect of L6b silencing on sleep. Our results show a reduction in sleep spindle activity during NREM sleep. This suggests that L6b may be involved in the network for sleep spindle generation and maintenance, a specific component of sleep linked to memory consolidation, sensory disconnection during sleep and neural plasticity.

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Poster

223. Sleep Regulation and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 223.04

Topic: F.07. Biological Rhythms and Sleep

Title: Circuit Basis of Cortical Theta Oscillations during REM Sleep

Authors: *A. TAKSOKHAN, J. FRAIGNE, J. PEEVER;
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Abstract: REM sleep is characterized by rapid-eye movements, vivid dreaming, motor atonia, and cortical theta oscillations (4-8Hz). Although theta oscillations during REM sleep are associated with facilitating learning and memory, virtually nothing is known about the circuits that regulate theta oscillations during REM sleep. Therefore, this project aims to identify the mechanisms and pathways which underlie the regulation of theta oscillations during REM sleep. My **working hypothesis** is that the glutamate sublaterodorsal tegmental nucleus (SLD^{GLU}) neurons which generate the state of REM sleep, communicate with the GABA medial septum (MS^{GABA}) cells that generate theta oscillations, to selectively control theta oscillations during REM sleep. Here, we used genetically-assisted track tracing, electrophysiology, Ca^{2+} imaging, and optogenetic approaches to identify the circuit basis of cortical theta oscillations during REM sleep in freely behaving mice. First, we used Ca^{2+} imaging and fiber photometry to show that the activity of SLD^{GLU} are positively correlated with theta oscillations ($r=0.73$, $p<0.001$, $n=7$), suggesting that they have the potential to orchestrate theta oscillations during REM sleep. Next, our anatomical data indicated that SLD^{GLU} neurons do not directly project to MS^{GABA} neurons. Instead, they innervate glutamate neurons in the parabrachial nucleus (PB^{GLU}), which in turn project to MS^{GABA} neurons ($n=12$). Then, we used optogenetics to probe the function of the $SLD^{GLU-->}PB^{GLU-->}MS^{GABA}$ circuit in regulating theta oscillations during REM sleep. We confirmed that silencing MS^{GABA} neurons markedly decreased theta power during REM sleep (laser on vs laser off; paired t-test, $p<0.001$, $n=10$). Moreover, silencing SLD^{GLU} neurons significantly reduced theta power during REM sleep (laser on vs laser off: paired t-test, $p<0.01$, $n=5$), confirming that SLD^{GLU} neurons are important for theta oscillations. Finally, we showed that silencing the axon terminal of PB^{GLU} neurons in the MS significantly reduced theta power during REM sleep (laser on vs laser off; paired t-test, $p<0.001$,$n=8$), suggesting that PB^{GLU} neurons could function as a SLD-->MS relay center. Altogether, our study explores the function and pathways by which the SLD and MS nuclei communicate to regulate theta oscillations during REM sleep. These oscillations have important implications in learning and memory, and thus, identifying how the brain regulates them may enhance our understanding of these cognitive functions.

Disclosures: **A. Taksokhan:** None. **J. Fraigne:** None. **J. Peever:** None.

Poster

223. Sleep Regulation and Mechanisms

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Program #/Poster #: 223.05

Topic: F.07. Biological Rhythms and Sleep

Support: Middlebury College Undergraduate Collaborative Research Fund

Title: Local cortical sleep need can initiate homeostatic changes in sleep intensity or duration

Authors: A. GREEN[1], K. N. MONTEN[2], L. GRANGER[2], *M. B. DASH[3];
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Abstract: The homeostatic regulation of sleep ensures that sufficient sleep duration and/or intensity are produced to meet sleep needs generated during previous waking. Sleep need appears, at least in part, to be generated within local neuronal populations. Although these local needs may be met through local increases in sleep intensity (e.g. increased slow wave activity; SWA), it remains unclear how disparate sleep needs across local populations ultimately affect the homeostatic regulation of global behavioral state. To address this question, in freely-behaving rats we recorded local field potentials within the prefrontal and motor cortices to assess the homeostatic response to 1) 3hr global sleep deprivations, 2) 3hr, local cortical infusions of an artificial cerebral spinal fluid (ACSF) that significantly reduced the expression of local slow waves, and 3) 3hrs of simultaneous global sleep deprivation and local cortical ACSF infusion. Reduced global sleep duration was offset by homeostatic increases in local SWA and ultimately produced daily slow wave energy (SWE; a measure of total sleep efficacy) levels that were not significantly different from undisturbed baseline recordings. Reduced local SWA following ACSF infusion, by contrast, was offset by significant increases in total non-rapid eye movement sleep (NREM) duration that likewise restored SWE to baseline levels. Strikingly, only when both sleep duration and intensity were experimentally diminished, was the brain unable to maintain sleep homeostasis; here, significantly less daily SWE was observed as compared to baseline. Collectively, these results indicate that sleep intensity and duration may provide redundant mechanisms to maintain sleep homeostasis. Moreover, they provide novel evidence to indicate that sleep need within local cortical regions can modulate global sleep duration.

Disclosures: **A. Green:** None. **K.N. Monten:** None. **L. Granger:** None. **M.B. Dash:** None.

Poster

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Program #/Poster #: 223.06

Topic: F.07. Biological Rhythms and Sleep

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Title: Sleep Regulation by Multiplexing Neural Codes

Authors: *M. TABUCHI, A. N. HUTSON, Y. ZHANG, E. M. PAUL, E. E. FAULK, S. D. DANIELS, L. H. ZUKOWSKI;
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Abstract: Millions of Americans suffer from poor sleep quality including frequent brief awakenings during the night, which has a significant impact on their health and productivity. A better understanding of the molecular and cellular mechanisms underlying sleep quality may help promote the development of novel pharmacologic agents to alleviate the suffering associated with fragmented sleep. Drosophila is now well-established as a model organism for studying sleep, and my recent studies using Drosophila sleep have defined a new molecular pathway linking the circadian clock to temporal-specific neural coding to regulate sleep quality. Importantly, our results demonstrate that specific patterns of spiking activity in circadian clock neurons can induce a novel form of synaptic plasticity to regulate sleep quality. We have named this form of plasticity SPDP (Spike Pattern Dependent Plasticity). Moreover, we now found that SPDP synergistically interacts with timing information to alter sleep quality. We identified a novel sleep regulation mechanism by characterizing molecular and coding mechanisms mediating SPDP as well as its dynamic interaction with timing information. Specifically, we examined how distinct activity pattern of neural activity can trigger SPDP, how SPDP and timing information can synergistically interact to act as multiplexing neural codes, and how controlling such multiplexing neural codes can regulate sleep quality. Together, these studies lead to a better understanding of the molecular and multiplexing coding mechanisms of SPDP to regulate sleep quality and thus facilitate the potential development of a novel methodology for maintaining our healthy performance during wakefulness and for being a therapeutic target for the treatment of sleep disorders.

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Poster

223. Sleep Regulation and Mechanisms

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Topic: F.07. Biological Rhythms and Sleep

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22H04918

20H00567
JPMJCR1655

Title: Regulation of sleep amount by SIK3 in hypothalamic nuclei

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Abstract: Sleep amount is tightly regulated in a homeostatic manner. Although a number of sleep/wake switching neural circuits have been identified, the mechanisms by which these neural circuits contribute to homeostatic regulation of sleep remain to be elucidated. We previously found that a gain-of-function *Sleepy* (*Slp*) mutation in the *salt-inducible kinase 3* (*Sik3*) gene, which produces the mutant SIK3(SLP) protein, increases non-REM sleep (NREMS) and NREMS EEG delta density, a marker for the level of sleep need. However, it remains to be elucidated which cells and brain regions are responsible for the increased NREMS in *Sleepy* mutant mice. In this study, we aim to determine the cell type and neuronal groups that enhance quality and quantity of NREMS through SIK3(SLP) expression. First, we have generated *synapsin1*^{CreERT2}*;Sik3*$^{Slp\text{-}flox/+}$ mice that enable us to induce *Sik3(Slp)* in mature neurons upon tamoxifen injections. *Synapsin1*^{CreERT2}*;Sik3*$^{Slp\text{-}flox/+}$ mice administrated with tamoxifen at late infancy exhibited increased NREMS time and NREMS EEG delta density, compared to tamoxifen-administrated *synapsin1*^{CreERT2}*;Sik3*$^{+/+}$ mice. In addition, simulated sleep-homeostatic Process S of *Sik3*$^{Slp/+}$ and *synapsin1*^{CreERT2}*;Sik3*$^{Slp\text{-}flox/+}$ mice with tamoxifen administration showed that increased ratio between the rates of sleep-need accumulation and dissipation than *Sik3*$^{+/+}$ and *Synapsin1*^{CreERT2}*;Sik3*$^{+/+}$ mice, respectively. These results suggest that SIK3 in mature neurons plays an important role in regulating sleep amounts and sleep need. Next, we have explored neuronal groups through which SIK3(SLP) expression increases NREMS, by AAV-mediated expression of SIK3(SLP) in the hypothalamus. We found that SIK3(SLP) expression in the medial part of the hypothalamus increased NREMS compared to kinase-dead mutant SIK3(T221A) expression. This result implies that SIK3(SLP) expression in specific brain regions directly or indirectly modulates the activity of neurons executing the switch of sleep/wake states, which results in increased NREMS amounts. We expect that SIK3 takes part in cellular signaling conveying seep need. This will be an important step to connecting the intracellular signal representing sleep need with the circuit-level mechanism for sleep/wake switch.

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Poster

223. Sleep Regulation and Mechanisms

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Program #/Poster #: 223.08

Topic: F.07. Biological Rhythms and Sleep

Support: Tiny Blue Dot Foundation
NIH NCCAM P01AT004952-10

Title: Neural signatures of conscious experience during sleep: a serial awakening study using high-density EEG

Authors: *A. SELTE, T. VANASSE, B. SEVAK, K. GJINI, B. RIEDNER, T. ALAUDDIN, G. TONONI, M. BOLY, A. STRUCK;
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Abstract: Rationale: Sleep represents a key condition to study the neural correlates of consciousness, because of the frequent alternations between conscious and unconscious epochs alternating within the same behavioral state. Serial awakening paradigms coupled with high-density EEG (HDEEG) allow to identify differences in brain activity between conscious and unconscious sleep epochs thanks to the collection of subjective reports about subjective experiences just prior to awakening, while discarding behavioral confounds. Methods: 256 electrode HDEEG recordings were performed in 115 healthy subjects at the University of Wisconsin-Madison sleep laboratory. The subjects were awakened by the examiner to report about their experiences about 10 times per night (n= 1389 awakenings). The awakenings were divided into conscious experience (CE) and no conscious experience (NCE) based on questionnaires. Five minutes of HDEEG data pre-awakening were bandpass-filtered between 1-55 Hz, with visual rejection of noisy epochs and channels, after which Independent Component Analysis (ICA) was performed. One minute of HDEEG data prior to awakening was used for the final analysis. We used data of CE and NCE epochs originating from non-REM sleep stage 2 for our statistical analysis (64 subjects, 296 awakenings) using a linear mixed effect model. HDEEG topographies of power in delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz), gamma (30-50 Hz) bands, as well as Lempel Ziv complexity (LZC), sample entropy and spectral exponent were compared between conditions. We also compared the HDEEG topography of a unique measure - the Cho-Gaines distance, which is the Euclidean distance between the observed data distribution and an ideal Benford's distribution in time domain - for CE and NCE sleep epochs. False discovery rate (FDR) was used to correct for multiple comparisons across analyses. Results: Although the CE group showed increased LZC and spectral exponent in the posterior regions, increased sample entropy in the central regions, and decreased delta and theta power overall, these differences were not significant after correction with FDR. The most consistent difference between CE and NCE conditions was found to be significantly higher Cho-Gaines distance in the posterior region. Conclusions: Our results suggest that Cho-Gaines distance, comparing the EEG signals to a a distribution following Benford's law, reliably distinguished between CE and NCE epochs during non REM sleep. Follow up work will attempt

to design multivariate algorithms incorporating EEG power and Cho-Gaines features to detect covert consciousness in patients with severe brain damage in the future.

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Poster

223. Sleep Regulation and Mechanisms

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Topic: F.07. Biological Rhythms and Sleep

Support: Wu Tsai Human Performance Alliance

Title: Impact of Alzheimer's disease on non-visual light perception and SCN connectivity

Authors: *H. CALLIGARO[1], B. KHOV[1], K.-Y. KIM[2], W. K. JU[3], M. H. ELLISMAN[4], S. PANDA[1];
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Abstract: Circadian disruption, and specifically sleep disruption, is an early indicator of Alzheimer's disease (AD), occurring before the onset of more commonly known neurodegenerative symptoms such as memory loss. The suprachiasmatic nucleus (SCN), the central circadian clock in the brain responsible for regulating the timing of many biological rhythms, receives direct retinal input through melanopsin-expressing retinal ganglion cells (mRGCs) to synchronize with the environment light cycle. The anatomical basis for circadian disruption in the SCN of those with Alzheimer's disease is largely unknown. Here, we sought to identify the cellular basis of the observed circadian perturbations by studying in parallel the impact of AD on SCN and retinal melanopsin cells at different ages in an AD mouse model.
To study the connectivity with the SCN, we generated image volumes from serial block-face scanning electron microscopy of 3-month and 8-month APP/PS1 mice. We observed changes in neurons and neurites morphology, associated with neuronal loss. By quantifying connectivity parameters, we noticed an increase in bouton frequency on axons with an increase in their synaptic partners in the 8-month SCN compared to the 3-month SCN. Moreover, the dendro-dendritic network that we had previously assumed to be important for its proper functioning seems to be almost absent in the aged mouse SCN.
In addition, we recorded the electrical response to light of mRGCs in APP/PS1 mice aged 3 to 8 months. We observed hyperactivity associated with increased sensitivity of mRGCs of 3 months AD mice. However, at 8 months, mRGCs of AD mice showed a reduced discharge rate compared to WT mice.
Together, these results suggest that AD induces crucial changes in both the retina and the SCN at

the early stages of the disease that may be responsible for circadian disruption in those with Alzheimer's disease. The next step will be to identify specific changes in mRGCs connectivity within the SCN to elucidate the role of retinal input in the onset of circadian symptoms of AD.

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Poster

223. Sleep Regulation and Mechanisms

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Program #/Poster #: 223.10

Topic: F.07. Biological Rhythms and Sleep

Support: R01 NS051305
R01 NS076980

Title: Mapping sleep circuits in Drosophila using gaboxadol, a potent sleep promoting drug

Authors: K. MELNATTUR[1], Z. MARSHALL[2], Z. LI[3], M. FORD[3], E. PERIANDRI[3], B. VAN SWINDEREN[4], ***P. SHAW**[3];
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Abstract: The GABA-A agonist Gaboxadol increases sleep in *Drosophila*. The sleep induced by Gaboxadol exhibits all the molecular, physiological, and functional hallmarks of spontaneous sleep. Importantly, Gaboxadol-induced sleep restores short-term memory and long-term memory to classic memory mutants underscoring its importance as a sleep promoting agent. In mammals and flies, Gaboxadol impacts sleep via specific GABA-A receptor subunits. In the fly, Gaboxadol acts primarily via the *Glycine receptor* (*Grd*) and *Ligand-gated chloride channel homolog 3* (*Lcch3*) GABA-A receptors. To identify the neural circuits impacted by Gaboxadol to promote sleep, we conducted an unbiased screen by knocking down the *Grd* receptor using over 40 GAL4 lines. Surprisingly, knocking down *Grd* in octopaminergic, dopaminergic, or serotonergic neurons did not attenuate the ability of Gaboxadol to increase sleep. However, we have identified ~14 unique GAL4 lines, including specific subsets of neurons implicated in learning and memory, that attenuate Gaboxadol-induced sleep. These results will allow more precise investigations into the relationship between sleep and memory.

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Poster

223. Sleep Regulation and Mechanisms

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Topic: F.07. Biological Rhythms and Sleep

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Title: Effect of total sleep deprivation on the sleep-wake cycle and the taiep rat: a model of tubulinophaty

Authors: *K. ESPINOZA[1], C. CORTES[2], A. B. SILVA[3], J. R. EGUIBAR, Sr.[4];
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Abstract: The *taiep* rat is a mutant of the tubulin β 4A gene (TUBB4A) characterized by initial hypomyelination followed by progressive demyelination of the central nervous system. *Taiep* rats are the only animal model of the disease called hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC). Additionally, *taiep* rats had a disorganized sleep-wake pattern and had immobility episodes (IEs) with similar characteristics to rapid eye movement (REM) sleep. Our group showed that IEs had behavioral and electroencephalographic characteristics similar to cataplexy episodes in narcolepsy type 1, however, they had a normal number of orexin neurons in the lateral posterior hypothalamus. This study aims to evaluate the effect of periods of short and long-duration sleep deprivation on the sleep-wake cycle in the *taiep* rat. We used 12 male *taiep* rats, using stereotaxic surgery, we implanted electrodes in the cerebral cortex, neck muscles, and the orbit of the eye. All subjects were maintained under standard conditions. We did 24h control records coupled with video-recordings. The subjects were divided into periods of short (6h) and long (12h) of total sleep deprivation by the gentle handling method. The records were evaluated by classifying the stages of the sleep-wake cycle and the IEs. Our results showed that there was a significant difference in the frequency and duration of awake and non-REM bouts of sleep in the dark with respect to the light phase ($p<0.05$). Six hours of total sleep deprivation induced a significant increase in non-REM sleep in the recovery phase ($p<0.05$), importantly there was a reduction in the number of REM-sleep bouts and increased immobility episodes. In conclusion, *taiep* rat a model of tubulinopathy had a sleep rebound instead of the demyelination in the central nervous system.

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Poster

223. Sleep Regulation and Mechanisms

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Program #/Poster #: 223.12

Topic: F.07. Biological Rhythms and Sleep

Support: HD081168

Title: Moving While Sleeping: On the Paradoxical Co-occurrence of Muscle Atonia and Twitching

Authors: Z. YOU, G. SOKOLOFF, *M. BLUMBERG;
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Abstract: During active (or REM) sleep, discrete, jerky movements called myoclonic twitches occur against a "background" of muscle atonia. The neural mechanisms that allow these two seemingly contradictory phenomena to co-occur are not known. One view holds that twitches are produced when a large descending motor signal overpowers the inhibition on spinal motor neurons. An alternative view is that atonia and twitching are coordinated by the same brainstem network that regulates active sleep. One such structure is the sublaterodorsal tegmental nucleus (SLD), which plays a key role in adult rats in the production of atonia, but whose contribution to twitching has not been examined. Here, we investigated the relationship of SLD neurons to atonia and twitching during a period in early development when twitching is abundant. We recorded extracellular neural activity from the SLD in 12-day-old head-fixed rats as they cycled freely between sleep and wake (n=8). To assess the relationship between twitches and neural activity, we also recorded limb movements using high-speed video and tracked the movements using DeepLabCut. Consistent with the adult literature, the majority of SLD neurons (35/56) were significantly more active during periods of sleep-related atonia. Interestingly, for a subset of the atonia-on neurons (n=27), we saw increased activity around the onset of a burst of twitches. Moreover, these neurons were most active during bursts of twitches that involved movements of all four limbs. The exact role of the SLD in the production of twitching is not yet clear. Nonetheless, the discovery of twitch-related neurons in a structure that plays a causal role in the production of muscle atonia suggests that twitching is coordinated within the brainstem's sleep-regulatory network. These findings open new avenues for further research into the neural mechanisms that coordinate twitching and atonia during active sleep.

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Poster

223. Sleep Regulation and Mechanisms

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Topic: F.07. Biological Rhythms and Sleep

Support: Lundbeck Foundation Grant R313-2019-735

Title: Regulatory pathways underlying nocturnal increase in cerebrospinal fluid

Authors: *B. L. E. HENRIKSEN[1], A. B. STEFFENSEN[1], D. BARBUSKAITE[1], S. N. ANDREASSEN[1], M. H. OLSEN[2], N. MACAULAY[1];
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Abstract: The cerebrospinal fluid (CSF) dynamics may fluctuate during the day-night cycle and could, as such, modulate metabolite clearance in the brain, the intracranial pressure (ICP), and other sleep-related physiological processes. We here determined ICP and CSF dynamics during day and night in humans and rats. Through a combination of patient data obtained from the Neurointensive Unit at Rigshospitalet, Denmark, and *in vivo* telemetric probe measurements in adult male rats, we demonstrate that the ICP increases at night (in the dark phase), independently of activity level in the nocturnal rats. This increase aligns with elevated nightly CSF collection in patients and CSF production rate in rats, the latter obtained with the ventriculo-cisternal perfusion assay, modified to accommodate measurements in non-anaesthetized, freely moving rats. The raise in the rat CSF production rate at night was, in part, assigned to increased transport activity of the choroid plexus Na^+,K^+,$2Cl^-$ cotransporter (NKCC1), which is implicated in CSF secretion by this tissue. RNA sequencing of rat choroid plexus demonstrated differential nightly expression of other choroid plexus transport mechanisms that may contribute to CSF secretion alongside NKCC1. These results suggest that CSF secretion, and thus ICP, increases at night in man and rat, irrespective of their diurnal/nocturnal lifestyle, in part due to altered transport activity of a choroid plexus transport mechanism. Our findings thus indicate that CSF dynamics might not be sleep dependent, but rather coupled to the light/dark period.

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Poster

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Support: Israel Science Foundation
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Title: Investigating modularity in locus coeruleus norepinephrine regulation of sleep and wakefulness

Authors: *N. MATOSEVICH, N. REGEV, Y. NIR;
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Abstract: The locus coeruleus (LC) is the main source of brain norepinephrine (NE) and is involved in multiple functions including regulation of vigilance states. However, we only have limited understanding of the downstream pathways through which the LC regulates arousal and sleep awakenings. In adult male c57bl/6j mice, we set out to test the possibility that LC-NE activities in forebrain and brainstem targets may be partially distinct and potentially biased to mediate either sensory or motor aspects of arousal, respectively. First, we tested anatomical modularity by examining the extent to which LC neurons projecting to the basal forebrain (BF) or the brainstem pontine reticular nucleus (PRN) comprise distinct subpopulations. To this end, we injected retrobeads to either BF (n=7) or PRN (n=8), and blindly analyzed expression patterns in the LC. We found that the location of PRN-projecting LC neurons was significantly biased to ventral locations compared with BF-projecting LC neurons located more dorsally (Monte-Carlo permutation test, p=0.0007). Second, we validated setups that would allow to investigate potential physiological markers of this modularity, by monitoring calcium activities in LC subpopulations (with retrograde virus injections and GCaMP monitoring) and by measuring extracellular NE dynamics in target regions (using $GRAB_{NE}$). Using fiber photometry of LC calcium activity with GCaMP6s we first validated that, under anesthesia, LC activity induced by noxious stimulation coincides with pupil dilation (n=7, p=0.031 Wilcoxon signed rank test). Next, combining fiber photometry with EEG/EMG/video monitoring during 24-hours of undistributed wakefulness and sleep, we observed higher calcium activity in LC in wakefulness compared to sleep, in line with electrophysiology literature. Similar patterns were observed when monitoring LC subpopulations attesting to robust signals. We are now exploring the associations between activities in distinct LC subpopulations and different indices of arousal. Finally, we validated measurements of extracellular NE levels in forebrain regions using $GRAB_{NE}$ by establishing their parametric modulation by LC optogenetic stimulation and correlation with pupil diameter under anesthesia. We are now extending $GRAB_{NE}$ measurements to natural sleep and wakefulness to examine modulation by vigilance states and how NE signaling acting at distinct target regions relates to different indices of arousal.

Disclosures: N. Matosevich: None. **N. Regev:** None. **Y. Nir:** None.

Poster

223. Sleep Regulation and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 223.15

Topic: F.07. Biological Rhythms and Sleep

Title: Caudate Volume and Sleep Duration

Authors: N. JONES, ***T. IKUTA**;
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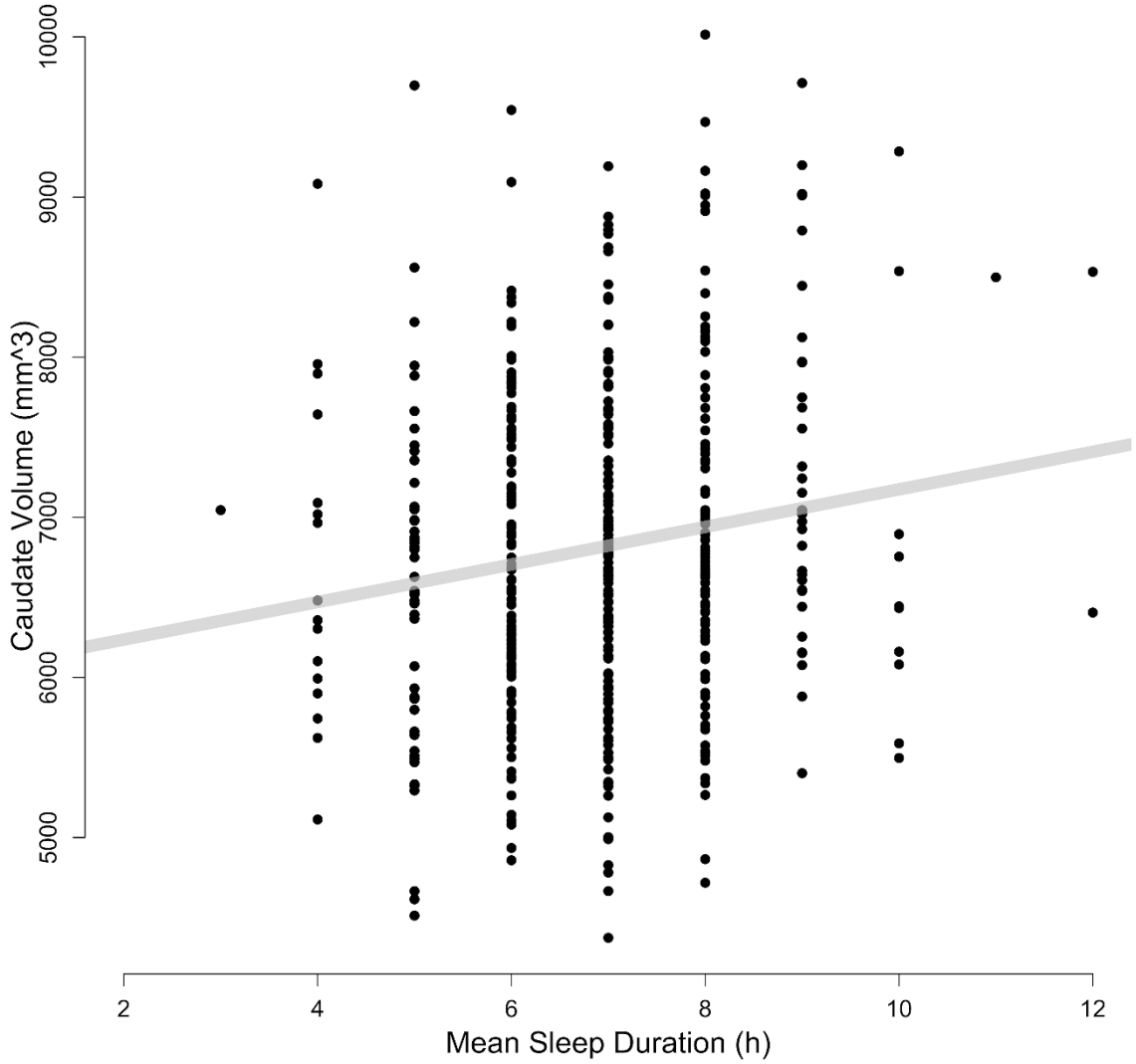
Abstract: The role of the caudate nucleus in sleep has been implicated. Previous literature showed that the caudate volume is associated with longer habitual sleep duration in older adults.

However, the association between sleep duration and caudate volume remains unknown in the younger population. In this study, we examined the caudate volume in youth to older adults (10 to 85 years old) with a greater sample size (N=463). The volumetric size of the caudate nucleus showed a significantly positive association with habitual sleep duration. Sleep duration showed a significant association with executive function performance. However, caudate volume did not significantly predict executive function. There was a non-linear association between age and sleep duration and between age and caudate volume, where sleep duration and caudate volume declined toward middle age. The association between caudate volume and sleep duration was only found in the younger population, failing to replicate previous findings. While our results suggested that sleep duration is associated with the caudate volume and executive function, it is also suggested that there may be some external mechanisms that modulate executive function, which prevent the caudate-sleep relation's effect on executive function.



Disclosures: **N. Jones:** None. **T. Ikuta:** None.

Poster

223. Sleep Regulation and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 223.16

Topic: F.07. Biological Rhythms and Sleep

Support: ERC STg-715933 iNAPS to RHW

Title: Sleep deprivation methodology and cortical dynamics

Authors: *C.-L. LEU[1,2], M. HESSEL[1], E. VOGGENREITER[1], M. MILLER[1], R. H. WILLIAMS[1];
[1]Helmholtz Zentrum München, Neuherberg, Germany; [2]Tech. Univ. of Munich, Munich, Germany

Abstract: Sleep is an important behavior that all animals need to sustain life. Sleep is regulated by a two-process model, the circadian rhythm and the sleep homeostat. In sleep homeostasis, the drive to sleep increases with sustained arousal and dissipates with sleep onset. Circadian rhythm affects when sleep onset can easily occur. Our understanding of circadian components for sleep is evolving yet how the brain senses sleep need or which brain regions are directly involved in the sleep homeostat is more elusive. Nevertheless, to study the sleep homeostat researchers must induce sleep loss, normally in experimental animals. The gold standard method is gentle handling by a trained researcher for a defined period of time to evoke sleep deprivation (SD). However, the technique varies between sleep laboratory, involves significant researcher - animal interaction which can lead to stress effects on the animal or diminished concentration of the researcher. We therefore examined how manual SD compared a treadmill-induced sleep loss in C57Bl6J mice (male and female).
Mice were implanted with abdominal telemeters (HD-X02, DataSciences Inc) for wireless EEG and EMG capture. Following post-operative recovery, mice were subjected to 4h and 6h SD periods manually or by walking on a treadmill (speed ≤ 3cm/s). Assessment of total SD and delta power in recovery sleep were carried out. Additionally we performed further technical comparisons to assess the similarity of SD modalities, namely 1) whole brain activity mapping using c-FOS expression as an indicator for cellular activity, 2) calcium imaging of cortical neurons via microendoscope recordings, and 3) PiezoSleep screen to compare pressure sensor detection of vigilance states to EEG. Through these different analyses we found that while trained researchers can ensure consistent sleep loss during sleep deprivation periods (90-95%), treadmill evoked sleep loss was more efficient and easier to implement. Secondly, whole brain mapping revealed similarities in brain regions activated in SD between manual- or treadmill-induced sleep loss but also subtle differences. Nevertheless, overall the robustness and ease of treadmill-induced sleep loss may prove an important methodology for adoption for researchers of sleep homeostasis.

Disclosures: **C. Leu:** None. **M. Hessel:** None. **E. Voggenreiter:** None. **M. Miller:** None. **R.H. Williams:** None.

Poster

223. Sleep Regulation and Mechanisms

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Program #/Poster #: 223.17

Topic: F.07. Biological Rhythms and Sleep

Support: ERC STg-715933 iNAPS to RHW

Title: Cortical astrocytes and sleep homeostasis

Authors: *M. HESSEL[1], C.-L. LEU[1,2], M. MIęKUS[1,2], E. VOGGENREITER[1], R. H. WILLIAMS[1];
[1]Inst. of Neurogenomics, Helmholtz Zentrum München, Neuherberg, Germany; [2]Tech. Univ. of Munich, Munich, Germany

Abstract: The activity of cortical astrocytes contributes to fluctuations in sleep-wake behaviour. Here we elucidate the role of astrocytes within the anterior cingulate cortex in sleep homeostasis by assessing how activation of astrocytes contribute to sleep loss and cortical excitability. Using Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-based chemogenetic tools we specifically activated cortical astrocytes in freely behaving female mice. Animals bilaterally injected with pAAV-GFAP-hM3D(Gq)-mCherry (Addene code: 50478, stereotactic coordinates related to bregma: AP: (- 0.02)-(-0.1) mm, ML: ± (0.6-0.8) mm, DV: 1.5 mm) underwent 4h sleep deprivation (SD) starting at ZT0 or left undisturbed. At the end of SD or ZT4, animals were injected with either CNO (0.3 mg/kg, i.p.) or vehicle (saline, i.p.) in a crossover randomised design. Vigilance state was assigned using a PiezoSleep screen to differentiate movement and breathing activity. In a separate cohort, mice were also stereotaxically injected with AAV1.Camk2a.GCaMP6f (Inscopix, Mouse Neocortex, ID: 1000-002613) and a GRIN lens implanted to observe *in vivo* calcium imaging of cortical neurons and an abdominally implanted transmitter (HD-X02, DataSciencesInc) for EEG analyses. Quantification analyses were performed on histological expression of astrocytes in Aldh1l1-creERT:Ai14 mice in different sleep conditions (4h SD, 4h SD & 2h recovery sleep or undisturbed time-matched controls). We observed a reduction in the number of fluorescently-tagged cortical astrocytes in mice that were sleep deprived. Chemogenetic activation of cingulate/motor cortical astrocytes reduced total sleep time only in undisturbed mice for 4h post-dose yet had no effect when activated at the end of SD. Our data support a role for cortical astrocytes in sleep-wake states. When sleep-drive is high, such as following sleep loss, supplemental activation of astrocytes is no longer effective in modifying sleep-state. This may be due to a change in astrocyte functionality.

Disclosures: **M. Hessel:** None. **C. Leu:** None. **M. Miękus:** None. **E. Voggenreiter:** None. **R.H. Williams:** None.

Poster

223. Sleep Regulation and Mechanisms

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Program #/Poster #: 223.18

Topic: F.07. Biological Rhythms and Sleep

Support: Natural Sciences and Engineering Research Council of Canada USRA
Carleton University International Research Seed Grant
European Research Council STG-715933

Title: Characterization of amygdala neurons activated by sleep deprivation

Authors: *N. K. KOZIEL LY[1], C.-L. LEU[2,3], R. H. WILLIAMS[2], M. J. CHEE[1];
[1]Neurosci., Carleton Univ., Ottawa, ON, Canada; [2]Inst. of Neurogenomics, Helmholtz Zentrum München, Neuherberg, Germany; [3]Tech. Univ. of Munich, Munich, Germany

Abstract: Chronic sleep deprivation (SD) is associated with worsened health outcomes, including increased risk for mental health disorders. The amygdala is a stress and emotional-processing center, thus it may link mood deficits seen with SD. The mouse amygdala is activated by sleep loss, and our preliminary findings show increased c-FOS expression following SD relative to well-rested control mice. However, the spatial distribution and electrical properties of SD-activated amygdala cells are yet unresolved. In order to characterize the amygdalar nuclei activated by SD or that support SD recovery, male wildtype mice (n = 3 per group) underwent SD for 4 h (SD group), SD for 4 h then 2 h of recovery sleep (RS group), or slept undisturbed for 4 h (control group). We quantified the number of c-FOS+ cells in all mice from each group using Nissl-stained coronal sections to guide our parcellation of amygdalar nuclei. After SD, we observed significantly more c-FOS+ cells in the basolateral (BLA; 7-fold higher), central (CEA; 5-fold higher), and medial amygdala (MEA; 5-fold higher). Interestingly, the number of c-FOS+ cells in the BLA remained elevated after RS but was lower in the CEA and MEA. Next, to assess if the increase in c-FOS activity marker corresponded with functional neuronal activation, we performed whole-cell patch-clamp recordings from acute amygdala slices of control and SD mice. The properties of BLA and CEA cells following SD support their excitability, which may be expressed in different ways. BLA cells from SD mice required less current to elicit action potential firing (SD: 37 ± 7 pA, n = 11; control: 80 ± 9 pA, n = 10; $p = 0.002$) and exhibited more spike activity (SD: 20 ± 5, n = 11; control: 3 ± 2, n = 7; $p = 0.020$). CEA cells did not exhibit increased firing following SD, but they did exhibit reduced current flow at negative voltage potentials (SD: -165 ± 23 pA, n = 6; control: -283 ± 57 pA, n = 5; $p < 0.001$) consistent with the dampening or closure of ion channels related to membrane hyperpolarization. Interestingly, we did not detect changes in the excitability of MEA cells after SD. Taken together, we show that SD-mediated excitation corresponded with the increase in c-FOS marker

within the amygdala, but the mechanisms of activation may differ in a region- or cell-type specific manner. Robust changes in excitability, such as that seen in the BLA, may reflect sustained c-FOS activity even following RS, and ensuing studies will assess if RS would differentially reverse the excitability of amygdalar cells in a region-specific manner. These findings point to novel amygdalar candidates engaged by mounting sleep pressure and that may mediate heightened arousal during SD.

Disclosures: **N.K. Koziel Ly:** None. **C. Leu:** None. **R.H. Williams:** None. **M.J. Chee:** None.

Poster

223. Sleep Regulation and Mechanisms

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Program #/Poster #: 223.19

Topic: F.07. Biological Rhythms and Sleep

Support: ERC STg-715933 iNAPS to RHW

Title: Anas: amygdala neurons activated by sleep deprivation

Authors: D. D. LAM[1,2], **C.-L. LEU**[1,2], M. HESSEL[1], I. K. DELIGIANNIS[3], E. VOGGENREITER[1], C. P. MARTINEZ-JIMENEZ[3,2], *R. H. WILLIAMS[1];
[1]Helmholtz Zentrum München, Neuherberg, Germany; [2]Tech. Univ. of Munich, Munich, Germany; [3]Helmholtz Pioneer Campus, Neuherberg, Germany

Abstract: Given the ubiquity of sleep loss and its profound negative effects on health, well-being, and productivity, there is a pressing social and economic need to develop new therapeutic strategies to improve the quality of sleep. Effective sleep aids require understanding which brain centres contribute to sleep homeostasis and govern the delicate balance between sleep need and sleep drive.
We applied whole brain activity mapping from C57Bl/6J mice (male and female; n=8/cohort, age 24 ± 9 weeks old) subjected to 4h manual sleep deprivation (SD), 4h SD and 2h recovery sleep (RS), or undisturbed time-matched controls (C4, or C6 respectively). Brain regions were parcellated based on the Allen Brain Atlas and the number of c-FOS+ nuclei in each brain region computed. Next single nucleus RNA seq (snRNA-seq) was performed on cortical and amygdala samples from male C57Bl/6J mice (n= 6; 2 pooled replicates) after 6h manual SD, 6h SD and 30 min RS or undisturbed time-matched control at C6. After sample processing, cells are clustered and annotated to a reference atlas. Expression of Fos and other immediate early genes between cells were compared across sleep conditions using two-tailed Kruskal-Wallis tests with Dunn's post-test analyses.
Quantification of c-FOS expression after optical clearing indicated increased expression patterns during SD in the amygdala and specific subregions compared to RS or control groups. We did not observe statistically significant changes in any brain region following RS. These initial results permitted follow-up snRNAseq on amygdala and cortical regions to identify precisely

molecularly defined cell types activated by perturbations of sleep homeostasis. Functional follow-up *in vivo* will reveal which are likely to be involved in sensing or responding to these perturbations to affect behaviour and vigilance state.

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Poster

223. Sleep Regulation and Mechanisms

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 223.20

Topic: F.07. Biological Rhythms and Sleep

Support: MOST-110-2636-B- 002-020

Title: Acute light-induced calcium responses of suprachiasmatic nucleus cells are correlated to circadian time in anesthetized mice

Authors: *P.-T. YEH[1,2], K.-C. JHAN[3], S.-C. WU[3], S.-K. CHEN[1];
[1]Dept. of Life Sci., Natl. Taiwan Univ., Taipei City, Taiwan; [2]Taiwan Intl. Grad. Program in Interdisciplinary Neurosci., Academia Sinica, Taipei City, Taiwan; [3]Dept. of Engin. and Syst. Sci., Natl. Tsing Hua Univ., Hsinchu City, Taiwan

Abstract: Creatures on our planet actively adjust their endogenous clocks so that their physiological and behavioral functions could act in concert with the periodic changes of days and nights. Light exposure entrains the circadian rhythms in a discrete manner, that light could advance or delay the endogenous clocks depending on the circadian time of the light stimulation, which could be plotted as phase-response curves (PRC). In mammals, the suprachiasmatic nucleus (SCN) lies at the bottom of the hypothalamus serves as the central pacemaker that coordinates the circadian rhythms, as well as receives the light information transmitted through the retinohypothalamic tract (RHT). Furthermore, previous studies indicated that those RHT neurons innervate a large volume and diverse parts of the SCN, suggesting multiple light reception and entrainment hubs may exist in the SCN. We hypothesized that the advance and delay phases of PRC are driven by different light entrainment hubs in the SCN. In addition, we hypothesized that the acute light responses of those entrainment hubs are correlated to the circadian time of light stimulation. To test our hypotheses, we combined a set of techniques including calcium imaging, gradient-index (GRIN) lens optics, and two-photon microscopy. The regions of interest were identified and aligned in images acquired from different circadian time points so that the cellular light responses could be temporally compared. More than 100 units per animal could be collected and analyzed between different circadian times. Here we found that SCN neurons were acutely responsive to light onsets and offsets. Analysis of the cellular light responses showed distinct groups of “hub” neurons, which had increased correlations of activity patterns to other neurons. Moreover, those hub neurons were responsive to light stimulations at

specific circadian times. Our findings may give a hint to the nonlinear nature of circadian photoentrainment.

Disclosures: **P. Yeh:** None. **K. Jhan:** None. **S. Wu:** None. **S. Chen:** None.

Poster

223. Sleep Regulation and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 223.21

Topic: F.07. Biological Rhythms and Sleep

Support: Fund for Research on Anesthesia at the Hebrew University of Jerusalem
Hebrew University's Seymour and Cecile Alpert Chair in Pain Research to author MD
Author MB is recipient of the Dr. Willem Been Legacy Fellowship

Title: Anesthetic loss of consciousness induced by chemogenetic excitation of mesopontine effector neurons.

Authors: *M. BARON[1], M. DEVOR[2];
[1]Hebrew Univ. of Jerusalem, Hebrew Univ. of Jerusalem, Jerusalem, Israel; [2]Hebrew university of Jerusalem, Jerusalem, Israel

Abstract: Although general anesthesia is normally induced by systemic dosing, an anesthetic state can be induced in rodents by microinjecting minute quantities of GABAergic agents into the brainstem mesopontine tegmental anesthesia area (MPTA). Correspondingly, lesions to the MPTA render rats relatively insensitive to standard anesthetic doses delivered systemically and insomnia. Using a chemogenetic approach we have identified and characterized a small subpopulation of neurons restricted to the MPTA which, when excited, render the animal anesthetic by sensorimotor (immobility) and electroencephalographic (EEG) criteria. These "effector-neurons" do not express $GABA_A\delta$-Rs, the likely target of GABAergic anesthetics. Rather, we report a distinct sub-population of nearby MPTA neurons which do. These likely excite the effector-neurons by disinhibition. Within the effector population ~70% appear to be glutamatergic, ~30% GABAergic and ~40% glycinergic. Most are projection neurons that send ascending or descending axons to distant targets associated with the individual functional components of general anesthesia: atonia, analgesia, amnesia, and loss-of-consciousness. Identifying the MPTA “effector-neurons” opens the way to determining how GABAergic agents excite them in situ and how this excitation alters neural activity in their projection targets such that bistable transitioning between conscious and unconscious brain states occurs. Functional differentiation of effector pathways might be exploited by selective recruitment of a subset of effector-neurons. Driving descending connectivity, for example, might permit surgical quality pain control without sedation. Likewise, it might prove possible to reanimate a comatose patient by selectively recruiting appropriate ascending pathways.

Disclosures: **M. Baron:** None. **M. Devor:** None.

Poster

223. Sleep Regulation and Mechanisms

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Program #/Poster #: 223.22

Topic: F.07. Biological Rhythms and Sleep

Support: VA BX000798
VA 1K6BX004216

Title: Zona incerta Lhx6 neurons are most active during NREM and REM sleep and after prolonged waking

Authors: *P. SHIROMANI[1], C. A. BLANCO-CENTURION[2], A. VIDAL-ORTIZ[1];
[1]Ralph H Johnson VA Med. Ctr., Charleston, SC; [2]Psychiatry and Behavioral Sci., Med. Univ. of South Carolina, Mount Pleasant, SC

Abstract: To determine how a waking brain falls asleep we are monitoring the activity of brain neurons. We focus on GABA neurons in the zona incerta (ZI) because transfer of the orexin gene into ZI neurons blocks cataplexy (Liu et al., JNeurosci, 2011) and vGAT GABA neurons in the ZI anticipate the onset of NREM (Blanco-Centurion et al., SLEEP, 2021). As there are multiple subtypes of GABA neurons in the brain, we now examine the activity of neurons containing the transcription factor, Lhx6. This study tested the hypothesis that for GABA neurons to drive sleep their activity should be further increased after prolonged waking. In Lhx6-cre mice (mice=4; all females; 4-5 mo), rAAV-DIO-GCaMP6s was delivered stereotaxically to the ZI (isofluorane anesthesia) and a GRIN lens, along with EEG and EMG electrodes were implanted. 21d later a miniscope (INSCOPIX) recorded fluorescence in individual Lhx6 neurons for 4h (baseline). On another day, the mice were kept awake for 6h (gentle handling; 9a-3p) and fluorescence in Lhx6 neurons was recorded for 2h during recovery sleep. The imaged data from the two recording periods (baseline and recovery sleep) was combined into a single data file and the change in fluorescence was determined against the mean image frame (F0). Previously, we and others found that the GCaMP6 calcium fluorescence is a direct measure of action potentials and serves as a marker of activity. 97 neurons were automatically extracted (PCA-ICA analysis; blinded without knowledge of sleep state). In 66 neurons (68%) the average fluorescence was significantly higher during REM, NREM, or both, compared to waking (Mixed Model ANOVA; SPSS25; $P<0.01$). In this population, the fluorescence was significantly higher during recovery sleep compared to baseline ($P<0.001$) indicating increased activity of the sleep-active neurons during recovery sleep. With ensuing sleep, the increase in fluorescence gradually returned to baseline levels, attesting to the fluorescence as a marker of homeostatic sleep pressure. In 14 neurons (14%), fluorescence was highest in waking as compared to the other states during baseline, and in these neurons, fluorescence did not increase after sleep loss. Interestingly, six neurons (6%) were most active in waking and NREM but silent in REM (REM-off). This is the

first study to measure fluorescence in individual neurons after sleep loss. We found that the fluorescence in two-thirds of ZI Lhx6 neurons was tightly linked to sleep and that the average fluorescence was further increased after prolonged waking. Microendoscopy follows each neuron longitudinally and provides a better gauge of sleep pressure versus indirect measures such as c-FOS or photometry.

Disclosures: P. Shiromani: None. **C.A. Blanco-Centurion:** None. **A. Vidal-Ortiz:** None.

Poster

223. Sleep Regulation and Mechanisms

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Program #/Poster #: 223.23

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant R01NS094571
NIH Grant R35NS122181
NIH Grant NS112266

Title: A Local Clock Mechanism in the Lateral Amygdala Coordinates Rhythmic Touch Sensitivity and Anxiety

Authors: *Q. LIU, D. KIM, S. LEE, J. XIONG, M. KELES, B. BELL, C. ALEXANDRE, S. BLACKSHAW, A. LATREMOLIERE, M. WU;
Johns Hopkins, BALTIMORE, MD

Abstract: The suprachiasmatic nucleus (SCN) organizes the circadian timing of behaviors and internal states. However, local brain clocks outside the SCN also exist, but their functions remain unclear. Here, we identify and characterize a local clock mechanism in the lateral amygdala (LA) that coordinates sensory perception and emotional state in a time-dependent manner. The LA has previously been suggested to house a local brain clock, and in support of this notion, immunostaining reveals that the core clock protein Per2 cycles in this region. Next, we focused on mWAKE, a conserved clock output molecule that acts to suppress excitability at night and is postulated to label rhythmic neural circuits in the brain. mWAKE is specifically expressed in the LA, and its levels cycle in this region, peaking at dusk. We used mWAKE-Cre mice, in combination with stereotaxic viral injections, to label and manipulate mWAKE^{LA} neurons. We find that mWAKE^{LA} neurons project to secondary somatosensory cortex (S2) and nucleus accumbens (NAc). Optogenetic activation of mWAKE^{LA} terminals at S2 or NAc reduces touch sensitivity and increases anxiety-like behavior, respectively. In wild-type mice, these behaviors are rhythmic in constant darkness, with increased touch sensitivity and reduced anxiety observed during the subjective night. To address whether these rhythms are under local clock control, we generated a novel Cre-dependent Clock-dominant negative virus. We used this tool to disrupt the local LA clock selectively in mWAKE^{LA} neurons, which led to loss of cycling of touch perception and anxiety-like behaviors. Similar phenotypes were observed following conditional

knockout of mWAKE in the LA, suggesting that mWAKE acts downstream of the local clock in the LA to promote these behavioral rhythms. Whole cell patch-clamp slice recordings from mWAKE^{LA} neurons reveal that their intrinsic excitability cycles, with reduced excitability at night. In *mWake* mutants, this electrical cycling is lost, with mWAKE^{LA} neurons exhibiting increased excitability at night. Thus, mWAKE levels in the LA are higher at night, and this leads to suppression of mWAKE^{LA} neuron activity. This in turn results in greater touch sensitivity and reduced anxiety at night when mice are active and exploring; conversely, this mechanism reduces touch perception and increases anxiety during the day, when mice may hide in a burrow to sleep. In summary, we define a novel circuit that coordinates sensory perception and internal emotional state in a time-dependent manner and demonstrate a key role for a local brain clock and mWAKE in this process.

Disclosures: **Q. Liu:** None. **D. Kim:** None. **S. Lee:** None. **J. Xiong:** None. **M. Keles:** None. **B. Bell:** None. **C. Alexandre:** None. **S. Blackshaw:** None. **A. Latremoliere:** None. **M. Wu:** None.

Poster

223. Sleep Regulation and Mechanisms

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Program #/Poster #: 223.24

Topic: F.07. Biological Rhythms and Sleep

Support: Howard Hughes Medical Institute
Brain and Behavior Research Foundation Young Investigator Grant 29767

Title: Rhythmic Transcriptome Network and a Significant Connection between the Rhythmic Transcriptome and Autism Spectrum Disorder

Authors: *C. LEE[1], J. S. TAKAHASHI[2];
[1]The Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [2]Chair, Dept. of Neurosci., Univ. of Texas Southwestern Med. Center, Howard Hughes Med. Inst., Dallas, TX

Abstract: Autism spectrum disorder (ASD) has had significant impacts on medical and social care world-wide with approximately 1 case in every 68 people. ASD is known to be driven by changes in the transcriptome and is also associated with impacts on circadian rhythms which are controlled by the internal, biological clock. One such impact is that well over 80% of ASD patients have been documented to suffer from sleep disorders. However, the connection between the disruption of sleep rhythms and ASD has not yet been clearly identified at the transcriptomic level. Here, rhythmic transcriptome studies in nine wild-type mouse brain regions and liver tissues have identified differences in phase and amplitude. Results from the SCN and liver tissues showed a distinct phase advance in their transcriptomes as compared to those of other brain regions. Within the other brain regions, we were also able to identify rhythmic transcriptome networks many of which still showed strong connections to the main network despite the differential phase and amplitude. The exception being the transcriptomes of the

olfactory bulb (OB) and cerebellum (CB) which exhibited weak connections to the rhythmic transcriptome network and thus generated unique expression patterns. Both the OB and CB exhibited genes with phase shifts between day and night of which they were enriched with fear response genes in neuronal synapses. Additionally, the rhythmic genes peaking in the evening within the SCN were significantly enriched for ASD-risk genes similar to the rhythmic genes with peak phases in the other brain regions at night. These genes are strongly enriched for sleep deprivation genes suggesting a connection to the ASD sleep phenotype. Since the frontal cortex is the most disrupted brain region in ASD patients, I further studied its particular transcriptome using single nucleus RNA-seq. The identified cell numbers were from high to low in the following the order: neuron, astrocyte, oligodendrocyte, microglia, and endothelial. Among them, neurons were the only cell type driving this rhythmic gene expression in brain and that this unique rhythmic expression has exceptionally strong enrichment with ASD-risk genes. Taken together, genome-wide transcriptome analyses have shown a significant connection between the rhythmic transcriptome and ASD in neuronal cells.

Disclosures: C. Lee: None. **J.S. Takahashi:** None.

Poster

223. Sleep Regulation and Mechanisms

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Program #/Poster #: 223.25

Topic: F.07. Biological Rhythms and Sleep

Support: ERC Starting Grant (No 802923) awarded to TSK

Title: Sensory inputs to the circalunar clock of Clunio marinus

Authors: *D. BRISEVAC, T. S. KAISER;
Max Planck Inst. for Evolutionary Biol., Ploen, Germany

Abstract: Circalunar clocks are endogenous time-keeping mechanisms, which allow many marine organisms to synchronize behaviour and reproduction with lunar phase. Their molecular components are thus far poorly understood. Here we study the circalunar clock in natural populations of the marine midge *Clunio marinus* (Chironomidae: Diptera). The circalunar clock is synchronized by moonlight and tidal cycles of mechanical agitation, and it controls development of the *Clunio* larvae. Interestingly, one out of ten investigated populations has lost the sensitivity to moonlight, and two have lost sensitivity to mechanical agitation. Crossing experiments between the insensitive and sensitive strains showed that sensitivity to a synchronizer is a genetically determined trait. To further uncover the molecular components of the sensory inputs, we combined quantitative trait loci (QTL) mapping and genome-wide association mapping. QTL mapping suggested an oligogenic origin for the phenotypic loss, with one prevalent additive locus in two out of three insensitive strains. Genome-wide analysis brought to light a few candidate genes with known functions in the development of the sensory

and central nervous system as well as light perception in the moonlight-insensitive strain. The vast majority of the mutations associated with the phenotypic loss fall in intergenic and regulatory regions of the genome. We then asked: Which genes have altered expression upon the loss of sensitivity to moonlight? To provide an answer, we performed RNA sequencing of embryos and larvae of the moonlight-sensitive and -insensitive strains. We screened for genes identified as causal by QTL and association mapping, whose expression was significantly altered in the moonlight-insensitive strain. Taken together, our results reveal a few candidate genes with known roles in light detection. Finally, in order to investigate these candidate genes in the context of the sensory neuronal networks, we are currently applying in situ hybridization. By localizing cells expressing identified candidate genes we hope to trace the sensory inputs into the circalunar clock and finally uncover the cellular components of the circalunar clock.

Disclosures: D. Brisevac: None. **T.S. Kaiser:** None.

Poster

223. Sleep Regulation and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 223.26

Topic: F.07. Biological Rhythms and Sleep

Title: Melatonin analogue activated ROR-α dependent circadian reprogramming ameliorates glioma pathogenesis via PGC1α/SIRT1/NFκB axis.

Authors: *G. BATRA[1], A. R. SHARMA[2], M. MODI[2], A. PRAKASH[4], B. MEDHI[3]; [1]PGIMER, PGIMER, CHANDIGARH, India; [2]Neurol., [3]Pharmacol., PGIMER, Chandigarh, India; [4]Univ. Inst. of Pharmaceut. Sci. (UIPS), Univ. Inst. of Pharmaceut. Sci. (UIPS), Chandigarh, India

Abstract: Introduction: Disrupted circadian rhythm alters the various signalling pathways regulating the cellular proliferation and growth of glioma cells. In this study, circadian rhythm was targeted via activating the RAR-related orphan receptor alpha (ROR-α) using melatonin analogue, UCM1037. Activated ROR-α regulates mitochondrial biogenesis and NFκB induced cytokine release by decreasing SIRT1 and PGC1α. This is the first study that identified the mechanistic link between ROR-α dependent circadian reprogramming and glioma pathogenesis via PGC1α/SIRT1/NFκB axis.**Methods:** *Insilico* docking and molecular dynamic simulation (MD) of ROR-α with UCM1037 and melatonin was performed using the crystal structure from protein database. Further, effect of melatonin analogue was observed in C6 and U87 glioma cell lines. UCM1037 (100µM, 300µM, 600µM, 800µM, 1mM) treated cells were checked for cell viability, apoptosis, ROS production, autophagosomes formation, immunoflouresence, gene expression. Xenograft model was made by ICV injection of rat C6 glioma cells in the brain of Wistar rats. Glioma model was validated via actophotometer, gripstrength, seizue scoring, RT-PCR, H&E and IHC of cancer markers. Animals were divided into Control, Sham, Vehicle (1% DMSO), TMZ (5mg/kg), and UCM1037 (10mg/kg, 20mg/kg, 30mg/kg; i.p.) groups, n=6 per

group. Different parameters like locometer, seizure scoring, RT-PCR, H&E, and IHC, genes expression, and oxidative stress were measured for all groups. All experiments were performed in triplicates. Data was represented in mean±SEM and analyzed by one way ANOVA.**Results:** UCM1037 had strong binding to ROR-α than melatonin with a docking score of -59.72kcal/mol. UCM1037 showed improved pharmacokinetic properties and BBB permeability. UCM1037 treated cells showed cell intrinsic ROR-α dependent significant increase in apoptosis, autophagosomes formation and other cancer associated genes in a dose dependant manner as compared to TMZ treated/control cells. In glioma C6 rat model, UCM1037 (20 mg/kg) showed significant improvement in locomotor parameters, reduced seizure occurrence, reduced oxidative stress. It also increased ROR-α dependent circadian core gene reprogramming leading to decrease in PGC1α and SIRT1 and nuclear localization of NFκB thereby increasing the cancer cell apoptosis.**Conclusion:** ROR-α is a potential target to treat glioma via targeting circadian rhythm. UCM1037, an ROR-α activator leads to increase in apoptosis and decrease in cell growth. Exploration of other mechanistic pathways linking disrupted circadian rhythm and cancer will help in identifying novel targets for the treatment of cancer.

Disclosures: G. batra: None. **A.R. Sharma:** None. **M. Modi:** None. **A. Prakash:** None. **B. Medhi:** None.

Poster

224. Motivation and Food

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 224.01

Topic: G.02. Reward and Appetitive Learning and Memory

Support: 1R15MH122729-01

Title: Sex-dependent effects of cannabidiol (CBD) on habit formation

Authors: C. MOREHOUSE[1], C. MADDOX[1], R. VAN DER MERWE[1], R. MCLAUGHLIN[1], J. SCOTT[1], M. GHANEM[1], E. RAMSSON[2], C. HOWARD[1];
[1]Neurosci., Oberlin Col., Oberlin, OH; [2]Biomed. Sci., Grand Valley State Univ., Allendale, MI

Abstract: Habits are inflexible behaviors that persist despite changes in outcome value. While habits allow for efficient responding, neuropsychiatric diseases such as drug addiction and obsessive-compulsive disorder are characterized by overreliance on habits. Recently, the commercially popular drug cannabidiol (CBD) has emerged as a potential treatment for addictive behaviors, though it is not entirely clear how it exerts this therapeutic effect. As brain endocannabinoids play a key role in habit formation, we sought to determine how CBD modifies goal-directed behaviors and habit formation. To explore this, we trained mice on random interval (RI30/60) or random ratio (RR10/20) schedules designed to elicit habitual or goal-directed lever pressing, respectively. Mice were tested for habitual responding using probe trials following reinforcer-specific devaluation as well as omission trials, where mice had to withhold responding

to earn rewards. Mice were administered either CBD (20 mg/kg i.p.) or vehicle as a control before all behavioral sessions. We found that while CBD had no effect in the reward devaluation task, CBD inhibited goal-directed behavior in a sex-specific and context-dependent manner during the omission task. In the RI experiment, CBD-treated mice demonstrated a decreased ability to update lever-pressing and head entries during the omission task, and this effect was more pronounced in CBD-exposed male mice. Likewise, CBD inhibited omission in female mice trained on the RR schedule. Beyond drug treatment, we found a robust sex effect during training, reward devaluation, and omission. In light of recent interest in CBD's potential to reduce habit-based behaviors, this work provides evidence that CBD has no effect on habit formation in a reward devaluation paradigm. However, the omission results suggest that CBD may slow learning of novel action-outcome contingencies and decrease goal-directed behaviors. This work calls for further examination of sex-dependent outcomes of CBD treatment and highlights the importance of investigating sex effects in habit-related experiments.

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Poster

224. Motivation and Food

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 224.02

Topic: G.02. Reward and Appetitive Learning and Memory

Support: The University of Massachusetts Amherst Department of Biology

Title: Sex differences in adrenergic α1 regulation of reinforcement behavior

Authors: *E. M. RODBERG[1], S. Y. YU[2], E. M. VAZEY[1];
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Abstract: Humans and animals need to exhibit appropriate behavioral responses to environmental cues, both to obtain resources and avoid threats. Appropriate behavioral responses are mediated in part by brainwide norepinephrine (NE) levels and locus coeruleus (LC) neural activity. LC-NE has been shown to have an inverted-U shaped effect on task performance where performance decreases when NE levels are outside an optimal range, either too high or too low. In addition to the relationship between NE and behavior, previous research has identified sex differences in the size, structure, and stress responsivity of LC. Independently, sex-differences have been found in positive and negative reinforcement learning.
To probe the role of α1 adrenergic signaling on reinforcement behavior, we administered an α1 agonist (cirazoline; low dose 0.1mg/kg, high dose 0.3mg/kg) and antagonist (prazosin; low dose 0.5mg/kg, high dose 1mg/kg) in adult Sprague Dawley rats (n=22, 12 female and 10 male) during an active avoidance and reward seeking task. During this task, rats learned to press a lever

in response to a cue for a liquid sucrose reward (reward seeking trials) and press an opposing lever after a different cue to avoid a foot shock (0.25mA, 500ms; active avoidance trials). We found that the ability to appropriately respond to positive and negative reinforcement cues is sensitive to α1 adrenergic signaling in a sex-specific manner. On active avoidance trials, high doses of both α1 agonists and antagonists decreased accuracy in males whereas females were only affected by α1 agonists. This decreased accuracy was driven by increased omitted responses in both males and females. Reward seeking trials were less sensitive to α1 manipulation and accuracy only decreased after high doses of cirazoline in males. Overall, reinforcement behavior in males, particularly to cues predicting a potential threat, was sensitive to α1 manipulation and followed a Yerkes-Dodson relationship as predicted for LC-NE. Females were more resilient to α1 manipulations during this task which may be due to different behavioral strategies or NE receptor distribution. Results from this study indicate that cirazoline and prazosin, by increasing and decreasing α1 noradrenergic signaling respectively, can impair reinforcement behavior and the degree to which α1 modulation impacts behavior differs based on sex.

Disclosures: E.M. Rodberg: None. **S.Y. Yu:** None. **E.M. Vazey:** None.

Poster

224. Motivation and Food

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 224.03

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA 1F31DA05477-01
NIDA 5R01DA042475

Title: Multi-site modulation of extended amygdala activity and affective behaviors via the mGlu8 receptor

Authors: *B. P. NABIT[1], A. A. HUSSAIN[2], D. G. WINDER[3];
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Abstract: Altered neuronal activity within the extended amygdala is implicated in driving drug relapse behaviors and other psychiatric disorders characterized by increased negative affect (or valence) and stress responses. In particular, signaling within the Bed Nucleus of the Stria Terminalis (BNST), specifically by G-protein coupled receptors (GPCR), is thought to mediate neuronal signaling responsible for negative affective behavioral states and stress-induced reinstatement of previously extinguished cocaine-conditioned place preference in rodents. Thus targeting GPCRs coupled to stress-related signaling provides unique opportunities to control relapse-relevant behaviors and signaling pathways. One such receptor is the metabotropic glutamate receptor subtype 8 (mGlu8) - a heterogeneously expressed, G_i-coupled GPCR in the

CNS. Prior studies utilizing constitutive *grm8* knockout animals identified mGlu8 as an interesting target in behavioral neuropharmacology due to altered anxiety behaviors and neuronal signaling patterns in the contexts of stress and drugs of abuse. Furthermore, numerous recent human genetics reports have implicated mutations within the *grm8* gene in driving multiple, relevant psychiatric disorders. Thus, we have developed, and are characterizing, a floxed *grm8* mouse line, $Grm8^{fl/fl}$, to identify cell type- and circuit-specific functions of mGlu8 signaling. Initial data suggest that mGlu8 receptors expressed within the BNST or on efferent BNST terminals do not modulate gross BNST-associated anxiety behaviors in the absence of stress following virally-mediated knockdown. Previous work from the lab has indicated a role for mGlu8 in suppression of excitatory transmission in BNST, suggesting extrinsic mGlu8-modulated afferents could play a role in BNST-mediated behaviors. Future studies will aim to determine the electrophysiological profiles, origins and cell-type specificity of mGlu8-regulated terminals arriving in the extended amygdala and the roles of these circuitries in driving drug-related and related behavioral phenotypes.

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Poster

224. Motivation and Food

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 224.04

Topic: G.02. Reward and Appetitive Learning and Memory

Support: DA016285-05

Title: Role of serotonin 1A and 1B receptors in motivation of male and female rats to respond for sucrose

Authors: *J. W. GRIMM, E. SPAULDING, K. GRIFFIN, F. SAUTER, M. HARDY, R. MARX, A. GILSRUD;
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Abstract: Sucrose self-administration behavior of rats has been used as a model to better understand food or drug-directed behaviors in humans including substance use disorder. The progressive ratio (PR) schedule of reinforcement provides a measure of motivation to take a substance and may be interpreted as a measure of reinforcement efficacy. Several neurotransmitter systems contribute to the reinforcing efficacy of food and drugs including dopamine and glutamate. The present study evaluated a role for serotonin by targeting 1A and 1B receptors that function as hetero- and autoreceptors. Adult male and female Long-Evans rats learned to respond for 10% sucrose solution in daily 3h access sessions. Female rats responded at higher rates than males. In three experiments rats were then challenged 15 min pre-session (SC injection) with either a serotonin 1A/1B mixed agonist (RU24969 .3, 1 mg/kg), a 1A agonist (8-OH DPAT .03, .1, .3 mg/kg), or a 1B agonist (CP94253 2.5, 5, 10 mg/kg). The 1A/1B mixed

agonist decreased responding in both males and females but was more effective in males. The 1A agonist did not affect responding. The 1B agonist decreased responding of both males and females. These findings demonstrate the effectiveness of a 1B agonist to decrease sucrose taking on the PR schedule of reinforcement and build on previous findings where a 1B agonist reduced food (sucrose) and drug (cocaine) seeking behavior (PMID: 15680183) but increased cocaine taking on the PR schedule of reinforcement (PMID: 9822762).

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Poster

224. Motivation and Food

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Program #/Poster #: 224.05

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Lundbeck foundation grant R210-2015-2982
Wellcome Trust grant RG74877

Title: Serotonergic modulation of reversal learning and reinforcement learning processes

Authors: *M. E. HERVIG[1,2], M. SELIN[2], S. F. OLESEN[2], K. ZÜHLSDORFF[2], B. PHILLIPS[2], T. BOŽIč[2], M. B. POZO[2], S. DEWAN[2], R. N. CARDINAL[3], J. ALSIÖ[2], T. W. ROBBINS[2]; [1]Dept. of Neurosci., Univ. of Copenhagen, Copenhagen N, Denmark; [2]Dept. of Psychology, [3]Dept. of Psychiatry, Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Background: Cognitive flexibility, the fundamental ability to adapt behavior in response to a changing environment, is disrupted leading to compulsivity in several neuropsychiatric disorders including obsessive compulsive disorder (OCD). In OCD patients, compulsive behavior has been linked to reduced serotonergic transmission and is typically treated with selective serotonin reuptake inhibitors (SSRIs). However, exactly how flexible behavior is modulated by serotonin and its action on specific serotonergic receptors is unknown and needs to be further investigated. **Objective:** To investigate the effects of serotonin reuptake inhibition, as well as 5-HT2_C and 5-HT2_A receptor blockade, on cognitive flexibility and underlying reinforcement learning mechanisms. **Methods:** Male rats were trained on the valence-probe visual discrimination (VPVD) task with reversal probing negative and positive feedback strategies during reversal learning. We evaluated the effects of systemic treatments with the SSRI citalopram as well as 5-HT2_A and 5-HT2_C antagonists on overall reversal learning performance as well as performance on probe trials. During probe trials the correct or incorrect stimulus was presented with a third, probabilistically rewarded (50% of trials), and thus intermediately valenced, stimulus providing individual learning curves for positive and negative feedback processes. Computational reinforcement learning modelling with a hierarchical Bayesian model (HBM) approach was applied to VPVD choice data. **Results:** Low dose (1

mg/kg) citalopram had no effects on reversal learning, but high dose (10 mg/kg) citalopram improved reversal learning overall, at least partly due to improved learning from positive feedback. 5-$HT2_A$ receptor antagonism impaired reversal learning by impairing the late learning phase - while having no significant effects on feedback sensitivity. 5-$HT2_C$ receptor antagonism impaired reversal learning across sessions by impairing learning from positive feedback without affecting negative feedback sensitivity. Computational modeling further differentiated the effects in a dose- and drug-dependent manner. In particular, citalopram affected both learning rate and explore/exploit tendencies. **Conclusion:** These data indicate that the effects of citalopram to enhance cognitive flexibility may be mediated, at least partly, through actions at 5-$HT2_A$ and 5-$HT2_C$ receptors.

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Poster

224. Motivation and Food

Location: SDCC Halls B-H

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Program #/Poster #: 224.06

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Shionogi B.V
Cambridge Trust

Title: Opponent modulation of attentional performance on a signal detection task by D1 and D2 receptors in the medial prefrontal cortex but not in the nucleus accumbens core

Authors: *L. WILOD VERSPRILLE[1], C. MCKENZIE[1], J. ALSIO[2], K. YANO[1], J. W. DALLEY[3], T. W. ROBBINS[4];
[1]Univ. of Cambridge, Cambridge, United Kingdom; [2]Cambridge Electronic Design Ltd., Cambridge, United Kingdom; [3]Univ. Cambridge, Cambridge CB2 3EB, United Kingdom; [4]Psychology, Univ. Cambridge, Cambridge, United Kingdom

Abstract: The role of dopamine (DA) in executive functions such as attention and working memory is well established. Such effects are thought to follow an inverted U-shaped function consistent with the Yerkes-Dodson principle with low to moderate activation of D1 and D2 receptors improving and impairing attentional performance, respectively. The present study investigated the generality of these effects by infusing selective D1 and D2 agonists into two forebrain regions previously implicated in the control of attentional performance, the medial prefrontal cortex (mPFC) and the nucleus accumbens core (NAcC). Rats were trained to criteria on a Signal Detection Task (SDT) to detect and respond to the absence or presence of visual light in order to obtain food reward. Following acquisition of the task, the indirect DA agonist, d-amphetamine (1; 3; 10 µg/hemisphere), and direct D1 receptor (D1R) and D2 receptor (D2R)

agonists, SKF81297 (0.3; 3 µg/hemisphere) and quinpirole (0.3; 3 µg/hemisphere), respectively, were locally administered into the NAcC (n=12) and mPFC (n=13). Different doses of each compound were administered according to a Latin square design with a washout period of 1-week between each compound. We found that low-dose d-amphetamine (1 µg/hemisphere) improved performance while higher doses (dose) impaired performance on the SDT when given into either the mPFC or NAcC ($p = 0.01$*). The D1R agonist SKF81297 had a tendency to improve attentional accuracy when administered in the mPFC ($p = 0.076$) but impaired accuracy following infusions into the NAcC ($p = 0.038$). In contrast, the D2R agonist quinpirole significantly impaired attentional accuracy when given in both mPFC and NAcC ($p = 0.03$* and $p = 0.003$**), respectively.These findings indicate that D1 agonists have cognitive enhancing effects when administered in the mPFC, which may be counteracted by opposing effects in subcortical regions such as the NAcC. The general impairing effects of quinpirole are consistent with our hypothesis of opposing interactions between D1R and D2R and suggest that the impairing effects of d-amphetamine in the mPFC may be mediated by D2R activation in this region.

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Poster

224. Motivation and Food

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Program #/Poster #: 224.07

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH R21 MH124036-01A
Pearson Center for Alcoholism and Addiction Research
NIH/NIAAA Institutional Training Grant T32 AA007456
National Institutes of Health Clinical Translational Science Award (NIH CTSA)
STSI TL1 Training Program TR002551

Title: Trapping anterior insula neurons during bingeing on palatable food

Authors: *G. MACEDO[1], S. BAGSIC[1], B. HUI[2], C. WILLIAMS[2], J. HUANG[2], A. KREISLER[2], E. ZORRILLA[1,2];
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Abstract: Loss of control (LOC) eating, is a hallmark of binge eating disorders. Many models of binge-like eating have been developed that study overeating within a brief time; however, less work has examined the substrate underlying loss of control, modeled as eating compulsively persisting despite adverse consequences or incorrect outcomes. The anterior insular cortex (AIC) subserves visceral-emotional functions, including taste processing, and is implicated in drug

craving and relapse. The AIC sends inputs to the nucleus accumbens core (AcbC), which subserves reward and reinforcement. AIC-AcbC projections have been implicated in compulsive-like drinking in a model of alcohol addiction and in compulsive-like food self-administration in our model of binge eating. Previous data in our laboratory showed that rats with intermittent access to a preferred palatable (P) food developed increased PR breakpoints and footshock punishment-resistant FR food self-administration, validating this as a model of compulsive consumption. Here, we have adapted this model to C57BL/6J mice and are applying it for neuronal activity-dependent analysis. Male and female mice were allowed to acquire fixed ratio (FR1, FR2, FR3 and finally FR5) 5TUM chow self-administration over 2 weeks via nose poke responses in daily sessions at dark onset. Matched for weight, daily chow intake, and baseline FR5 self-administration, mice then were assigned to 1 of 3 groups: *ad lib* chow (C), *ad-lib* Palatable food (P; high-sucrose, chocolate flavored 5TUL)), 3) or intermittent Palatable (INT) mice that received 24 hr access to P on 3 non-consecutive days each week (Monday, Wednesday, Friday), with chow otherwise available. All mice received operant self-administration sessions at dark onset on access days. Over subsequent weeks, INT mice developed cycling food intake and body weight in relation to the availability of P food, reduced latencies to respond for food, increased FR5 self-administration, increased PR breakpoints, and increased responding during extinction, as compared to *ad lib* fed controls. Now, we are using neuronal activity-dependent ribosomal profiling in H2B-TRAP mice to identify AIC ensembles that are activated by binge eating in INT mice that developed substantially increased PR breakpoints. The results inform the role of intermittent access in the etiology and modeling of behavioral LOC for translational studies. Molecular identification of LOC-associated AIC ensembles may identify novel targets and will enable future causal study of specific cell types via combinatorial genetic approaches.

Disclosures: **G. Macedo:** A. Employment/Salary (full or part-time):; Scripps Research Institute. **S. Bagsic:** None. **B. Hui:** None. **C. Williams:** None. **J. Huang:** None. **A. Kreisler:** None. **E. Zorrilla:** A. Employment/Salary (full or part-time):; Scripps Research Institute.

Poster

224. Motivation and Food

Location: SDCC Halls B-H

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Program #/Poster #: 224.08

Topic: G.02. Reward and Appetitive Learning and Memory

Support: DGAPA UNAM IN232120
L.M.R.-S. received a postdoctoral grant from Dirección General de Asuntos del Personal Académico, Universidad Nacional Autónoma de México

Title: Regulation of palatable food intake by CB2 cannabinoid receptors of the anterior cingulate cortex

Authors: *L. RODRIGUEZ-SERRANO[1], J. RODRÍGUEZ-AGUILAR[2], E. HERNÁNDEZ[3], F. CORTÉS SALAZAR[4], J. MANCILLA-DIAZ[5], R. ESCARTIN-PEREZ[6];

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Abstract: People suffering from obesity express elevated plasma endocannabinoid tone, among other alterations. According to the experimental evidence, there are alterations in endocannabinoid concentration in particular areas of the brain related to experimentally induced binge-like behavior or obesity in rodents. The anterior cingulate cortex (ACC) sends projections to regions such as the nucleus accumbens shell (NAcS) to provide the information required to integrate an emotional component into the consequences of behavior. Furthermore, the NAcS and the CCA express CB2 cannabinoid receptors (CB2R), and the mRNA of CB2R has been found in the CCA, suggesting a potential modulatory role of the CCA projections to the NAcS mediated by CB2R in behavioral and motivational processes of non-homeostatic feeding. The present study aimed to evaluate the contribution of CCA CB2R in the consumption of two different palatable foods (PF). We assessed the effects of CB2R activation in the CCA on PF intake using two behavioral procedures with male Wistar rats. The first protocol aimed to induce binge-like behavior by providing limited access to a palatable liquid diet (10% sucrose evaporated milk, 1h/day) for 21 days. The second protocol consisted of an operant task (reinforcement schedule progressive ratio) evaluating changes in motivation (breaking points, BP) for the PF (45 mg chocolate-flavored sucrose pellets). Different rats were used to induce binge-like behavior and to assess BP. In the operant behavior protocol, we use a sequence of conditioning programs. The training started with fixed ratio programs 1 and 5 (FR1 and FR5), and finally, we exposed the subjects to the progressive ratio program (PR, response ratio= [5e(0.2 × trial number)] - 5) and measured BP. After completing the corresponding training sessions, we implanted stereotaxically unilateral injection cannulas in the CCA. Following the recovery period (5-7 days), rats were assigned to different groups and received local injections of the vehicle (DMSO 5% in 0.9% saline solution) or the selective CB2R agonist (GW838972A, 0.25-0.5 µg/µl). Our results showed that CB2 receptor activation in the CCA produces differential effects on the consumption of palatable diets depending on the behavioral protocol. The activation of CB2R in the CCA decreased the response rate and breaking points in the operant task, while the binge-like behavior remained unaffected by the pharmacological activation of the CB2R. Our findings suggest that CB2 receptor-mediated cannabinoid regulation in the CCA preferentially affects the rewarding process associated with the consumption of PF when the delivery of an operant response is required.

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Poster

224. Motivation and Food

Location: SDCC Halls B-H

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Program #/Poster #: 224.09

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NSF GRFP
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NIH Grant DA051014

Title: The role of neuropeptides and the estrous cycle in mediating the effect of stress on Pavlovian conditioning.

Authors: *M. P. JOHNSTON[1], B. HAMAUEI[2], K. F. ALEXANDRE[3], S. K. PATEL[3], M. J. WANAT[1];
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Abstract: Stress is an unavoidable and universal experience among all living organisms. Both acute and chronic stress can produce long-lasting behavioral changes. We have found that a single stressful experience elicits a persistent increase in Pavlovian conditioned responding in male rats. This stress-enhancement on responding is accompanied by an elevation in reward-evoked dopamine release in the ventral lateral striatum (VLS). However, it is unclear (i) what neural mechanisms underlie this phenomenon, (ii) whether sex or the estrous cycle affects how stress influences Pavlovian learning, and (iii) how chronic prior stress impacts conditioned responding and dopamine release in the VLS. Corticotropin-releasing factor (CRF) is released into the ventral tegmental area (VTA) in response to stress and mediates many of the behavioral effects of stress. Furthermore, NMDA receptors on VTA dopamine neurons are involved in synaptic changes that facilitate learning and changes in behavior. Taken together, we hypothesized that the long-lasting behavioral effects of stress are mediated by CRF release and NMDA receptor activation in the VTA. To address this, we antagonized either CRF or NMDA receptors in the VTA prior to exposing rats to an acute restraint stress. Our findings indicate that antagonizing either CRF or NMDA receptors blunts stress-enhanced behavioral responding to a reward-predictive cue. Preliminary analyses indicate the stage of the estrous cycle when stress is administered influences the magnitude of conditioned responding in subsequent Pavlovian training sessions. Ongoing experiments are investigating how chronic prior stress influences Pavlovian conditioned responding and VLS dopamine release. The results of this project will provide valuable insights to the mechanisms underlying how stress produces long-lasting changes in behavior.

Disclosures: M.P. Johnston: None. **B. Hamauei:** None. **K.F. Alexandre:** None. **S.K. Patel:** None. **M.J. Wanat:** None.

Poster

224. Motivation and Food

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 224.10

Topic: G.02. Reward and Appetitive Learning and Memory

Support: RO1-DK111475
GFED PD# 55346

Title: Phenotypic Differences to Pharmacological Manipulations of the Dopamine Receptor in Binge Eating Prone and Resistant Female Rats

Authors: *J. R. LEE, N. FOWLER, A. PATARO, K. KLUMP, C. SISK, A. W. JOHNSON; Michigan State Univ., Michigan State Univ., Lansing, MI

Abstract: Binge eating (BE) is a pathological eating behavior that can manifest in several eating disorders in humans. The dopamine system contributes to the regulation of eating behavior, therefore in this study we examined whether differential palatable food (PF) consumption was associated with stimulation and blockade of dopamine receptors in an animal model of individual differences in binge eating behavior. Sixty adult female Sprague Dawley rats were given intermittent access to PF 3x/week for 2 weeks to identify binge eating prone (BEP; consistently high levels of PF intake) and non-BEP rats (all other rats). Next, all rats received intraperitoneal injections of either vehicle, low, or high doses of the dopamine D1R agonist SKF 38393, D1R antagonist SCH 23390, D2R agonist quinpirole, and the D2R antagonist raclopride followed by PF and chow intake tests. The findings revealed that BEP rats displayed enhanced responsivity to the pharmacological action of the D2R agonist and anatagonist, whereas D1R-dependent manipulations had similar effect of PF intake in both BEP and BER rats. To examine whether this enhanced responsivity to pharmacological manipulations of dopamine receptors reflected individual differences in receptor expression, D1R and D2R mRNA in the nucleus accumbens was quantified in a subset of BEP and non-BEP rats. Collectively, our findings suggest that alterations in dopaminergic reward circuitry may underlie increased PF intake in BEPs.

Disclosures: J.R. Lee: None. **N. Fowler:** None. **A. Pataro:** None. **K. Klump:** None. **C. Sisk:** None. **A.W. Johnson:** None.

Poster

224. Motivation and Food

Location: SDCC Halls B-H

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Program #/Poster #: 224.11

Topic: G.02. Reward and Appetitive Learning and Memory

Support: BP-ENDURE NIH R25NS080686
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New York University’s Dean’s Undergraduate Research Fund

Title: Individual differences in the positive outcome from adolescent ketamine treatment in a female mouse-model of anorexia nervosa involves drebrin A at excitatory synapses of the medial prefrontal cortex

Authors: *R. TEMIZER, Y.-W. CHEN, C. AOKI;
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Abstract: Anorexia Nervosa (AN) is a mental illness with the highest rates of mortality and relapse, and no approved pharmacological treatment. Using an animal model of AN, called activity-based-anorexia (ABA), we showed earlier that a single intraperitoneal injection of ketamine at a dose of 30 mg/kg, but not 3 mg/kg, has a long-lasting effect upon adolescent females of ameliorating anorexia-like symptoms through the following changes: enhanced food consumption and body weight; reduced running and anxiety-like behavior. However, there were also individual differences in the drug's efficacy. We hypothesized that individual differences in ketamine's ameliorative effects involves drebrin A, an F-actin-binding protein known to be required for the activity-dependent trafficking of NMDA receptors (NMDARs). We tested this hypothesis by electron microscopic quantifications of drebrin A immunoreactivity at excitatory synapses of pyramidal neurons (PN) and GABAergic interneurons (GABA-IN) in deep Layer 1 of prefrontal cortex (PFC) of these mice. Results reveal that (1) the areal density of excitatory synapses on GABA-IN is greater for the 30 mg/kg than 3 mg/kg group; (2) the proportion of drebrin A+ excitatory synapses is greater for both PN and GABA-IN of 30 mg/kg than 3 mg/kg group. Correlation analyses with behavioral measurements revealed that (3) 30 mg/kg's protection is conferred through reduction of drebrin A in the cytoplasm of GABA-IN and increase at extrasynaptic membranous sites of PN and GABA-IN; (5) altogether pointing to 30 mg/kg-induced homeostatic plasticity that engages drebrin A at excitatory synapses of both PN and GABA-IN.

Disclosures: R. Temizer: None. **Y. Chen:** None. **C. Aoki:** None.

Poster

224. Motivation and Food

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 224.12

Topic: G.02. Reward and Appetitive Learning and Memory

Support: PAPIIT Grant IA206521

Title: Evaluation of muscarinic receptors in the amygdaloid nuclei during incidental taste memory formation.

Authors: *V. TORRES-GARCÍA, E. RODRÍGUEZ-NAVA, R. ALCÁNTARA-RIVAS, G. ROLDÁN-ROLDÁN, J.-P. MORIN;
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Abstract: The sense of taste provides information regarding the nutrient content, safety, or potential toxicity of an edible. In addition, learning the positive or aversive consequences associated with novel taste has a strong importance for an animal's survival. Attenuation of neophobia (AN) is a type of learning that occurs when a new taste has no negative consequences and becomes recognized as familiar and safe, leading to an increase in its consumption. This form of incidental learning represents a process that is impaired in many psychiatric disorders. Recent evidence suggests that cholinergic signaling in the amygdala, a structure implicated in storage of emotional memories, as well as in acquiring a sense of familiarity, plays an important role in learning and storage of approach or avoidance behaviors. The present study aims to determine the role of muscarinic receptors in basolateral amygdala (BLA) and central amygdala (CeA) in the task of AN by infusing the antagonist scopolamine before or after novel taste presentation as well as before the presentation of the familiarized taste. Fifty four adult male Wistar rats (250−300g) were used as experimental subjects. Animals were implanted bilaterally with cannulas aimed at either the BLA or CeA. After recovery, rats were randomly assigned to either vehicle or drug (eg.: scopolamine) group, for each experiment. Animals were presented with a novel taste solution (saccharin 0.3% in tap water) in 5 burettes and after two days they were again presented with 5 burettes containing water and 5 containing saccharin to evaluate long-term memory. The drugs were administered 1) 5 min before novel taste presentation; 2) immediately after novel taste presentation; 3) 5 min before retrieval. Rats infused with scopolamine before or after novel taste presentation markedly differed from those infused with the vehicle solution (Two-way ANOVAs; $F(1,12)= 41.14$, $p<0.0001$ in the "before" condition and $F(1,21)=32.87$, $p<0.0001$ in the "after" condition). Intriguingly, post-hoc Sidak test showed that rats infused with scopolamine in the BLA before or after novel taste presentation developed a strong aversion for the taste ($p<0.001$ and $p<0.0001$, respectively). This drug-induced taste avoidance was not observed in animals pre-exposed to the taste, indicating that it was novelty-dependent. Finally, as it is the case in gastric malaise-induced taste avoidance paradigms such as conditioned taste aversion, repeated non-contingent exposures to the taste led to the extinction of the intra-BLA scopolamine-induced taste avoidance.

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Poster

224. Motivation and Food

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Program #/Poster #: 224.13

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R01 NIMH126178

Title: Decreasing reward value ameliorates pathological impulsivity in mice lacking the serotonin 1B receptor

Authors: *S. S. DESROCHERS, D. Y. COMPAORE, K. M. NAUTIYAL;
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Abstract: Impulsivity is a key characteristic of many psychiatric disorders, however, our understanding of its behavioral and biological mechanisms remain incomplete. Our previous research has highlighted a particular role for the serotonin 1B receptor (5-HT1BR) in the control of impulsive action (reduced ability to withhold responding), as well as motivation for reward, in a reversible knockout mouse model. In the present study, we showed that an absence of the 5-HT1BR in mice also increased taste reactivity for palatable substances in a brief access lickometer test, suggesting heightened reward 'liking'. Normal 'liking' can be restored to control levels with adult rescue of receptor expression (confirmed with mRNA quantification). This suggests that the role of 5-HT1BRs in reward-related neural pathways is adult mediated. We considered whether the increased subjective reward valuation was causal to the increased impulsivity. To do this, using the lickometer, we determined a reward concentration for the 5-HT1BR knockout mice that was of equivalent subjective value to the maximum concentration in controls. This 'normalized' reward value was then used as the outcome in a series of operant conditioning experiments. Interestingly, we found that performance in effort-based random ratio schedules is influenced by satiety effects such that mice pressed more for lower concentrations of reward, with 5-HT1BR knockout mice showing overall increased motivation. Then, in a Go/No-go task measuring impulsive action, we found that mice lacking the 5-HT1BR had increased impulsivity which was reduced to the level of controls with the lower 'normalized' reward concentration. This supports the idea that increased subjective reward valuation is indeed causal to disordered impulsivity in these mice, and suggests that the neural circuits underlying reward and impulsivity may be convergent. Current experiments are using viral-mediated projection specific knockdown of 5-HT1BR to examine the neural circuits through which serotonin influences reward processing and/or impulsivity. Overall, our data indicate that changes in reward processing could be a mechanism through which serotonin modulates impulsive behavior.

Disclosures: S.S. Desrochers: None. **D.Y. Compaore:** None. **K.M. Nautiyal:** None.

Poster

224. Motivation and Food

Location: SDCC Halls B-H

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Program #/Poster #: 224.14

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Impact of neonatal escitalopram on affective and social behaviors in adolescence

Authors: *J. C. BEZENAH, A. N. TEJADA, D. GARCIA, C. CUETO, M. GONZALES, J. RICHIE, L. R. AMODEO;
Psychology, CSU San Bernardino, San Bernardino, CA

Abstract: Selective serotonin reuptake inhibitors (SSRIs) are frequently used during pregnancy, and while SSRIs are considered safe during this period, there is limited knowledge of the long-term consequences of neonatal SSRI exposure on cognitive and behavioral development. SSRIs increase extracellular serotonin, which contributes to their clinical effectiveness in major depression and anxiety-related disorders. However, this hyper-serotonergic state during pregnancy can be transferred from the mother to the fetus through the placenta and breastmilk, causing potential alterations in neurotransmission and neural functioning. Further, recent human studies have demonstrated that prenatal exposure to SSRIs in humans may increase susceptibility to developmental delays and autism spectrum disorder (ASD). In a recent systematic review and network meta-analysis comparing the efficacy and acceptability of antidepressant drugs, escitalopram was shown to be one of the most effective antidepressants. However, as one of the newer SSRIs on the market introduced in 2002, there is still a lack of information on its safety profile during pregnancy and lactation. While prior studies conducted by this lab have found increases in compulsive/anxiety-like behaviors when administration occurs late in pregnancy, this study focuses on the behavioral impact following administration during the neonatal period in rats, as this period is equivalent to the third trimester through toddlerhood in humans. For this study, escitalopram (0 or 10 mg/kg, s.c.) was administered to lactating Long-Evans rat dams from postnatal day 1-10. In adolescence (P35-55) offspring were then assessed for changes in social play, taste novelty learning, and inhibitory/anxiety-like behavior using an open-field conflict test. These preliminary results suggest that neonatal escitalopram can have an effect on behaviors that commonly emerge during adolescence.

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Poster

224. Motivation and Food

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Program #/Poster #: 224.15

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Wake Forest University

Title: Injections of nociceptin into the nucleus accumbens core decrease appetitive but not consummatory motivation in an effort-based choice task

Authors: *L. WILSON, M. KLAUSNER, Q. LOY, S. CHUANG, S. PATEL, W. E. PRATT; Dept. Psychology, Wake Forest Univ., Winston-Salem, NC

Abstract: The nucleus accumbens (NAc) is a critical region for regulating the appetitive and consummatory aspects of motivated behavior. Previous work has shown differential effects of NAc μ-, δ-, and κ- receptor stimulation on free food intake as well as shifting motivation within an effort-based choice (EBC) task. However, the specific effects on motivation remain unknown

for the ORL1 receptor, a fourth member of the opioid receptor family. The goal of these studies was therefore to characterize the effect of NAc injections of nociceptin, the endogenous ligand for ORL1, on consummatory and appetitive motivation. Two groups of male Sprague-Dawley rats received bilateral guide cannulas targeting the NAc core prior to being tested in a palatable feeding assay or an EBC task. For both experiments, nociceptin was injected at 0.0, 4.0 and 10.0 nmol/0.5µl/side, and all rats received each dose randomly counterbalanced across days. Injections were separated by at least 48 hrs. In the first experiment, rats (N=7) received nociceptin injections into the NAc over the course of 3 treatment days, and were tested in 2-hr sessions with free access to a palatable sweetened-fat diet. 10 nmol of nociceptin increased consumption in the first 30 minutes, but this increase was not sustained through the end of the 2-hr session. In the EBC task, rats (N=11) were presented the choice either to eat freely available rat chow or to work for sugar pellets on a progressive ratio 2 reinforcement schedule during a 1-hr session. Behavior was examined both under *ad libitum* conditions and following 23-hr food deprivation, resulting in a total of 6 treatment days. Here, nociceptin did not affect the amount of chow rats consumed, but did significantly decrease breakpoint for sugar pellets. Breakpoint was also more strongly inhibited by nociceptin treatment in the food deprived condition than in the *ad libitum* condition, as evidenced by a nociceptin X deprivation interaction that approached significance ($p = .055$). Overall, these data suggest that NAc ORL1 stimulation inhibits appetitive motivation but does not have strong effects on consummatory motivation. This pattern of results differs from those previously obtained in the EBC task by NAc stimulation of the other opiate receptors. Specifically, µ-opioid receptor stimulation was found to increase free-feeding while decreasing breakpoint; δ-receptor stimulation increased both free-feeding and breakpoint; and κ-receptor stimulation affected neither free-feeding nor breakpoint. Together, these data suggest that these four opioid receptor classes each serve a unique role in modulating food-directed motivation within the nucleus accumbens core.

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Poster

224. Motivation and Food

Location: SDCC Halls B-H

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Program #/Poster #: 224.16

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Wake Forest University
Psi Chi Undergraduate Research Grant

Title: Stimulation of nucleus accumbens serotonin 5-HT_{1A}, 5-HT_3, or 5-HT_6 receptors affects food-directed motivation in an effort-based choice task

Authors: *W. E. PRATT, G. E. ANDERSON, Q. LOY, S. CHUANG, S. PATEL, M. KLAUSNER, A. SHARP, L. WILSON;
Dept. Psychology, Wake Forest Univ., Winston Salem, NC

Abstract: The nucleus accumbens (NAc) receives information regarding both energy need and the hedonic properties of food, and influences both appetitive and consummatory motivation. Previously, we examined the effects of serotonin (5-HT) receptor stimulation of the NAc and found that the subclasses of 5-HT receptors have differential effects on food intake. For instance, stimulation of NAc shell 5-HT_{1A} receptors reduces chow intake in restricted rats, and stimulation of the 5-HT_3 or 5-HT_6 receptors increases food intake under similar conditions. Despite these receptors having clear influences on behavior, it is unclear whether they may preferentially affect the appetitive or consummatory phases of motivation. To examine this question, we tested whether stimulation of NAc shell 5-HT_{1A}, 5-HT_3, or 5-HT_6 receptors alters behavior in an effort-based choice task that simultaneously presented rats with the choice to eat freely-available rat chow or to work for a preferred sugar pellet. Male Sprague-Dawley rats (n=12/group) were food restricted to 90% *ad libitum* weight and trained on a 30 min progressive-ratio 2 (PR2) schedule of reinforcement. Following the training, food was returned to the rats, and they were surgically implanted with bilateral guide cannulas targeting the medial NAc shell. After one week of surgical recovery, rats returned to the operant chambers. Session length increased to 1 hr, and rats also had free access to rat chow while lever pressing for sugar on the PR2 schedule. After at least 10 days of training on this effort-based choice task, separate groups were tested following NAc 5-HT_{1A} receptor stimulation (with 0.0, 2.0, 4.0, 8.0 μg /0.5 μl/ side 8-OH-DPAT), 5-HT_3 receptor stimulation (with 0.0, 10.0, 20.0 μg /0.5 μl/ side m-CPBG), or 5-HT_6 receptor stimulation (with 0.0, 1.0, and 4.0 μg/ 0.5 μl/side EMD 386088). Individual rats in each drug group randomly received all doses of a single drug across multiple test days. Stimulation of NAc 5-HT_{1A} receptors led to a decrease in appetitive motivation (defined as a decrease in the breakpoint on the progressive ratio schedule) and an increase in consummatory motivation (as measured by the intake of the freely-available rat chow). NAc 5-HT_3 stimulation had no impact on appetitive motivation for sugar but increased chow consumption. Stimulation of 5-HT_6 receptors in the NAc shell increased both appetitive and consummatory motivation. These data add to a growing literature suggesting that serotonin signaling in the NAc regulates food-directed motivation and that individual 5-HT receptors serve differential roles in regulating appetitive and consummatory motivation.

Disclosures: W.E. Pratt: None. **G.E. Anderson:** None. **Q. Loy:** None. **S. Chuang:** None. **S. Patel:** None. **M. Klausner:** None. **A. Sharp:** None. **L. Wilson:** None.

Poster

225. Reward Seeking and Motivation, Neuronal Circuits

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 225.01

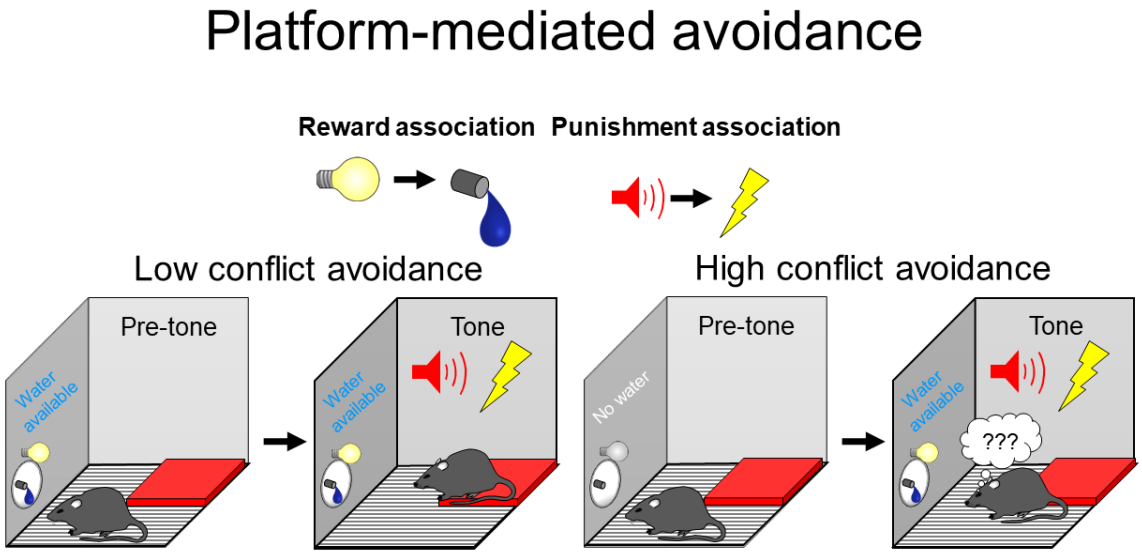
Topic: G.03. Motivation

Support: MH123495
MH058883
NS115917
MH108924

Title: Sex differences in reward approach/punishment avoidance conflict in mice.

Authors: A. G. VEGA-REYES[1], M. F. BONILLA-GUTIÉRREZ[1], L. J. RAMIREZ-SANCHEZ[2], S. SANTOS DE LEÓN[1], M. CRUZ-BERRÍOS[1], S. E. BOYLE[2], B. LI[2], *C. BRAVO-RIVERA[1];
[1]Univ. of Puerto Rico Sch. of Med., Univ. of Puerto Rico Dept. of Psychiatry and Anat. & Neurobio., San Juan, PR; [2]Neurosci., Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Reward is often present in risky environments, requiring individuals to weigh the benefits of rewards against associated risks. There are individuals that are unable to choose an appropriate response during risky reward opportunities and thus exhibit extreme avoidance or risky behaviors that can severely impair quality of life or endanger people. It is therefore necessary to characterize how neurons mediate reward approach and threat avoidance conflict. Here, we used a novel approach-avoidance conflict task to characterize individual differences in behavior and neuronal activity in mice. Here, we adapted the platform-mediated avoidance conflict task (Bravo-Rivera et al 2014; Bravo-Rivera et al 2021), such that water-deprived mice could nose-poke for a light-signaled water reward (3 uL, 4 sec ITI) and avoid a tone-signaled (20 sec, 70 dB) foot-shock (0.2 mA, 2 sec co-terminating) by stepping onto a safety platform away from the reward port. Mice were trained in two different conflict contingencies; in low conflict, reward was available during safety periods (inter-tone intervals) and during the warning tone, whereas in high conflict, reward was available only during the warning tone. All mice (n = 10 males, 10 females) learned to actively avoid the signaled shock in >90% of trials by the tenth day of low conflict training. Interestingly, females stepped on the platform earlier than males after tone onset (5 sec vs 10 sec) and had a longer latency to leave the platform after tone offset (16 sec vs 10 sec) in low conflict. Females also mounted the platform earlier than males after tone onset (15 sec vs 17 sec) in high conflict. Males received more shocks than females (5 vs 2 out of 20) and received more water reward (759 ul vs 609 ul) than females by the end of high conflict training. Moreover, females exhibited more tone-induced freezing (33% vs 15% of tone duration) and exhibited more frequent darting (73% vs 51% of trials) than males. These results suggest that females exhibit more avoidance behavior and less reward approach than males in the face of approach/avoidance conflict.



Disclosures: **A.G. Vega-Reyes:** None. **M.F. Bonilla-Gutiérrez:** None. **L.J. Ramirez-Sanchez:** None. **S. Santos de León:** None. **M. Cruz-Berríos:** None. **S.E. Boyle:** None. **B. Li:** None. **C. Bravo-Rivera:** None.

Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Program #/Poster #: 225.02

Topic: G.03. Motivation

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Title: Hippocampus-dependent calibration of context-evoked feeding by a genetically defined lateral septum to hypothalamus circuit

Authors: *T. D. GOODE[1,2,3,4], D. CHIZARI[4], A. BESNARD[1,2,3,4], T. KAMATH[4], M. KRITZER[1,2,3,4], E. MACOSKO[4], A. SAHAY[1,2,3,4];
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Abstract: Context-appropriate initiation or termination of motivated behaviors, including feeding, is thought to depend on the successful recognition, linkage, and relay of internal states (e.g., hunger vs. satiation), external cues (e.g., novelty vs. familiarity), and outcomes (e.g., food consumption) to and from the brain's hippocampus (HPC) to goal-relevant subcortical structures. Upstream to hypothalamic regions known to be central to consummatory behaviors, the long-range inhibitory (GABAergic) network of the lateral septum (LS) is well-positioned to integrate environmental and contextual cues from its dense HPC afferents for modulation of situation-specific feeding. Leveraging single-cell transcriptomics and multiplex fluorescent *in situ* hybridization, we have uncovered a genetically distinct and topographically restricted subpopulation of inhibitory LS cells, defined in both male and female mice by the expression of the neuropeptide, prodynorphin (Pdyn). Neural tracing of LS(Pdyn) cells revealed dense input from CA3/2 of the dorsal HPC. In turn, these LS(Pdyn) cells were found to significantly innervate the lateral hypothalamus (LH). Accordingly, we tested whether LS(Pdyn) neurons critically regulate context-dependent food-seeking. Activity-related experiments showed that LS(Pdyn) neurons are engaged by feeding, while optogenetic interrogation of LS(Pdyn) cells was found to bidirectionally modulate spontaneous food consumption in a familiar place. Moreover, inhibition of LS(Pdyn) neurons eliminated context-specificity in a task of context-dependent overconsumption. Finally, inhibition of dorsal HPC terminals in the dorsal LS, where LS(Pdyn) cells are located, was found to control context-induced changes in feeding. In total, these experiments identify a previously uncharacterized neuropeptidergic cell population in the dorsal LS, which may link context signals of the dorsal HPC with consumption-regulating cells of the LH.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Topic: G.03. Motivation

Support: NSF DGE 1256260
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T32 - DA07268

Title: Chemogenetic and optogenetic manipulation of neuronal projections from the lateral hypothalamus to the paraventricular nucleus of the thalamus impact cue-motivated behaviors

Authors: *A. G. IGLESIAS[1], S. R. WESTBROOK[2], S. E. CHANG[2], S. B. FLAGEL[2];
[1]Neurosci. Grad. Program, [2]Michigan Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: Environmental cues can influence behavior in an adaptive manner by signaling the availability of valuable resources. For some individuals, however, such cues attain inordinate control and promote maladaptive behavior. In rodents, individual differences in cue-motivated behaviors can be captured using a Pavlovian conditioned approach (PavCA) paradigm, wherein presentation of a cue (lever) is followed by delivery of a food reward. Following PavCA training, two distinct phenotypes emerge – goal-trackers (GT) and sign-trackers (ST). While both GT and ST attribute predictive value to the cue, ST also attribute incentive value to the cue. ST and GT rely on distinct neurobiological mechanisms and the paraventricular nucleus of the thalamus (PVT) has emerged as a neural hub that mediates their characteristic differences in associative learning. Prior findings have led us to postulate that bottom-up projections to the PVT relay the incentive value of reward-associated cues, with the lateral hypothalamus (LH), which sends dense orexinergic projections to the PVT, acting as a critical neural node. Here we investigate the role of the LH-PVT pathway in encoding the value of reward cues via (1) chemogenetic inhibition and (2) optogenetic excitation. In the chemogenetic study we utilized a dual-vector approach to selectively express an inhibitory (Gi) DREADD virus in the LH-PVT pathway in outbred Sprague-Dawley male rats. Following PavCA, rats developed a conditioned response and received either vehicle or clozapine-N-oxide (CNO; 5 mg/kg) to activate the Gi-DREADDs. Selective inhibition of the LH-PVT pathway decreases the expression of goal-directed behaviors for GTs, without affecting the behavior of STs. In the optogenetics study we utilized transgenic orexin (OX)-Cre Long Evans male and female rats, which express Cre-recombinase in OX neurons, and infused a retrograde Cre-dependent excitatory optogenetic (channelrhodopsin, ChR2) virus into the anterior PVT. Rats received laser-induced excitation of LH-PVT neurons during cue (lever) presentation early in Pavlovian training. Preliminary results indicate that selective stimulation of OXergic neurons in the LH-PVT pathway increases goal-tracking behavior. Thus, manipulation of the LH-PVT pathway appears to selectively affect goal-directed behaviors, and this is true both early in training and after a conditioned response has been acquired. Ongoing studies are further investigating a potential role for the LH-PVT pathway in incentive learning processes.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Topic: G.03. Motivation

Support: National Institute of Mental Health R01MH110594
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McKnight Foundation award

Title: Neuronal mechanisms of novelty seeking

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Abstract: Humans and other primates interact with the world by observing and exploring visual objects. In particular, they often seek out the opportunities to view novel objects that they have never seen before, even when they have no extrinsic primary reward value. However, despite the importance of novel visual objects in our daily life, we currently lack an understanding of how primate brain circuits control the motivation to seek out novelty. We found that novelty-seeking is regulated by a small understudied subcortical region, the zona incerta (ZI). In a task in which monkeys made eye movements to familiar objects to obtain the opportunity to view novel objects, many ZI neurons were preferentially activated by predictions of future novel objects and displayed burst excitations before gaze shifts to gain access to novel objects. Low intensity electrical stimulation of ZI facilitated gaze shifts, while inactivations of ZI reduced novelty-seeking. Surprisingly, additional experiments showed that this ZI-dependent novelty seeking behavior is not regulated by canonical neural circuitry for reward prediction. The habenula-dopamine pathway, known to reflect reward predictions, was relatively inactive during novelty-seeking behavior in which novelty had no extrinsic reward value. Instead, high channel-count electrophysiological experiments and anatomical tracing identified a prominent source of control signals for novelty seeking in the anterior ventral medial temporal cortex (AVMTC), a brain region known to be crucially involved in visual processing and object memory. In addition to their well-known function in signaling the novelty or familiarity of objects in the current environment, AVMTC neurons reflected the predictions of future novel objects, akin to the way neurons in reward-circuitry predict future rewards in order to control reward-seeking. Our data uncover a network of primate brain areas that regulate novelty-seeking. The behavioral and neural distinctions between novelty-seeking and reward-prediction highlight how the brain can accomplish behavioral flexibility, providing a mechanism to explore novel objects.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Title: Striatal acetylcholine reports distinct update signals during flexible multi-step decision making

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Abstract: The striatum plays a critical role in coordinating reward guided decision making. It also has one of the highest concentration of markers for cholinergic transmission. Striatal acetylcholine (ACh), which is mainly supplied by a small population of cholinergic interneurons with extensive local arborization, exerts a powerful influence over neurotransmission and plasticity. A handful of electrophysiological studies show rewards and reward-predictive cues elicit ACh responses in simple behavioral tasks, and temporally coarse manipulations of striatal ACh suggest a role In rapid behavioral flexibility. However, historical technical challenges in measuring and precisely manipulating acetylcholine release in vivo have hampered the ability to refine our understanding of how striatal ACh shapes more complex behavior, such as when animals need to update sequential decisions in a structured environment. The recent advent of genetically encoded tools enabling measurement and manipulation of ACh levels with high temporal precision has rekindled interest in this area. Here we used the recently developed GRABACh3.0 sensor to characterize rapid ACh fluctuations in the nucleus accumbens core (NAc) and dorsomedial striatum (DMS) during a sequential reward guided decision-making task in mice. Probabilistic reward delivery enabled us to determine how ACh levels were shaped by reward expectations, and the action-state transition structure allowed us to measure ACh fluctuations while navigating changing action plans. Both NAc and DMS ACh carried time-locked information about (i) reward and reward expectations (though only by reward omission in DMS), (ii) value updates (inverse “reward prediction error”), (iii) action updates, and (iv) movement (at distinct timepoints in DMS and NAc). These signals co-occurred with sustained information reflecting the recent local reward rate. Together, these findings suggest that NAc and DMS striatal ACh differentially contribute to flexible decision making by signaling unexpected changes in the environment.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Topic: G.03. Motivation

Support: NIMH R01 MH123661

Title: Sex-specific stereotyped behavior in a mouse model of 16p11.2 hemideletion

Authors: E. GIGLIO[1], G. R. ROJAS[1], M. MERFELD[2], S. AVILES[1], *N. GRISSOM[1];
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Abstract: Neurodevelopmental disorders like autism spectrum disorder (ASD) have strong male biases in diagnosis, but the underlying mechanism the way sex differences interact with genetic variation remains unclear. A key diagnostic feature of ASD is the development and expression of stereotypic motor behaviors, which employ the same neural systems involved in social and non-social reward learning. We use a mouse model of human 16p11.2 hemideletion (del/+) - a copy number variation linked to neurodevelopmental diagnoses including ASD - to delve into these interactions. 16p11.2 del/+ mice have previously been demonstrated to have changes in their striatal molecular function and learning deficits as well as sex-divergent deficits in motivational behaviors. Dopinaminergic signaling is involved in repetitive locomotor behavior, motivation, and decision-making, and amphetamine locomotor sensitization is a well-established paradigm of probing dopamine function. We have previously observed that male del/+ mice rotate rapidly in response to increasing doses of amphetamine, whereas male wildtypes sensitize through increased gross locomotion. These effects were not seen across female del/+ and wildtype, but female and male mice sensitize to amphetamine at different rates and doses regardless of genotype. We are now investigating the emergence and development of hyperactivity and male-biased stereotyped locomotor behaviors using pose estimation software (SLEAP). Preliminary results examining c-fos expression in cleared brains following locomotor sensitization suggest there are significant reductions and/or imbalances in dopamine function in the striatum which could drive the expression of stereotypic/repetitive behaviors and bias reward guided behaviors. These results have important implications for the role of dopamine in the co-regulation of movement and motivation in a mouse model of 16p11.2 del+/.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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University of Bordeaux

Title: Feeding and exercise essential values in cannabinoid type-1 (CB_1) receptor mutant mice under closed economy conditions

Authors: *I. HUREL[1,2], B. REDON[1,2,3], E. MESGUICH[1,2], L. BELLOCCHIO[1,2], D. GISQUET[1,2], F. JULIO-KALAJZIć[1,2], G. MARSICANO[1,2], F. CHAOULOFF[1];
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Abstract: Body homeostasis is regulated by the balance between feeding and activity. Dysregulations in this balance, as observed in obesity or anorexia nervosa, find their roots at the motivation level. This led us to develop an operant conditioning protocol wherein wheel-running motivation, palatable food motivation and preference for either reward in a choice task can be assessed. The use of this protocol, combined with pharmacological and genetic (i.e. constitutive and conditional mutant mice for cannabinoid type-1 (CB_1) receptors) approaches, has revealed the involvement of CB_1 receptors located on GABAergic neurons in the choice between palatable feeding and wheel-running (Muguruza et al., JCI Insight 2019). Our protocol however suffers two main limitations which are respectively related to the facts that (i) mice were daily exposed to the operant chambers, albeit for a limited time (1h), and (ii) home cages were provided with (unlimited) food but not with a running wheel. Thus, the drives for palatable food and wheel-running might have been respectively underestimated and overestimated. Accordingly, we next investigated the role of CB_1 receptors in a closed-economy choice setting wherein mice living in the operant chambers were able to feed a standard diet and to run, doing so under fixed ratio (FR) reinforcement schedules (FR1, FR3, FR10 and FR30). Demand curves for food and wheel running allowed the calculations of their respective essential values. We found that CB_1 receptors located on GABAergic neurons in adult male/female mice (n=5-7 mice/genotype/sex) positively control the essential value for wheel-running. As opposed to the role exerted by CB_1 receptors on palatable feeding motivation, the full deletion of CB_1 receptors proved inefficient on standard food motivation, even after successive changes in the price of food. Using conditional male/female mice expressing CB_1 receptors exclusively in GABAergic neurons, we next asked if the essential value for running was rescued in this group. Although such a CB_1 receptor re-expression allowed an increase in the essential value for running, its level did not fully reach that measured in wild-type mice. This finding indicates that CB_1 receptors on GABAergic neurons are necessary, but only partly sufficient, for running motivation under closed economy conditions. Overall, this model provides a new framework for studying genetic and environmental causes of obesity and anorexia nervosa.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Support: NIMH 1F30MH129055-01
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Title: Synaptic characterization of cholinergic interneurons in the nucleus accumbens core

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Abstract: The nucleus accumbens (NAc) is a critical center for reward-related and motivated behaviors and becomes disrupted in neuropsychiatric disorders such as drug addiction and depression. The core subregion of the NAc (NAcCore) lies between its surrounding shell and the dorsal striatum, each integrating distinct inputs from many brain areas. Within the NAc, cholinergic interneurons (CINs) are uniquely non-GABAergic and instead release acetylcholine to modulate the local network. However, the ability of CINs to receive and process different long-range inputs is largely unexplored in the NAcCore. Using rabies cell-type-specific tracing, we identify the major inputs to CINs in the NAcCore. With whole-cell voltage clamp recordings and optogenetics, we examine the strength and dynamics of cortical and thalamic glutamatergic inputs onto CINs in the NAcCore. Switching to current-clamp recordings, we also determine how cortical and thalamic inputs influence CIN firing activity. Lastly, we assess the roles of different glutamate receptors in generating distinct modes of CIN firing. These experiments provide insights into the synaptic organization of CINs in the NAcCore, including how different inputs shape patterns of activity. This work furthers our understanding of how the NAcCore integrates inputs containing information about motivational drive, reward value, and attention, and how circuitry in this key reward center may be disrupted in neuropsychiatric disorders.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Topic: G.03. Motivation

Support: Samsung Science and Technology Foundation under Project No. SSTF-BA1301-53.

Title: An inhibitory medial preoptic circuit mediates innate exploration

Authors: *K. SHIN, J. RYOO, S. PARK, J. LEE, Y. LEE, D. KIM;
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Abstract: Animals have an innate motivation to explore objects and environments with unknown values. To this end, they need to activate neural pathways that enable exploration. Here, we reveal that photostimulation of a subset of medial preoptic area (MPA) neurons expressing the vesicular-GABA transporter gene (vgat+) and sending axonal projections to the ventrolateral periaqueductal gray (vPAG) increases exploration in a chamber but causes no place preference when tested there without photostimulation. Photoinhibition of MPA^{vgat}-vPAG projections leads to no emotional changes as measured by normal activity in an open field assay. Electrophysiological recordings revealed that most GABAergic vPAG neurons are inhibited by MPA^{vgat} neurons. In contrast to a previous report that suggested that MPA^{vgat}-vPAG neurons may impart positive valence to induce place preference, our results suggest that these neurons can increase innate exploration.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Topic: G.03. Motivation

Support: Sainsbury Wellcome Centre Core Grant from the Gatsby Charitable Foundation and Wellcome 090843/F/09/Z

Title: Subcortical circuits for balancing exploration and exploitation

Authors: *M. AHMADLOU, M. Y. SHIRAZI, J. DZIUBEK, I. ROGERS, S. ZHANG, S. B. HOFER;
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Abstract: Animals have to constantly decide whether to explore new resources and thus acquire novel information, or to keep exploiting known resources. Previous studies have mainly focused on value calculations and optimal decision strategies during explore/exploit trade-offs and the representation of these computations in the forebrain. However, much less is known about the brainstem circuit-mechanisms that implement behavioural switching between exploitatory and exploratory strategies. Making the decision of whether to exploit or explore necessitates information about the valence and familiarity of different options as well as a modulation of engagement and arousal state. We hypothesised that the median raphe nucleus (MRN) may play a key role in keeping the balance between exploration and exploitation since it has the capacity to integrate the necessary information through major inputs from areas such as the hypothalamus, the interpeduncular nucleus (IPN), and the lateral habenula (LHb). We examined this hypothesis, using a combination of neural tracing, optogenetic manipulations and calcium recording techniques during a battery of behavioural tests in freely moving mice performing instinctive and

learnt behaviours. Our results show that GABAergic neurons in the hypothalamus send motivational signals with strong positive valence to MRN, biasing mice towards exploitation of a known resource and sustainment of current behaviour. Interpeduncular nucleus input to MRN conveys saliency information and glutamatergic input from the lateral habenula to MRN modulates general engagement state required for either strategy. MRN neurons integrate these signals and we find that GABAergic and glutamatergic, but not serotoninergic neurons in MRN are required for switches between explore/exploit strategies. Our study therefore provides a novel brain pathway underlying the balance between exploration and exploitation to implement animals' behavioural strategies during instinctive and learnt behaviours.

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Poster

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Title: Social threats reduce sociability and increases arousal responses

Authors: *G. VELAZQUEZ-HERNANDEZ[1], M. ORTIZ-JUZA[2], V. R. CURTIS[3], S. S. MOY[4], N. C. PEGARD[5], J. RODRIGUEZ-ROMAGUERA[6];
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Abstract: Anxiety disorders are often characterized by excessive behavioral and physiological arousal responses to stimuli.In the case of social anxiety disorders, little is known about the complex neural interactions needed to regulate physiological arousal responses and how these regulate ongoing socially motivated behaviors in the presence of social threats. To study social threats, we employed a social fear conditioning task that pairs contact with a same-sex social stimulus to an aversive foot shock (Toth et al., 2012). On day 1, mice were placed in a chamber (with a floor equipped to deliver foot shock) that contains a window to allow social interaction with a stimulus mouse (inside a small enclosure at the other side of the window). When the two

mice make nose contact for a brief period of two seconds (assessed by individual photobeam breaks in both sides of the window), the experimental mouse receives a 0.3 mA foot shock. We find that mice exhibit both freezing and avoidance behavior to the social threat immediately after receiving a single foot shock, in comparison to a social stimulus that did not receive a foot shock. On day 2, we observed that mice can recognize social threats in a distinct context (three-chamber social interaction assay) and exhibit reduced sociability and freezing behavior in the presence of the social threat. To assess arousal responses to a social threat, we monitored pupil size in head-fixed mice exposed to a freely moving social threat. The threat mouse was allowed to move towards and away from the head-fixed mouse in an L-shaped maze and pupil size was assessed in comparison to a baseline acquired before the stimulus mouse was introduced. We found that arousal responses are higher in the presence of the social threat, as compared to control mice that did not receive a foot shock. Furthermore, social arousal responses were highest when the social threat made physical contact with the head fixed experiential mouse. Taken together, our data suggest that social fear conditioning paradigms are suitable to assess both decreases in social motivation and increases in arousal responses to social threats.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Title: Biomimetically patterned optogenetic stimulation of GABAergic ventral tegmental area axon terminals synapsing onto the brainstem tegmental pedunculopontine nucleus is sufficient to produce artificial naive opiate reward

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Abstract: Morphine injections into the Ventral Tegmental Area (VTA) produce rewarding effects in place preference paradigms. Lesions of the Tegmental Pedunculopontine Nucleus (TPP) in the brainstem selectively block these rewarding effects in previously drug naïve animals. Disruption of dopamine function selectively blocks the VTA-mediated rewarding effects of morphine only in opiate dependent animals undergoing opiate withdrawal. This double dissociation suggests that two different VTA output pathways underlie the motivational effects of morphine in previously drug naïve versus opiate dependent and withdrawn animals. Given that only VTA GABAergic neurons (and not VTA dopamine neurons) have downstream projections to the TPP, we hypothesized that TPP-projecting VTA GABA neurons produce

morphine reward in previously opiate naïve animals. In order to test this, we optogenetically activated VTA GABA neuron cell bodies, as well as the axon terminals of those same neurons in the TPP of separate mice. Mice expressing a GABA-neuron specific Cre construct also had a high fidelity double-floxed channelrhodopsin(ChETA) viral construct injected into the VTA in order to convert each laser pulse into a neuronal action potential. To obtain optogenetic stimulation patterns that would mimic the opiate naïve state, we recorded an exact trace of VTA GABA neuron firing from mice after their first morphine injection. This neuron firing trace then was represented to new mice as pulses of light, spaced apart in time in such a way as to mimic the action potentials seen in the original trace. Previous work in our lab has shown that this biomimetic optogenetic pattern leads to reward behaviour within both real time place preference (RTPP) conditioning and conditioned place preference (CPP) tasks when administered to VTA GABA neuron cell bodies only. To selectively target the VTA GABA pathway projecting to the TPP in mice, ChETA was expressed in VTA GABA neurons and a fiber optic cable capable of delivering the laser "pattern" was implanted over the TPP. Optogenetic triggering of the pattern was thus restricted to GABAergic axons and terminals extending from the area of injection within the VTA and localised to the stimulation-intensity defined region below the fiber optic tip in the TPP. The mice (n=7) displayed significant reward(Student's t-test, $p<0.01$) behaviour on a CPP test following a RTPP paradigm, indicating that selectively stimulating VTA GABA terminals that synapse in the TPP is sufficient to evoke a reward response. Thus, direct projections from VTA GABA neurons to the TPP may underlie the rewarding effects of morphine in previously opiate naïve mice.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Title: Selective reduction in loss-related behavior during subthalamic deep brain stimulation in Parkinson's disease

Authors: *C. M. MERRICK[1], B. BLAIN[2], J. L. SUN[1], G. W. FENG[3], L. H. HAMMER[1], J. SAAL[5], R. B. RUTLEDGE[4], S. LITTLE[1];
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Abstract: Behavioral symptoms such as apathy and impulsivity represent a prevalent and disabling feature of Parkinson's disease (PD), yet there exist limited treatments for these symptoms. Currently available therapies for PD to treat motor symptoms such as deep brain stimulation (DBS) and dopaminergic medication may even exacerbate these behavioral symptoms. To better understand how apathy and impulsivity might be affected by DBS, we recruited fourteen patients with PD implanted with the Percept™ PC device targeting either the subthalamic nucleus (STN; $n = 6$) or the globus pallidus internus (GPi; $n = 8$). While subcortical intracranial and EEG data was recorded, patients played multiple rounds of a value-based decision-making task while DBS was ON or OFF, with the stimulation condition order counterbalanced. For each trial, the participant chose between a gamble option or a guaranteed option to win points. There were three different trial types: trials in which the participant has the potential to lose points (Loss trials), gain points (Gain trials) or to either gain or lose points (Mixed trials). Here, our main dependent variable is the percentage of trials in which the patients chose to gamble. Our secondary dependent variable is a momentary happiness measure in which patients rated their subjective happiness on a visual analog scale from 0-100 after every 2-3 choice trials. Using a mixed effect model, we found a significant interaction between DBS target (STN vs. GPi) and stimulation (ON vs. OFF) for Loss trials ($\chi^2(1) = 8.12$, $p = .004$), suggesting that the behavioral effect of stimulation differs between the two DBS targets. Specifically, for Loss trials, patients with STN DBS gambled more ON stimulation ($M = 41\%$) compared to OFF stimulation ($M = 25\%$; $\chi^2(1) = 10.22$, $p = .001$), but patients with GPi DBS did not differ in their gambling across stimulation condition, choosing risky options 36% ON vs. 39% OFF stimulation ($\chi^2(1) = 0.58$, $p = .445$). Interactions between DBS target and stimulation were not observed for Mixed trials ($\chi^2(1) = 0.005$, $p = .946$), or Gain trials ($\chi^2(1) = 0.845$, $p = .358$) and there were no main effects observed for stimulation (all p's > .666) or DBS target (all p's > .249). Patients with STN DBS also reported higher happiness scores after gambling on Loss trials when STN stimulation was ON ($M = 60$) compared to when stimulation was OFF ($M = 52$; $\chi^2(1) = 6.92$, $p = .008$). This difference was not observed after gambling for Loss trials in patients with GPi DBS, with average happiness rating of 66 ON vs. 63 OFF stimulation ($\chi^2(1) = 1.21$, $p = .272$). Our results suggest that DBS in the STN but not the GPi leads to changes in loss-related behavior and affect.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Title: Subjective food evaluation recruits hypothalamic interactions with reward-related regions

Authors: A. OGAWA[1], T. OSADA[1], M. TANAKA[1], A. SUDA[1], K. NAKAJIMA[2], S. OKA[1], K. KAMAGATA[1], S. AOKI[1], Y. OSHIMA[2], S. TANAKA[2], N. HATTORI[1], S. KONISHI[1]; [1]Juntendo Univ., Tokyo, Japan; [2]The Univ. of Tokyo, Tokyo, Japan

Abstract: The reward-related brain regions in humans (i.e., the midbrain, ventral striatum, orbitofrontal cortex, and ventromedial prefrontal cortex) evaluate and compare various types of rewards given to the organisms. Accumulating evidence has suggested that autonomic factors modulate the reward system via the hypothalamus, a central hub of autonomic functions, consisting of numerous nuclei performing different functions related to food. In decision-making for rewards, interoceptive states such as hunger and thirst influence decision processing. However, it remains unclear how hypothalamic nuclei functionally interact with reward-related brain regions while processing dietary rewards. Higher-resolution images of the participants (N = 25) were collected to investigate the effective interaction between the human hypothalamic nuclei and the regions in the reward system during the subjective evaluation of foods in a reward task that involves reward evaluation as a whole, which is thought to activate the brain regions in the reward system. We also collected the resting-state functional images to parcellate the medial hypothalamus into individual nuclei. The brain activity in individual hypothalamic nuclei was examined during the evaluation of food rewards compared to monetary rewards. The neural pathways toward the reward-related regions were then explored. By analyzing the individual nuclei of the human hypothalamus, we found a significant brain activity decrease in the paraventricular nucleus (PVH) and lateral area (LHA) in the hypothalamus in food evaluation compared with monetary evaluation. A psychophysiological interaction analysis revealed dual interactions between the PVH and the midbrain region and the ventromedial prefrontal cortex (VMPFC) in the reward system. Effective connectivity analyses further revealed the causal interaction from the PVH to the midbrain region and VMPFC during the subjective evaluation of food. These results suggest dual hypothalamic pathways from the PVH to the midbrain region and to the VMPFC in the human reward system that may modulate reward evaluation for decision making.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Title: Effect of optogenetic deep brain stimulation of the supramammilo-septal pathway on reward-seeking behaviors

Authors: *N. WESTCOTT, A. J. KESNER;
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Abstract: Reward seeking is fundamental in the onset and maintenance of substance abuse disorders. It has been shown that the Supramammilary region (SuM) and Medial Septum (MS) play an important role in reward seeking and processing. We have previously demonstrated that optogenetic stimulation of neurons in the SuM-MS pathway is reinforcing, and this effect is mediated by glutamate (GLU) neurons. In addition, we found that SuM^{GLU} neurons form monosynaptic connections with MS^{GLU} neurons, which in turn project to the VTA to influence DA release in the ventral striatum. Further characterization of this novel reward system implicated it in information seeking motivation. Neuroplasticity, or the ability for synapses to have potentiated or depressed responses to stimuli, has also shown to play a large role in addictive disorders as the brain modifies in response to drugs of abuse. The current study aims to follow up on these previous findings by using optogenetics to induce potentiation or depression at the SuM to MS synapse. Our goal was to develop an optogenetic brain plasticity protocol that can drive plasticity in the SuM to MS pathway in order to examine the effect of such manipulation on motivated behavior. To confirm plasticity prior to testing mice in operant sucrose-seeking behavior paradigms as well as open field behaviors, we compared optogenetically driven local field potentials before and after a plasticity protocol that mimics optogenetic stimulation trains used in our prior studies that are known to be reinforcing. In preliminary studies, significant decreases in local field potential were detected post-plasticity protocol, indicating that the procedure successfully altered synapse activity in the SuM-MS pathway. The animals did not alter basic operant responding for sucrose reward in an fixed-ratio-1 schedule, but did augment effort exerted to earn the same sucrose rewards in a progressive ratio test. Open field testing showed a significant increase in average velocity between plasticity and control groups. These findings are an important step in understanding the role of this understudied pathway in motivational processes and could lead to therapeutic interventions for treating psychiatric disorders related to maladaptation in motivated behaviors via techniques like electrical deep brain stimulation or transcranial magnetic stimulation.

Disclosures: N. Westcott: None. **A.J. Kesner:** None.

Poster

225. Reward Seeking and Motivation, Neuronal Circuits

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 225.16

Topic: G.03. Motivation

Support: NIMH R01MH123544.

Title: The role of CRF+ neurons in the anterolateral bed nucleus of the stria terminalis in motivated behavior

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Abstract: The bed nucleus of the stria terminalis (BNST), a highly heterogenous region of the extended amygdala, is densely populated by GABAergic interneurons expressing the neuromodulator Corticotropin-Releasing Factor (CRF). These neurons, and the BNST in general, is sexually dimorphic in both rodents and humans, and may be linked to sex differences in stress-related mood disorders. CRF-expressing neurons in the BNST are known for their role in promoting fear learning and avoidant behavior, via indirect activation of the Hypothalamo-Pituitary Adrenal axis, which drives the stress response. CRF-expressing BNST neurons also project to brain areas involved in reward and motivated behavior. Here we used chemogenetic approaches to investigate how activation of CRF+ BNST neurons in both male and female C57BL/6J mice alters motivated behavior.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Topic: G.03. Motivation

Support: NSF IOS 1557987
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Title: Nucleus accumbens acetylcholine receptors differentially modulate the updating of sign tracking responses

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Abstract: Reward predictive cues can acquire their own motivational value, leading to cue-triggered Pavlovian conditioned approach, or sign-tracking. In dynamic environments, persistent responding to cues that no longer predict reward is maladaptive; thus, animals must learn to flexibly respond to changes in associative structures. While there is strong evidence that animals adjust their sign-tracking behavior after introduction of an omission schedule, where deflection of a lever-cue cancels delivery of the reward, the neural mechanisms underlying this flexibility remain elusive. One area of intrigue is the nucleus accumbens core (NAcC), whose role in

governing sign-tracking behavior has been extensively studied. Within the NAcC, there is a small population of cholinergic interneurons that have historically received little attention despite their modulatory power over dopaminergic input that is essential for the maintenance of motivated behaviors. Recently, these cells have been studied with respect to other flexible behaviors, but it is unknown what role cholinergic transmission plays in the regulation of sign-tracking behavior during the omission schedule. To address this, I evaluated the role of local cholinergic transmission in the NAc following acquisition of sign-tracking training behavior during an omission schedule. Either nicotinic receptors (nAChR) or muscarinic receptors (mAChR) in NAcC were inhibited via infusion of mecamylamine or scopolamine, respectively, prior to each of the first five sessions of omission. In the omission schedule, nAChR blockade reduced the rate at which rats altered their sign-tracking responses to meet the new contingency change in comparison to controls; although nAChR inhibition did not entirely prevent acquisition of the adjusted responses and induced increases in reward port entries. Blockade of mAChRs during omission results in a moderate opposing trend, in which rats may begin to learn the new contingency more quickly than controls. Altogether, these results indicate that activity at NAcC ACh receptors may differentially regulate behavioral flexibility in response to new information about reward-predictive cues.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Topic: G.03. Motivation

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Title: Orexin/hyporetin neurons regulate reward-seeking behavior via the supramammillary nucleus

Authors: *J. MENDOZA, Y. ARIMA, S. IKEMOTO;
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Abstract: Hypothalamic orexinergic/hypocretinergic neurons are known to regulate motivation and reward-related behaviors. Although orexinergic projections to midbrain dopamine neurons are thought to be important in reward-seeking behavior, other target regions may also play a role. In particular, orexinergic neurons appear to provide dense projections to the supramammillary region (SuM), whose projections to the medial septum (MS) are recently found to play a role in reward-seeking behavior. In this study we first examined efferent projection of orexin neurons to the SuM-to-MS pathway by injecting AAV-FLEX-mGFP-syn-mRuby into the LHA and cholera toxin subunit B (CTB) into the MS in orexin-Cre mice. We confirmed dense projection fibers

and terminals in the SuM and found enlarged ending of a mRuby labeled axon terminals close proximity to CTB labeled neurons, suggesting that orexinergic neurons make synaptic contacts with SuM-to-MS neurons. Second, we examined whether optogenetic projections to SuM-to-MS neurons are involved in reward-seeking behavior using an intercranial self-stimulation (ICSS) test and real-time place preference (RTPP) test. We expressed channelrhodopsin-2 in orexin neurons in the LHA and implanted optic fibers in the SuM of orexin-Cre mice. Mice quickly learned to press the lever that activated orexinergic terminals in the SuM. Moreover, the mice showed preference to the compartment where they received orexinergic terminal stimulation at the SuM over the non-stimulation compartment. The results suggest that the stimulation of orexinergic terminals at the SuM instigates and reinforces reward-seeking behavior. In summary, orexinergic neurons appear to innervate SuM-to-MS neurons and modulate reward-seeking behavior. Future studies will examine how the interaction of orexinergic neurons with the SuM-to-MS pathway affects midbrain dopamine neurons in reward-seeking behavior.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Program #/Poster #: 225.19

Topic: G.03. Motivation

Support: DFG SFB 1089

Title: A basal forebrain to midbrain circuit drives exploratory locomotion

Authors: *P. MOCELLIN[1], K. LUXEM[1], H. KANEKO[1], O. BARNSTEDT[1], S. MIKULOVIC[2], S. REMY[1];
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Abstract: Animals move for different reasons and different higher-order brain areas control specific aspects of locomotion. Subcortical circuits modulate and select the appropriate locomotor responses based on the external demands and the internal state of the animal. Here we describe a glutamatergic ($VGluT2^+$) Medial Septum and Diagonal Band of Broca (MSDB) circuit which projections to the Ventral Tegmental Area (VTA) controls locomotor behavior. To investigate the role of MSDB $VGluT2^+$ projections to VTA, we used a combination of electrophysiology, fiberphotometry, pharmacology, and optogenetics. *In vitro* results confirmed the existence of a monosynaptic glutamatergic pathway and highlighted that $VGluT2^+$ MSDB inputs preferentially target $VGluT2^+$ VTA neurons. *In vivo* experiments supported the hypothesis of the VTA as a downstream area in the locomotor circuit and showed a frequency-dependent optogenetic modulation of the speed. An unsupervised machine-learning algorithm (VAME, Luxem et al., 2020) allowed us to extract the animal's pose dynamics and quantify the effect of the network manipulation on the animal's behaviour. We found that the glutamatergic MSDB-

VTA projections drive not only locomotor activity but also whisking and sniffing in head-fixed and rearing in freely moving animals; all of which is a part of the exploratory behavioral repertoire of rodents. Taken together, we identified a glutamatergic basal forebrain to midbrain circuit that is critically positioned at the interface of septo-hippocampal and VTA-striatal circuits. This MSDB-VTA pathway controls both locomotion and rearing, two key behavioral motifs of exploration.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Program #/Poster #: 225.20

Title: WITHDRAWN

Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

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Topic: G.03. Motivation

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Title: Brainwide tracing of monosynaptic inputs to ventral tegmental area glutamate-GABA co-transmitting neurons

Authors: *E. D. PRÉVOST[1], A. PHILLIPS[1], K. LAURIDSEN[1], D. J. MCGOVERN[1], C. MCNULTY[1], Y. S. KIM[2], L. E. FENNO[2], C. RAMAKRISHNAN[2], K. DEISSEROTH[2], D. H. ROOT[1];
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Abstract: The ventral tegmental area (VTA) is a heterogenous midbrain structure involved in the processing of motivated behaviors. With the advent of INTRSECT viral vectors allowing specific targeting of neurons defined by multiple genetic characteristics, the roles of molecularly diverse VTA cell-types have begun to be dissected. The recently discovered glutamate and GABA co-transmitting VTA neurons signal rewarding and aversive outcomes. However, the pattern of neuronal integration onto this unique population of cells is heretofore unknown. Using

a genetically modified mouse line that expresses Cre recombinase in cells expressing vesicular glutamate transporter type 2 (VGluT2) and Flp recombinase in cells expressing vesicular GABA transporter (VGaT), we have mapped brainwide synaptic inputs to glutamate-GABA co-transmitting neurons in the VTA. We infused eight VGluT2::Cre/VGaT::FlpO double transgenic mice with Cre- and Flp-dependent helper viruses (AAV8-nEF-Con/Fon TVA-mCherry and AAV8-Ef1a-Con/Fon oG) followed by a monosynaptic retrograde rabies virus (EnvA-ΔG rabies-GFP). Via high-throughput imaging and a novel tool for semi-automated brain registration (SHARCQ), we quantified the presynaptic input neurons by brain region according to a mouse brain atlas. Glutamate-GABA VTA neurons received the most inputs from the lateral hypothalamus, superior colliculus, periaqueductal gray, lateral habenula, VTA, and dorsal raphe. Cell-type identification and functional assessments of presynaptic neurons are ongoing and will be discussed at the meeting.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Title: A forebrain neural substrate for behavioral thermoregulation

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Abstract: Thermoregulatory behavior is a basic motivated behavior for body temperature homeostasis. Despite its fundamental importance, a forebrain region or defined neural population required for this process has yet to be established. Here, we show that *Vgat*-expressing neurons in the lateral hypothalamus (LH^{Vgat} neurons) are required for diverse thermoregulatory behaviors. The population activity of LH^{Vgat} neurons is increased during thermoregulatory behavior and bidirectionally encodes thermal punishment and reward (P&R). Although this population also regulates feeding and caloric reward, inhibition of parabrachial inputs selectively impaired

thermoregulatory behaviors and encoding of thermal stimulus by LH^{Vgat} neurons. Furthermore, two-photon calcium imaging revealed a subpopulation of LH^{Vgat} neurons bidirectionally encoding thermal P&R, which is engaged during thermoregulatory behavior, but is largely distinct from caloric reward-encoding LH^{Vgat} neurons. Our data establish LH^{Vgat} neurons as a required neural substrate for behavioral thermoregulation and point to the key role of the thermal P&R-encoding LH^{Vgat} subpopulation in thermoregulatory behavior.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Topic: G.03. Motivation

Title: In-vivo structural connectivity of the reward system along the hippocampal long-axis

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Abstract: The hippocampus and it's afferent and efferent projections are involved in a variety of adaptive behaviors processes including memory, reward-learning, and decision making. Furthermore, deficits in the hippocampal system have been implicated in maladaptive behaviors associated with psychopathology, like schizophrenia and depression. Animal histology research has shown that brain regions involved in reward processing and motivation (i.e. vmPFC, NAc, VTA) are interconnected with the hippocampus, primarily the ventral (anterior) region. However, evidence of this structural connectivity in humans is lacking, precluding the ability to assay how structural connectivity with the hippocampus contributes to adaptive behavior. The present study used diffusion MRI and probabilistic tractography to quantify structural connectivity of the hippocampus with reward regions. Using a large sample of subjects (N=200) from the human connectome dMRI data release, we found that the dopaminergic midbrain (VTA) has stronger connectivity with the anterior versus posterior hippocampus, while the other regions had a more heterogeneous connectivity profile along the hippocampal long-axis. These efforts provide the foundation for generating a probabilistic atlas of the hippocampus organized around its structural connectivity with reward-related networks.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Title: The ventral midbrain commonly representing approach and avoidance motivations encodes future force generation

Authors: *S. K. SUGAWARA[1,2,3], Y. NAKAYAMA[1], T. YAMAMOTO[2], Y. H. HAMANO[4,2,5], M. FUKUNAGA[2,3], N. SADATO[2,3,6], Y. NISHIMURA[1];
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Abstract: Avoiding negative outcomes is essential for organisms to fulfill their own life. Although the dopaminergic midbrain is thought to be a center of motivation, it remains unclear whether the ventral midbrain is involved in avoidance motivation in humans. Using functional MRI, we aimed to investigate the functional role of the ventral midbrain for approach and avoidance motivation in humans.Twenty-nine healthy right-handed volunteers participated in a 3T MRI experiment. In the MRI scanner, participants performed a monetary incentive grip-force task with their right hand. In the behavioral task, they were asked to respond to the target stimulus following a cue stimulus as quickly as possible. To manipulate motivational states, there were five types of cue stimuli corresponding to different anticipatory content: high monetary gain, low monetary gain, no monetary incentives, low monetary loss, and high monetary loss. Then, the feedback message of "success" was displayed on the screen if the reaction time was faster than the pre-defined threshold, whereas the feedback message of "fail" was displayed if not. Performance measures were defined as reaction time and peak grip force in each trial.Behavioral data showed that reaction time and peak grip force were significantly improved depending on the amount of monetary incentives both in gain and loss conditions, confirming that motivational levels varied with conditions. To distinctly depict motivation-related and performance-predicting pre-movement activities, we estimated the contrast between conditions and the parametric modulation with trial-by-trial performance fluctuations. Consistent with the previous non-human primate study (Matsumoto & Hikosaka, 2009), we found two subdivisions within the ventral midbrain in which pre-movement activity was different for anticipating monetary losses. The dorsolateral part of the ventral midbrain was activated by anticipating both monetary gains and losses, while the ventromedial part was preferentially activated only by anticipating monetary gains. From the parametric modulation with the trial-by-

trial performance fluctuations, the dorsolateral part of the ventral midbrain was positively correlated with subsequent peak grip forces. In contrast, any part of the ventral midbrain was not correlated with the following reaction times. Taken together from these findings, we conclude that the dorsolateral part of the ventral midbrain, commonly involved in approach and avoidance motivation, also encodes future force generation in humans.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Title: Genetic disruption of dopamine beta hydroxylase confers a behavioral syndrome resembling toxoplasmosis in male and female mice

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Abstract: *Toxoplasma gondii* (TG) is associated with neuropsychiatric disorders and is highly prevalent within the human population. TG-infected rodents show a behavioral syndrome including reduced aversion to cat odors and neophobia, and massive suppression of *Dbh* expression in host organism (reducing NE and decreasing DA) may explain the striking reversal of innate behavior in infected hosts. The locus coeruleus (LC) is the primary source of NE in the brain, projecting extensively within forebrain circuits that govern arousal, stress responses, and motivated behavior. In this study, we examined abnormalities in defensive behaviors and arousal as well as neuronal activity induced by predator odor in *Dbh-/-* mice and *Dbh+/-* controls. *Dbh-/-* and control mice were exposed to a clean cage with a nestlet treated with predator odor (bobcat urine) for 90 min. Immediately after the test, mice were euthanized, and brains were processed for c-fos quantification in the LC and targets implicated in behavioral responses to predator odorants. *Dbh-/-* mice showed high levels of grooming and low levels of burying relative to controls. Controls sustained high levels of arousal throughout the predator odor test, but *Dbh-/-* mice fell asleep within 90 min. The odor-induced high grooming/low burying phenotype of the *Dbh-/-* mice was recapitulated in controls pretreated with a DBH inhibitor. Burying behavior was potently suppressed in control mice by drugs that reduce NE transmission and signaling, and excessive grooming behavior in *Dbh-/-* mice was blocked by a DA receptor antagonist.

Compared with control mice exposed to predator odor, *Dbh-/-* mice demonstrated increased c-fos induction in the LC and medial amygdala but decreased c-fos in the anterior cingulate cortex, lateral septum, periaqueductal gray, and bed nucleus of the stria terminalis. Moreover, using a novel chemogenetic activity-tagging tool (Fos-TRAP), we "captured" brain wide networks activated by bobcat exposure (Bob-TRAP) and reactivated them weeks later with CNO in an odorless environment. CNO reactivation of Bob-TRAPed neurons in the "safe" context induced robust freezing and increased time spent in corners. In conclusion, we suggest that *Dbh*-/- mice may represent a non-infected, monogenetic model of TG infection in rodents, and submit that central NE regulates both arousal and defensive responses to predator odor.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Title: Prepronociceptin-expressing neurons in the extended amygdala signal but are not necessary to drive avoidance behaviors to an aversive odor

Authors: *M. ORTIZ-JUZA[1], R. L. UNG[2], V. R. CURTIS[2], R. A. ALGHORAZI[2], G. VELAZQUEZ-HERNANDEZ[2], A. L. RING[2], R. A. GARCIA-REYES[2], G. D. STUBER[3], P. ZHOU[4], H. K. KATO[5], N. C. PEGARD[6], J. RODRIGUEZ-ROMAGUERA[7];
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Abstract: Atypical arousal responses are a core symptom in neuropsychiatric disorders and occur in parallel to maladaptive behaviors. However, arousal encoding within the context of motivated behavior is unknown. The bed nucleus of the stria terminalis (BNST), a subnuclei within the extended amygdala, is prominently studied for its role in neuropsychiatric disorders,

arousal encoding, and motivated behavior. We previously identified that neurons expressing the *prepronociceptin* gene in the BNST (*Pnoc*^{BNST}*)* encode rapid arousal responses to motivationally salient odors when performing two-photon microscopy simultaneously with pupillometry in head-fixed mice (Rodriguez-Romaguera et al., 2020). Here, we tested if *Pnoc*^{BNST} neurons also regulate distinct aspects of motivated behavior when mice freely explore the same salient odors as our previous study. We used miniaturized head-mounted one-photon microscopes capable of imaging *Pnoc*^{BNST} neurons in freely moving mice. We found that the population activity of *Pnoc*^{BNST} neurons selectively increased when mice approached an aversive odor but not a rewarding food odor. Analysis of individual *Pnoc*^{BNST} neuronal activity dynamics showed that neurons that were more excited to the aversive odor correlated with proximity to odor and the action to dart away from it. We next tested if inhibition of *Pnoc*^{BNST} neurons modulated these same behaviors. We found that bulk inhibition of *Pnoc*^{BNST} neurons did not modulate time mice spent near an aversive odor, distance to an aversive odor, or darting behavior. Our results demonstrate that *Pnoc*^{BNST} neurons signal the location of an aversive odor and the action to dart away from it, but do not regulate such behaviors. Our findings suggest that *Pnoc*^{BNST} neurons are specific to physiological arousal responses and do not play a role in modulating behavioral states.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

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Title: Identification of astrocyte and neuronal populations mediating recovery from a passive behavioral state

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Abstract: Animal behavior is modulated by both external signals in the environment and internal state. As animals switch between internal states, how they respond to a constant stimulus may change. For instance, larval zebrafish (*Danio rerio*) will swim in response to whole-field motion so they can stabilize their position in space. However, we previously established that fish will stop swimming for an extended period of time if they deem their swimming ineffective (i.e., if the swimming leads to no change in whole-field motion). While this demonstrated how fish enter a passive internal state brought about by the futility of their actions, it remains mechanistically unclear why they begin to swim again. Here, we found that the circuit mechanism that induces passivity (an increase in activation of astrocytes in the hindbrain) is inversely related to a mechanism that induces recovery from passivity (a decrease in activation of the same astrocytes). First, we noted that larval zebrafish remain passive for at least 12 seconds (normally, they swim in bouts approximately once per second); we call the active swimming periods that occur after a prolonged period of passivity "rare-swim active periods", or RAPs. We then analyzed whole-brain calcium imaging data to show that astrocytes in the hindbrain become less active in the lead-up to RAPs. This same population of astrocytes also becomes more active in the lead-up to the onset of passivity, suggesting that these cells also trigger passivity in the first place. We also analyzed whole-brain calcium imaging data in neurons and discovered that a brain-wide decrease in neuronal activity precedes each RAP. We next demonstrated that the activity of these astrocytes and neurons can be used to detect the onset of RAPs up to 7 seconds before they occur. Finally, we showed that we can use these astrocytes and neurons to classify individual imaging frames as non-RAPs or RAPs. To conclude, we found that the circuit mechanism responsible for triggering a futility-induced passive state in larval zebrafish may also be involved in exiting this very state. While glia seem to set the stage for recovery from passivity up to 7 seconds before it occurs, neurons may directly trigger swimming more closely in time.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 225.28

Topic: G.03. Motivation

Support: NIH Grant MH120136

Title: Functional properties of corticothalamic circuits targeting paraventricular thalamic neurons

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Abstract: Neurons in the paraventricular nucleus of the thalamus (PVT) receive extensive projections from prelimbic (PL) cortical areas that convey information relevant to decision-making and goal-oriented behavior. Similar to other corticothalamic circuits, PL neurons located in deep cortical layers target PVT via two functionally distinct pathways: i) monosynaptic projections that lead to PVT excitation, and ii) disynaptic PL projections via the anteroventral portion of the thalamic reticular nucleus (avTRN) that lead to feed-forward inhibition. However, it is unknown how the intrinsic neuronal properties and the synaptic dynamics of these two pathways control PVT neuronal output. Using a combination of whole-cell recordings, neuronal tracing, and optogenetics in brain slices of adult rats, we found that in the large majority of PVT neurons, depolarizing current steps or brief trains of PL-evoked synaptic excitation leads to a rapid decay of action potential amplitudes followed by depolarization block, thereby strongly limiting PVT maximum firing rates. Using an intersectional viral approach, we established that the same PVT neurons receiving monosynaptic PL innervation also receive disynaptic inhibition from the PL via the avTRN. Recordings in avTRN revealed that the majority of neurons generate spiking in tonic mode and fail to display rebound bursting, due to the absence of low-threshold calcium spikes. Activation of PL afferents with brief stimulus trains led to large-amplitude postsynaptic responses that displayed short-term facilitation, which in current-clamp led to precise and sustained action potential activity in avTRN. In turn, activation of avTRN afferents in PVT evoked short-term synaptic facilitation, thereby generating reliable $GABA_AR$-mediated inhibition at different frequencies. For PVT neurons that displayed current-step evoked depolarization block, photoactivation of avTRN inputs restored action potential activity. Performing single-unit recordings from PVT neurons *in vivo*, we found that for a subset of cells photoactivation of PL inputs at low frequencies led to sustained PVT firing, while higher stimulation frequencies only led to transient PVT activity, consistent with depolarization block. Taken together, our findings suggest that monosynaptic inputs from PL cause prominent depolarization block in PVT neurons and that highly reliable avTRN-mediated disynaptic inhibition is critical to maintaining PVT neuronal output over a range of PL afferent activity.

Disclosures: D. Jalloul: None. **G. Aquino-Miranda:** None. **X.O. Zhang:** None. **J. Campbell:** None. **F.H. Do Monte:** None. **M. Beierlein:** None.

Poster

225. Reward Seeking and Motivation, Neuronal Circuits

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 225.29

Topic: G.03. Motivation

Support: NIH Grant 5R00DA035251-05

Title: Beyond Reward: The Role of GABAergic Ventral Pallidum Neurons in Aversive Motivation

Authors: *E. M. RAMIREZ, M. X. MARTINEZ, V. A. VERA, M. R. FARRELL, C. M. RUIZ, S. V. MAHLER;
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Abstract: The ventral pallidum (VP) is a basal ganglia structure with a key role in motivated behavior and reward. The VP has an important role in food intake, "liking" of rewarding tastes, conditioned cue responses, and motivated responding for natural and addictive drug rewards. Recent work shows that VP contains at least 2 functionally distinct neuronal subpopulations, GABAergic and glutamatergic. In mice tested in simple behavioral situations, VP^{GABA} neurons seem to mediate reward seeking and approach, while $VP^{Glutamate}$ neurons instead seem to mediate aversion and withdrawal. Our recent work finds that VPGABA neurons in rats also play a key role in appetitive motivation across more complex behavioral situations, including motivated decision making tasks (Farrell et. al. 2021). However, we also found evidence for a role for VP^{GABA} neurons in motivated behavior to avoid a punisher, instead of to receive a reward. Here we followed up this finding using behavioral tasks of aversive motivational states, to better understand the exact circumstances in which VP^{GABA} neurons are required for adaptive behavior.We bilaterally injected a Cre-dependent inhibitory DREADD (hM4Di) vector into the VP of adult male and female GAD:Cre transgenic Long Evans rats, and control rats had no VP DREADDs. We then examined effects of inhibiting VP^{GABA} neurons prior to 1) exposure to a Pavlovian cue which was previously paired with footshock, and which elicits freezing responses, or 2) exposure to a metal probe which previously delivered shock when it was touched, which elicits defensive forepaw treading responses. These behaviors were chosen since they both involve shock punishment and require memory of a previously learned association, but they differ in other characteristics that may help inform the role of VP^{GABA} neurons in these processes. In a within-subjects design, counterbalanced CNO and vehicle injections preceded tests of the expression of previously-learned fear memories. For fear conditioning (FC), we paired tone cues with one of two different shock intensities (0.3 mA or 0.75 mA), and inhibited VP^{GABA} neurons while the tone was played 24 times in the absence of additional shock. FC and extinction videos were manually scored for measures of freezing, locomotion, sniffing and more. For the defensive burying task, responses to unelectrified probes that previously delivered 1.5mA shock were measured, with manual scoring of probe investigations and defensive treading/digging of bedding material, as well as bedding pile dimensions. Results presented in this poster will bring insight to the less-understood role of VP^{GABA} neurons in response to different types of fear-eliciting stimuli.

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Poster

225. Reward Seeking and Motivation, Neuronal Circuits

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 225.30

Topic: G.09. Drugs of Abuse and Addiction

Support: R01DA37207
Ancora Innovation, LLC

Title: Expression of M_5 muscarinic acetylcholine receptor mRNA in identified midbrain dopamine neurons projecting to the nucleus accumbens

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Abstract: Accumulating physiologic and behavioral evidence suggests that selective inhibition of the M_5 muscarinic acetylcholine receptor (mAChR) subtype may provide a novel approach for regulating disruptions in mesolimbic reward circuitry associated with substance use disorders. Previously, our group and others have demonstrated that selective inhibition of M_5 mAChRs using either the M_5 negative allosteric modulator (NAM) ML375 or the M_5 orthosteric antagonist VU6019650 attenuates cocaine, alcohol and opioid drug seeking behaviors in rodents. More recently, we have also demonstrated that the short-acting M_5 NAM VU6008667 can attenuate acquisition of oxycodone self-administration in opioid naïve rats over a 21-day period. In addition, we have reported that VU6019650 blocks the nonselective muscarinic agonist oxotremorine-M-induced increases in neuronal firing rates of ventral tegmental area dopamine neurons within the mesolimbic reward circuitry. In the present study, we conducted a series of anatomical studies to better understand the possible site(s) of action for the observed M_5 inhibitor effects on behavior and physiology by combining RNA in situ hybridization with a retrograde tracer (CTB-Alexa-Fluor 555) to identify the location of M_5 receptors on the different dopamine circuits projecting from the VTA, including the prefrontal cortex, nucleus accumbens (NAc) core and shell, and the dorsal striatum in male Sprague-Dawley rats. Preliminary results revealed that M_5 is primarily expressed on dopaminergic neurons in lateral regions of the VTA, including the parabrachial pigmented nucleus, which project primarily to the NAc, both core and shell. This provides a likely circuit through which M_5 inhibitors are exerting effects on opioid-related behaviors, via decreasing opioid-evoked dopamine increases in the nucleus accumbens core and shell.

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Poster

226. Appetitive Learning and Memory Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 226.01

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA Grant R21DA045277
University of Wyoming Biomedical Science Grant

Title: Sex and region-specific effects of junk food diet on perineuronal nets with the prefrontal cortex of rats

Authors: *G. E. KIRKPATRICK[1,2], B. SHAFAR[3], B. C. GASTON[3], Z. ZHANGE[3], C. R. FERRARIO[4], T. E. BROWN[1];
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Abstract: Obesity prevalence and associated health risks are a growing concern worldwide. Over the past two decades, severe adult obesity has risen from 4.7% to 9.2% in the United States (CDC, 2022). One of the key factors in the development of obesity is the overconsumption of palatable hypercaloric foods, which override homeostatic regulation of feeding to alter reward circuitry (Ferrario, 2020). Current literature suggests that consumption of palatable hypercaloric diets alters medial prefrontal cortex (mPFC) function, resulting in reduced executive control of eating behaviors (Lowe et al., 2019). The mPFC contains parvalbumin expressing, fast-spiking interneurons (PV+ neurons) that are surrounded by specialized extracellular matrix structures called perineuronal nets (PNNS). PNNs are known to limit synaptic plasticity and modulate PV+ neuron activity to maintain excitatory:inhibitory balance within local PFC circuits (Brown and Sorg, 2022). Our previous work showed that 21d consumption of 60% high fat diet altered the intensity of *Wisteria Floribunda Agglutinin* staining within the mPFC in both a sex and region-specific manner (Dingess et al., 2020). It is unknown if the changes we previously reported are widespread amongst palatable diets. To address this question, we exposed 60d male and female Sprague-Dawley rats to a "junk food" diet (14% protein, 58% carbohydrate, 28% fat, Oginsky and Ferrario, 2019) and assayed whether the presence and/or intensity of PNNs in the mPFC were altered. Rats were placed into one of two groups: *ad libitum* chow or *ad libitum* junk food. Following 30d exposure to either diet, we quantified the number and intensity of PNNs in the prelimbic (PL) PFC, infralimbic (IL) PFC, and the ventromedial orbitofrontal cortex (vmOFC). In both sexes there were no changes in PNN number in any region. In the PL-PFC there was no change in PNN intensity in males and females. In the IL-PFC, males had a significant reduction in PNN intensity (CH: 100.0±3.2, JF: 71.5±7.9), while the females showed no significant changes (CH:100.0±3.9, JF:102.5±6.0). In the vmOFC there was a significant decrease in PNN intensity for both male and female rats (Male-CH: 100.0±1.9, JF: 77.81±4.6; Female-CH: 100.0±3.5, JF: 71.6±5.4). Taken together, these results suggest that exposure to a junk food diet alters PNN remodeling in a sex and region dependent manner. In addition, the pattern of PNN changes was unique between junk food and previous findings for 60% high fat diet. Future studies will need to determine what dietary components are responsible for triggering PNN modifications and determine the significance of PNN plasticity on neuronal function and food seeking behaviors.

Disclosures: **G.E. Kirkpatrick:** None. **B. Shafar:** None. **B.C. Gaston:** None. **Z. Zhange:** None. **C.R. Ferrario:** None. **T.E. Brown:** None.

Poster

226. Appetitive Learning and Memory Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 226.02

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIMH Grant R01MH121546

Title: Disentangling Effort, Reward Valuation, and Reward Learning in Online Cognitive Testing

Authors: L. SARABIA[1], ***E. STURM**[3], E. DELAO[2], A. G. SARES[4], A. S. MENDEZ COLMENARES[5], J. DUFFY[4], J. R. TREGELLAS[1], M. THOMAS[4];
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Abstract: Reward learning is a fundamental aspect of cognition that is present across species and is important for understanding normal and abnormal human function, including mental illness. Research has found that probability and magnitude of a reward both contribute to subjective *value* and determine the amount of *effort* someone will expend. However, the relationship between *reward valuation*, *effort*, and *reward learning* is uncertain and difficult to disentangle in cognitive testing. To address the possible confound of effort on reward learning, this study aimed to determine 1) How are reward valuation and effort related; and 2) How do reward valuation and effort relate to reward learning? Participants were 77 members of an online crowdsourcing platform (64% female, 44% non-white), with an average age of 46 years. All participants completed the Effort Expenditure for Rewards Task (EEfRT) and Probabilistic Learning Task (PLT). The EEfRT presented participants with a reward level and win probability for completing an easy or hard trial. Hard trials required more button presses and offered more points than easy trials. The PLT required participants to choose between two stimuli with different probabilities of "correct" feedback (e.g., 60/40%). From the EEfRT, we extracted the proportion of hard trials chosen overall (overall score) and hard trials chosen at high probability and high reward (optimal score), to reflect effort. We then created a normalized score by dividing the optimal score by the overall score, to reflect reward valuation. Accuracy on the PLT was calculated as the proportion of trials where the participant correctly chose the higher probability stimuli, to reflect reward learning. The EEfRT overall and optimal scores were strongly correlated. Accuracy on the PLT was not correlated with the EEfRT optimal score, and the correlation with the overall score was negatively trending. The normalized score, by contrast, was positively trending with PLT accuracy. These results suggest that while reward valuation is associated with better reward learning, overall increased effort is not. Our findings imply that

reward valuation, reward learning, and effort are dissociable abilities. Consistent with these results, research has found separate brain areas are associated with reward learning and valuation. We also determined that to measure reward valuation, researchers should account for effort by using a normalized score. Multiple neuropsychiatric disorders involve deficits in reward processing, which lead to poor functional outcomes. Thus, a greater understanding of this tri-variate relationship could help explain unknown heterogeneity of mental illness.

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Poster

226. Appetitive Learning and Memory Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 226.03

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH K01 MH097091
NIH R01 MH067924
NIH R01 MH10095
NIH R01 MH048463

Title: Mechanisms for resolving the explore-exploit tradeoff in the human dorsal stream

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Abstract: Prior research has shown that, when exploring and exploiting a few discrete options, we rely on sampling and reinforcement histories encoded in our basal forebrain and prefrontal cortex. Additional demands, however, arise when we rapidly move though a continuous space, choosing when to harvest a reward, not unlike a primate moving through a forest. to explore rapidly changing, complex environments requiring fast and spatially precise actions, the primates rely on a phylogenetically newer system: the neocortical “where” stream or the dorsal attention network (DAN). A key DAN hub is the posterior parietal cortex (PPC), which contains world-centric maps incorporating visual information from temporo-occipital areas such as MT+ and parietal somatosensory information; PPC sends output to frontal dorsal and ventral premotor (PMd and PMv) cortex and the frontal eye fields (FEF). Using a previously validated reinforcement learning model (Hallquist & Dombrovski, 2019), we show that exploration/exploitation in this environment relies on dynamic maps in the human dorsal stream. BOLD signal in the DAN (discovery sample: n = 70, two replication samples: n = 146/152, all $p_{FDR} < .001$) and posterior cortical oscillations (n = 63, $p_{FDR} < .001$) revealed reward signals evolving faster than in Skinnerian conditioning yet integrating a longer reinforcement history than a working memory buffer. Exploitation scaled with updates to the dynamic map encoding

the total number of valuable options and spatially specific absolute reward prediction errors for the chosen option. These spatially specific updates modulated posterior beta1/alpha oscillations 500-800ms post-reinforcement and BOLD throughout the frontal and parietal DAN nodes. Exploration scaled only with reward prediction error signals. Finally, midline frontoparietal early theta oscillations were modulated by outcome valence. However, theta activity was not spatially specific. Contrary to previous reports, it did not scale parametrically with reward prediction errors and did not facilitate exploration. These findings were replicated out-of-session and, in case of BOLD signal, out-of-sample and with an enhanced version of the task that eliminated the potential novelty confound; they were robust to controlling for a number of potential behavioral confounds and to accounting for between-subject heterogeneity. These observations support the affordance competition view of human decision-making in a continuous, rapidly moving environment under time pressure and offer new insights into the dynamics of learning and the manner in which reinforcement encoding helps arbitrate among affordances.

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Poster

226. Appetitive Learning and Memory Mechanisms

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Program #/Poster #: 226.04

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01MH097767

Title: Neural response to monetary and social reward in adolescent girls

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Abstract: Research on the neural response to reward has largely been conducted by examining monetary gain and loss. Monetary reward is a convenient stimulus for research, but there are other types of reward that might be more salient to different populations. Notably, social reward has been theorized to have a large impact on motivation and behavior, particularly during adolescence. However, there is limited research on the similarities and differences in the neural response to monetary and social reward among adolescents. In addition, few studies have examined the extent to which different neuroscience measures of neural response, such as functional magnetic resonance imaging (fMRI) and event-related potentials (ERPs), of reward processing are correlated within adolescents, and whether that correlation may differ by the type of reward. In a sample of 145 14-22-year-old adolescent girls, the present study used well-matched monetary and social reward paradigms to assess neural response via fMRI and ERPs.

Both monetary and social reward elicited an increased neural response in the bilateral striatum as measured by the fMRI BOLD response and an increased neural response as measured by the ERP reward positivity (RewP). Striatal activation did not differ in response to monetary and social reward, but the RewP in response to monetary reward was greater than the RewP in response to social reward. Both monetary and social reward neural response was correlated within measures; however, only the neural response to monetary reward was correlated across fMRI and ERP measures. The present study suggests that, within measures, monetary and social reward elicit similar patterns of neural activation, but only monetary reward elicits converging neural activation across fMRI and ERP measures.

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Poster

226. Appetitive Learning and Memory Mechanisms

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Program #/Poster #: 226.05

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH P50 AA012870, NIH K01 AA027832

Title: Perceptual generalization of alcohol-related reward and loss among light and risky drinkers

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Abstract: Risky alcohol consumption is an increasingly prevalent public health problem that is challenging to treat. Understanding the neurocognitive mechanisms that drive consumption may highlight novel targets for intervention. One candidate mechanism is memory for drinking experiences. In particular, less precise, or more widely generalized, memories for drinking may cause a person to seek alcohol in situations that even vaguely resemble these past experiences. Indeed, dopaminergic and noradrenergic signaling, which influence memory generalization, are known to differ in risky drinkers. However, the precision of memory for alcohol-related associations in heavy drinkers has not yet been quantified. In a set of online experiments, we assessed generalization of alcohol-related rewards (Study 1) and losses (Study 2) among light and heavy drinkers using a novel translational paradigm.
Participants first completed a screening questionnaire to determine whether they met NIAAA criteria for light or risky (involving frequent binges and heavy weekly levels of consumption) drinking. Next, light and risky drinkers formed conditioned associations between cards portraying shapes (CS) and photographs (US) portraying alcoholic beverages or neutral objects in naturalistic contexts. In Study 1, US images were “tokens” (added to participants’ bonuses at the end of the study), whereas in Study 2, US images were “penalty tokens” (deducted from the

bonus). After conditioning, participants played a card game in which they chose to play with a shape card or a random card from a deck. Critically, the shapes shown were selected to vary in perceptual similarity to the original CS shapes, allowing us to compute a generalization gradient for associations with alcohol and neutral objects. Finally, participants completed a surprise recognition memory test for the US photographs.
We found conditioning effects in both drinking groups, such that participants in both groups learned to prefer (Study 1) or avoid (Study 2) CS shapes paired with alcohol-related or neutral US images. However, as hypothesized, we did find differences in how light vs risky drinkers generalized these associations, with stronger biases for alcohol-paired CS images among risky drinkers across studies. There is also preliminary evidence that risky drinkers preferentially recognized alcohol pictures during the surprise memory test. Together, these data highlight differences in how broadly conditioned alcohol-related rewards and losses drive subsequent behavior among risky drinkers, paving the way for investigations of neuromodulatory mechanisms shaping these cognitive biases.

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Poster

226. Appetitive Learning and Memory Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 226.06

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Center for Transformative Research on Health Behaviors at Virginia Tech

Title: Stress differentially modulates food reward in male and female mice

Authors: *A. DOFAT[1], K. RUNYON[1], M. TSYGLAKOVA[1], A. HARTLE[1], K. MARSCHALKO[1], R. JACOB[1], G. E. HODES[3], W. M. HOWE[2];
[2]Neurosci., [1]Virginia Tech. Neurosci. PhD Program, Blacksburg, VA; [3]Neurosci., Univ. of Virgina, Blacksburg, VA

Abstract: Acute and chronic stressors can robustly affect food preference, shifting it towards food items that may exacerbate cardio-metabolic risk associated with psychiatric illness. The neural mechanisms behind this relationship are unclear. Many models of food preference place emphasis on central representations of the sensory/hedonic qualities of a food as key determinants of choice. However, recent studies have shown that the nutritional profile of food also has a major influence on how the brain encodes its reward value. Specifically, peripherally-generated, post-ingestive signals of both fat and carbohydrate modulate midbrain dopamine (DA) systems via discrete pathways. Furthermore, food items made of combinations of fat and carbohydrate are over-valued, calorie-for-calorie, due in part to their unique effects on striatal DA release (See also Hartle et al.). Given the well-established effects of stress on DA systems, we hypothesized that stress-induced changes in post-ingestive modulation of mesostriatal DA

release result in maladaptive food choice. To test this hypothesis, we first confirmed our finding that when given free access, both male and female mice (n=40; 18 male, 22 female) preferentially consume food made of a combination of equal parts fat and carbohydrate compared to either macronutrient alone. Next, mice were subjected to either 6 days of variable stressors or control conditions, followed by a second preference test. We found that all male mice again preferentially consumed the CF stimulus in the second test (n=8), though stress further biased this preference (n=10). In contrast, female control mice gravitated toward the more caloric dense fat in the follow up test (n=12), although female mice exposed to stress continued to exhibit a strong preference for CF (n=10). Furthermore, combo food items were sufficient to blunt the inhibitory effect of a novel context on food seeking in female, but not male, mice. Combined, our results suggest that the macronutrient content of foods modifies its attractiveness, and that stress magnifies the appetitive qualities of combination foods in female mice. Our on-going experiments are exploring the mechanisms underlying these sex-specific effects of stress on meso-striatal DA encoding of food reward using multi-region photometry and fluorescent DA reporters.

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Poster

226. Appetitive Learning and Memory Mechanisms

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Program #/Poster #: 226.07

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Minducate - Science of learning research and innovation center
Israel Data Science Initiative

Title: Habit induction in the real world using a novel mobile app

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Abstract: Habits control a substantial part of our actions and thus constitute a prominent feature of behavior. Compared to animal research, the neurobehavioral mechanisms of habits in humans are relatively poorly characterized, in particular as there are no well-established procedures for habit induction in humans. We rationalized that the typical characteristics of laboratory timeframe constraints, artificial context and structured dense training sessions may not be optimal to study habit formation. We developed a novel mobile app, free of the traditional

laboratory constraints, yet well-controlled and informed by basic scientific principles. The app is implemented as a game in which participants enter the app freely and voluntarily 24/7 to virtually dig gold on a remote planet (reward). In a pre-registered and well-powered sample (n=133) we randomly assigned participants to three groups: a short training group (4 days; n=45) or one of two extensive training groups (11 days: n=45 and n=43) distinguished in the number of control manipulations they underwent. On the 3rd or 10th day of the short and extensive training groups, respectively, we devalued the reward (a virtual storage capacity ran out of space for the rest of the day) to test participants' sensitivity to reward devaluation, a behavioral marker to distinguish goal-directed form habitual behavior. Entries to the app despite reward devaluation are considered habitual responses. Our results indicate that the novel mobile app captures the individual variation in habit expression (mean habitual entries across all participants=7.75, standard deviation=20.3). Importantly, comparing changes in response rate following outcome devaluation and control manipulations pre- and post- devaluation between experimental groups has shown that participants were more likely to respond habitually following extensive training compared with a short training ($p=0.012$ of Group x Manipulation interaction; $p<0.008$ for the relevant simple interactions; obtained by a negative binomial mixed-model regression with a "quasi-Poisson" parameterization which was selected using leave-one-out cross-validation from models suitable for overdispersed data). Based on these promising results, the new experimental mobile app we have established is suitable for measuring habit acquisition and expression at the individual and group levels. Moreover, our method may provide a promising way to represent the related behavioral and cognitive processes in more naturalistic conditions. Future research may utilize this tool to further study the neural, behavioral and psychological characteristics of habit formation in humans.

Disclosures: R. Gera: None. **S. Barak:** None. **T. Schonberg:** None.

Poster

226. Appetitive Learning and Memory Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 226.08

Title: WITHDRAWN

Poster

226. Appetitive Learning and Memory Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 226.09

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Conditioned place preferences for virtual alcohol and cannabis cues

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Abstract: The conditioned place preference (CPP) paradigm is an experimental task that is commonly used to assess the motivational effects of contextual cues that have been associated with drug reward. However, the majority of studies to date have established a CPP effect using primary reinforcers such as addictive substances or food. Importantly, abundant evidence indicates that environmental cues that routinely accompany drug use (e.g., drug paraphernalia) can act as potent secondary reinforcers for addictive behaviors.
We used a human analogue of the CPP paradigm to investigate whether a CPP can be induced for a virtual reality (VR) room that previously contained virtual cues related to either alcohol (experiment 1) or cannabis (experiment 2), in the absence of any actual drug administration. In experiment 1, 298 undergraduates completed 6 conditioning sessions in which they were confined to 1 of 2 visually-distinct VR rooms (3 minutes per session); one of the VR rooms contained virtual alcohol cues while the other VR room was neutral. Both the VR room associated with alcohol cues and the order of conditioning sessions were counterbalanced across participants. Following conditioning, participants completed a 3-minute test session in which they had unrestricted access to both VR rooms and neither room contained any alcohol-related cues. Results indicated that during the test session, although no alcohol cues were present, participants with alcohol use (n = 248) spent significantly longer in the VR room that previously contained virtual alcohol stimuli relative to a neutral VR room compared to non-drinkers (n = 50; $F(1, 296)$ = 4.53, p = 0.03). That is, even in the absence of alcohol administration, participants with alcohol use demonstrated a significant CPP for a VR room that was previously paired with virtual alcohol cues, while non-drinkers did not.
Experiment 2 employs the same experimental design as experiment 1, except that we replaced virtual alcohol cues with virtual cannabis cues. Data collection remains ongoing, but pilot data suggests that participants with cannabis use also display a significant CPP for a VR room that previously continued virtual cannabis cues relative to a neutral VR room. To the best of our knowledge, these are the first studies to show that a CPP can be induced for alcohol-associated or cannabis-associated secondary reinforcers. These findings may have critical implications for the development of VR-assisted cue exposure therapies for problematic substance use among undergraduates.

Disclosures: **S. Sklenarik:** None. **C. Burrows:** None. **R.S. Astur:** None.

Poster

226. Appetitive Learning and Memory Mechanisms

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 226.10

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIMH 1P50MH119569-01A1

Title: A translational bandit task elicits time- and frequency-dependent neural value representations in humans

Authors: *E. RAWLS[1], C. D. TEICH[1], C. DEMRO[2], C. S. CHEN[2], N. M. GRISSOM[2], B. A. EBITZ[3], A. W. MACDONALD, III[2], S. R. SPONHEIM[4,1];
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Abstract: Progress in translational psychopathology research requires computationally valid tasks; that is, tasks that tap into the same computational process across multiple species. To examine reward valuation across multiple levels of translation, we report results from a human version of a Translational three-armed Bandit Task (TBT) that is validated for use in rodents and non-human primates. The reward probabilities of the three arms change slowly over time, generating robust learning following unexpected reward delivery or omission. We fit computational reinforcement learning (RL) models to the choice and reward delivery sequences of n = 16 healthy human subjects while EEG data were acquired concurrently with 3T fMRI. In line with prior research using non-human primate and rodent versions of the TBT, we found that subjects' behaviors were best described by weighted reward history (a Rescorla-Wagner delta-rule model) combined with a tendency to repeat previous choices, in comparison to simpler models. Using the best-fitting model parameters for each subject, we generated trial-level regressors consisting of signed positive and negative reward prediction errors (RPEs). We then used these regressors for single-trial model-based analysis of scalp EEG time series. This analysis revealed a robust parietal activity correlation with signed positive RPEs, peaking at ~350 ms post-feedback. Interestingly, even though 45% of feedback signaled reward omission, no correlations with negative RPEs survived corrections for multiple comparisons. To isolate task-responsive brain activity, we applied data reduction via group independent components analysis (G-ICA) to the combined EEG data. The highest-variance G-ICA component (29% ERP variance accounted for) exhibited a centro-parietal scalp topography and a time course resembling the reward positivity. Single-trial analysis of this component activity revealed enhanced signaling of signed positive RPEs compared to analysis of raw scalp time series, as well as significant positive low-frequency (~2 - 8 Hz) power correlations with signed positive RPEs that were not apparent at the scalp level. Even with the enhanced signal-to-noise ratio obtained by application of G-ICA, we detected no neural representations of signed negative RPEs on omission trials. We conclude that, despite necessary cross-species design differences, the human version of the TBT produces robust neural representations of signed RPEs, often considered a common currency underlying reinforcement learning.

Disclosures: E. Rawls: None. **C.D. Teich:** None. **C. Demro:** None. **C.S. Chen:** None. **N.M. Grissom:** None. **B.A. Ebitz:** None. **A.W. Macdonald:** None. **S.R. Sponheim:** None.

Poster

226. Appetitive Learning and Memory Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 226.11

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Bradley University Department of Psychology Summer Research Grant awarded to M. Traficanti

Title: Tracking the emergence of attentional capture by a subliminally-induced reward association: evidence from event-related potentials

Authors: M. TRAFICANTI, *J. HARRIS;
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Abstract: Reward, as a form of behavioral relevance, plays a key role in teaching attention to prioritize previously useful information. The effects of this mechanism are observed in the visual domain as capture of attention to previously reward-associated objects or features, reflected in faster and more accurate responses to task-relevant targets containing a reward associated visual element, and in an increased amplitude of the N2pc event-related potential component. Whether the reinforcement contingencies are explicit or learned over the course of an experimental task, these attentional capture effects appear relatively consistent. Whether a reward-association can be learned under conditions of complete unawareness of the reward associated feature itself is unknown. We sought to track the emergence of attentional capture by a previously subliminal reward associated feature. For this, participants completed a visual pop-out discrimination task, wherein they indicated the orientation of a circular Gabor patch presented on either the left or right side of the screen. In between these blocks of this task, they completed a continuous flash suppression task, in which they identified face or house targets in ongoing streams of visual distracters presented at a rate of ~11 Hz. Unbeknownst to them, Gabor patches of each target orientation were presented to the suppressed eye, with one being systematically associated with a 10 cent monetary reward. For the size pop-out discrimination task, mean amplitude N2pc measures of attentional deployment were submitted to a 3 by 2 repeated-measures ANOVA with the factors of task phase (before, during, and following exposure to subliminal reward-associated features) and target orientation (rewarded and unrewarded). A task phase by orientation interaction, driven by the emerging enhancement in the N2pc in response to targets of the rewarded orientation, demonstrates that a subliminal reward-associated feature can indeed teach attention.

Disclosures: M. Traficanti: None. **J. Harris:** None.

Poster

226. Appetitive Learning and Memory Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 226.12

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Reward learning induces structural plasticity along midbrain to nucleus accumbens white-matter tract

Authors: *S. OMYAN, E. H. ELLIS, J. K. LEONG;
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Abstract: We tested whether reward learning can induce structural plasticity in white-matter tracts converging on the Nucleus Accumbens (NAcc). Previous studies found that motor skill learning can change cortical gray matter and memory tasks can change hippocampal white matter. However, no research has found plasticity of subcortical white-matter tracts after reward learning in healthy adult humans. Since NAcc activity during reward anticipation and outcomes is caused in part by dopamine release from the Ventral Tegmental Area (VTA), we targeted the white-matter tract from the VTA to the NAcc. We measured 20 healthy adults (age mean = 28 years, age range = 18-40 years old, 9 females, all right-handed) at 2 scanning sessions separated by 3 days. At the first session, subjects completed the following procedure in order: Diffusion-Weighted Imaging (DWI) scan, T1 scan, 1 hour of reward learning tasks, repeat T1 scan, and repeat DWI scan. At the second session, subjects completed the same procedure in the same order. Bookending both sessions with DWI scans allowed us to demonstrate within-session reliability before testing between-session plasticity. Subjects completed 3 reward learning tasks: monetary incentive delay, monetary incentive learning with static reward contingencies, and dynamic reinforcement learning. Task order was counterbalanced between subjects, and also within subjects between the 2 sessions. The reward magnitudes in all tasks were -$5, $0, and +$5, and the tasks were fully incentivized on a trial-to-trial basis. To characterize the VTA-NAcc white-matter tract in each participant, we first identified the seed regions in subject space. We identified the NAcc with FreeSurfer's subcortical segmentation and the VTA with the Pauli atlas transformed from template space to subject space using Advanced Normalization Tools. We then performed constrained spherical deconvolution-based probabilistic tractography with MRtrix to visualize the VTA-NAcc tract in each hemisphere of every subject from all DWI scans. We measured Fractional Anisotropy (FA) along the trajectory of the VTA-NAcc tract, and compared tract FA between the first and last scans. Paired t-tests found increased FA along the tract near the NAcc endpoint in both hemispheres (left: $t=3.46$, $p<0.01$; right: $t=2.16$, $p<0.05$). These preliminary results provide the first neuroimaging evidence of white-matter plasticity along the VTA-NAcc tract in adult humans after reward learning.

Disclosures: S. Omyan: None. **E.H. Ellis:** None. **J.K. Leong:** None.

Poster

226. Appetitive Learning and Memory Mechanisms

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Topic: G.02. Reward and Appetitive Learning and Memory

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NCTIC (WFSOM CTSI)
NIH F31DA053174 (LPS)

Title: Reduced punishment learning and computational models that predict individual differences in subjective feelings characterize patients with treatment-resistant depression

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Abstract: Electroconvulsive therapy (ECT) is effective for 85% of patients with treatment-resistant depression, though it is unknown what differentiates and characterizes patients who will achieve full remission. To bridge this knowledge gap, we have deployed computational methods that link choice behavior, brain function, and clinical assessment data to pinpoint differences in 'positive and negative valence systems' that may correlate with degree of ECT success. We hypothesized that (i) prior to ECT, patients with treatment-resistant depression will show altered reward and punishment reinforcement learning compared to controls and (ii) parameters derived from computational reinforcement learning based models that are predictive of individuals' moment to moment changes in subjective feelings will correlate with clinical assessments of depression. In an ongoing study, we recruited patients who were naïve to, but consented for standard of care ECT (ECT: n=16), patients being treated for diagnosed depression (non-ECT: n=17), and participants without a diagnosis of depression (control: n=27). Participants completed a probabilistic reward and punishment task while being scanned with functional magnetic resonance imaging. Participants were also evaluated with Hamilton Depression Rating Scale (HAM-D) and Patient Health Questionnaire 9 (PHQ-9) assessments. Computational models that separate reward and punishment reinforcement revealed between-cohort differences in punishment learning rates; these were reduced for ECT compared to control groups ($F_{2,56}$=352.2, p=1.9e^{-32}; p-adj: 7.3e^{-12}). Further, the ECT cohort showed reduced BOLD response to punishment prediction errors which negatively correlated with HAM-D and PHQ-9 score in both the habenula and right dorsal striatum ($p<0.001$ small-volume uncorrected). Beta coefficients derived from models that predict participants' subjective feelings showed very strong positive associations with these same clinical scores (HAM-D: R=0.84, p=6.4e^{-16}; PHQ-9: R=0.81, p=2.5e^{-14}). Together, our results suggest that reduced neural and behavioral measures of punishment learning appear to characterize patients consented for ECT. Further, subjective feelings associated with decision-making outcomes show correlation with depression severity across cohorts. Future work includes obtaining similar measures after patients receive ECT to determine whether this treatment may alter components of the negative valence system or neural processes involved in representing subjective affect.

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Poster

226. Appetitive Learning and Memory Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 226.14

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01 DA034685
F32 DA038927
5F32MH119796

Title: Striatal dopamine influences the hemodynamic response in humans

Authors: *I. BALLARD[1], I. PAPPAS[2], D. FURMAN[3], A. S. BERRY[4], R. L. WHITE III[5], A. S. KAYSER[6], W. J. JAGUST[7], M. DESPOSITO[3];
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Abstract: The cerebral vascular response has a critical role in satisfying neuronal demands and is the basis for BOLD imaging. Dopamine and vasculature are closely associated as dopaminergic neurons innervate microvessels and dopamine agonists can elicit cerebrovascular dilations (Edvisson et al., 1985; Krimer et al., 1998). However, the relationship between hemodynamic responses and dopamine in humans is unclear.
In a group of 52 healthy male and female human subjects, we measured a proxy of hemodynamic delay using BOLD fMRI. We also measured dopamine synthesis capacity using the PET radioligand 6-[18F]fluoro-l-m-tyrosine ([18F]FMT). We found spatial differences of hemodynamic lags within the striatum. Hemodynamic responses in the nucleus accumbens, a major target of mesostriatal dopamine, peak an average of 2 seconds faster than more dorsal regions of the striatum and much of neocortex. Consistent with dopamine having a direct hemodynamic effect, we found a positive correlation between dopamine synthesis capacity as measured by PET and hemodynamic lag in the nucleus accumbens. In addition, in a separate cohort of 19 healthy male and female subjects performing a reward decision-making task, individuals, we found that lags in the hemodynamic response are reduced for positive relative to negative rewards, consistent with dopamine driving a faster hemodynamic response. In sum, we found converging evidence across three different datasets that dopamine release in the striatum leads to a faster hemodynamic response. Moreover, these results raise the possibility that hemodynamic lags in the nucleus accumbens may provide a proxy measure of dopaminergic tone in human subjects.

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Poster

227. Neural Mechanisms of Relevance to Psychiatric Illness and Treatment

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 227.01

Topic: G.05. Mood Disorders

Support: FAPESP 2018/04250-5

Title: Antidepressants trigger the p75 proteolytic pathway in addition to inducing p75-dependent behavioral changes and brain plasticity

Authors: *C. DINIZ[1], A. CRESTANI[1], A. BORGES-ASSIS[2], P. CASAROTTO[3], C. BIOJONE[3], F. WINKEL[3], C. CANNAROZZO[3], M. SAHU[3], B. WILTGEN[1], E. CASTRÉN[3], L. RESSTEL[2];
[1]Univ. of California, Davis, CA; [2]Univ. of São Paulo, Ribeirão Preto, Brazil; [3]Univ. of Helsinki, Helsinki, Finland

Abstract: Rationale: Despite all efforts, there are still huge gaps to be bridged up with regard to the mechanism of action of antidepressants. Based on the reasoning that p75 receptors are functionally important for some behavioral outcomes that overlap with antidepressant effects, we have studied how important are the p75 receptors for antidepressants. Indeed, I have previously conceptualized through what I called the continuum-sorting hypothesis that p75 might be relevant to the effects of antidepressants on cognitive flexibility and brain remodeling (Diniz et al 2018). I argued that the well-known action mechanism of such drugs involving BDNF/TrkB might spill over toward the non-canonical proBDNF/p75 pathway, a way to explain the great extent by which antidepressants fine tune neuroplasticity under broad demands. Methods: First, HEK cells were transfected to express p75. Cell surface p75 levels (chemiluminescence) and ECD (extracellular domain) shedding (ELISA) were verified. Next, western blotting was performed with lysed cells to measure the levels of CTF (carboxy-terminal fragment) and p75 dimers. ICD (intracellular domain) release was evaluated by using a specific approach based on the ability of the p75ICD bound-yeast transcription factor Gal4 (GVP) to express luciferase through the activation of an upstream activation sequence (UAS). ICD nuclear levels were checked through immunofluorescence and confocal imaging. Ocular dominance shift model was used to assess the effects of antidepressants on cortical neuroplasticity in p75KO mice. Still, a p75 blocker or a selective inhibitor of c-jun N-terminal kinase (JNK), either to treat mice intraperitoneally or the infralimbic (IL) of rats, were used to study whether antidepressants effects on extinction memory depend on p75 signaling. **Results:** Indeed, 15min or 6h of fluoxetine or ketamine treatment decreased cell surface p75 levels and increased ECD shedding. Fluoxetine for 6h, but not ketamine, increased p75 dimers and CTF levels. However, either treatment for 15min or 6h induced higher ICD release and nuclear localization. Besides, fluoxetine and ketamine induction of ocular dominance shift in eye deprived adult mice was gone with p75KO animals. In addition, indeed the effect of fluoxetine and ketamine in enhancing fear extinction depend on p75 signaling. Conclusion: Our data suggest that antidepressants as

diverse as the typical fluoxetine and the fast-acting ketamine are able to trigger the p75 proteolytic pathway in vitro and that p75 signaling is indeed pertinent for their in vivo effects.

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Poster

227. Neural Mechanisms of Relevance to Psychiatric Illness and Treatment

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Program #/Poster #: 227.02

Topic: G.05. Mood Disorders

Support: NIMH Grant MH097988

Title: Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) Infusion to the Medial Habenula Reduces Sucrose Preference in Rats

Authors: *N. R. FONTAINE[1], K. ABEDRABBO[2], M. AKTAR[1], M. N. BOUCHER[1], L. LEPEAK[1], V. MAY[2], S. E. HAMMACK[1];
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Abstract: We have previously demonstrated that the activation of central pituitary adenylate cyclase activating polypeptide (PACAP) systems plays key roles in mediating the consequences of stressor exposure. Moreover, chronic stress dramatically increases PACAP expression in the bed nucleus of the stria terminalis (BNST), and we have argued that this increase is critical for the behavioral and physiological consequences of stressor exposure. Using Cre-dependent reporter expression in PACAP-ires-Cre mice, we have identified a PACAP projection from the BNST to the medial habenula (MHb), and we have also demonstrated that MHb neurons contain PAC1 receptors. Anhedonia, or the lack of pleasurable responding to a normally pleasurable event, is a common symptom of depression, is a behavioral consequence of chronic stress in rodents, and may depend on MHb function. Using the sucrose-preference test, we report that adult male Sprague-Dawley rats that received bilateral infusions of PACAP to the MHb (n=15) exhibited significantly reduced sucrose preference scores compared to controls infused with vehicle (n=16). Moreover, we found that MHb infusion of vasoactive intestinal peptide (VIP) did not significantly alter sucrose preference scores, suggesting that the effects of MHb PACAP infusion are mediated by PAC1 receptors. These findings indicate that PACAP binding to PAC1 receptors in the MHb can induce anhedonia in male rats.

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Poster

227. Neural Mechanisms of Relevance to Psychiatric Illness and Treatment

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Program #/Poster #: 227.03

Topic: G.05. Mood Disorders

Support: 3R01MH120131-02S2
T32-MH076690-10

Title: Gaba and glutamate co-release in the lateral habenula affect risky decision-making

Authors: *N. RODRIGUEZ SOSA, S. SHABEL;
Univ. of Texas, Southwestern Med. Ctr., DALLAS, TX

Abstract: More than 300 million people in the world suffer from Major Depressive Disorder (MDD). For better treatment, we need an understanding of the circuits and mechanisms that underlie the disorder. Many brain regions contribute to the pathology of depression, but the lateral habenula (LHb) stands out because, in contrast to VTA dopamine neurons, it is activated by aversive stimuli and reward omission. Additionally, hyper-activation of the LHb has been implicated in depression in humans. Significant input to this region comes from the internal globus pallidus (GPi), which is thought to drive LHb activity during negative feedback (aversive stimuli) and reduce its activity during positive feedback (reward). Interestingly, although GPi-LHb projections are predominantly excitatory, they co-release GABA with glutamate. In a rat model of depression, the ratio of GABA-to-glutamate in GPi-LHb is reduced. In contrast, antidepressant treatment increased the ratio in mice. I hypothesized that the ratio of GABA-to-glutamate released from the GPi regulates neuronal activity in the LHb, and the inability to increase the ratio during excessive excitatory input results in LHb hyperactivity and increases in negative affective states. To test this hypothesis, I determined if reduced GABA or glutamate co-release from GPi-LHb projections increases or decreases negative affective behavior. Preliminary results suggest that reducing GABA co-release by knocking down the vesicular GABA transporter (VGAT) causes anhedonia in male mice and increased passive coping during the tail suspension test in female mice but does not change other negative affective behaviors. To test for a more specific role of GABA co-release in regulating behavioral sensitivity to positive and negative feedback, I tested another group of mice in a risky decision-making task. VGAT knockdown mice were risk-averse, consistent with altered sensitivity to positive and/or negative feedback. Surprisingly, preliminary results suggest that knockdown of glutamate co-release in GPi-LHb also causes risk aversion in female mice. Future experiments will focus on how GABA and glutamate co-release in GPi-LHb affect LHb activity and processing of positive and negative feedback.

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Poster

227. Neural Mechanisms of Relevance to Psychiatric Illness and Treatment

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Program #/Poster #: 227.04

Topic: G.05. Mood Disorders

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ONR 00014-19-1-2149
Pritzker Neuropsychiatric Research Consortium Fund, LLC
Hope for Depression Research Foundation

Title: Increased fibroblast growth factor-12 (FGF12) is associated with negative affect

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Abstract: The fibroblast growth factor (FGF) family has previously been implicated in mood disorders, especially Major Depressive Disorder (MDD). FGF12, one of the intracellular members, was increased in the anterior cingulate cortex of individuals with MDD (Evans et al., 2004). FGF12 is an intracellular FGF that binds to voltage-gated sodium channels to increase neuronal excitability. We sought to understand whether FGF12 was altered in the hippocampus of individuals with mood disorders, particularly MDD. We also analyzed FGF12 gene expression in the hippocampus of two rodent models of depression-like behavior. Finally, we sought to determine cell type expression of FGF12. In MDD, FGF12 gene expression was increased in the postmortem human hippocampus by qRT-PCR, and the anterior dentate gyrus by mRNA *in situ* hybridization. In rats, FGF12 gene expression was increased in the dentate gyrus of the hippocampus following chronic variable stress and subchronic social defeat in adulthood. FGF12 mRNA was present in glutamatergic and all subtypes of GABAergic neurons in the cortex and the dentate gyrus. Since intracellular FGF12 can increase neuronal excitability through its interaction with voltage-gated sodium channels, and there are considerably more glutamatergic cells in the dentate gyrus, FGF12 may bias the hippocampus towards increased excitability. This hypothesis remains to be tested. In conclusion, increased FGF12 is associated with depression in humans and stress exposure in rodents. Its potential role in altered excitation-inhibition balance in the hippocampus warrants further investigation.

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Poster

227. Neural Mechanisms of Relevance to Psychiatric Illness and Treatment

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 227.05

Topic: G.05. Mood Disorders

Support: NIH R01 MH124998

Title: Untangling how VTA-NAc firing rates mediate chronic stress-induced reward-seeking deficits

Authors: L. B. JOHNSTON[1], A. A. AMILCAR[2], I. B. BANDO[2], *A. HARRIS[3];
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Abstract: Anhedonia, defined as reduction in the pursuit of pleasure, is one of the core symptoms of depression. Stress, a risk factor for depression, decreases reward seeking in both humans and mice. We recently demonstrated that ventral tegmental area (VTA)-nucleus accumbens (NAc) activity underlies the reduction in anticipation seen after a single episode of restraint stress (Lowes et al., *Nature Communications* 2021). However, the precise nature of the behavioral changes (e.g., 'wanting' vs 'liking') that underlie deficits in reward seeking after chronic stress remain unknown. Moreover, the impact of chronic stress on reward circuit activity remains controversial, with studies reporting both increases and decreases in VTA dopamine firing rates (Tye et al. *Nature* 2013; Chaudhury et al. *Nature* 2013). Crucially, these findings were recorded in homecage or *in vitro* conditions, so it remains unclear how these changes in activity mediate altered reward processing. Here, we implanted chronic electrodes simultaneously in VTA and NAc to record both local field potential (LFP) and single unit activity as mice performed a cued gustometer task after undergoing chronic social defeat stress (CSDS; C57BL/6J males; n=5). Additional cohorts of female mice undergoing CSDS are currently underway. 24 hours after CSDS, we observe deficits in wanting and liking as measured by anticipatory lick rate and post-reward consumption lick rate, respectively. To determine the reward circuitry, we are sorting neurons into their putative identities (e.g., VTA dopaminergic, VTA GABAergic, NAc MSN, etc) and analyzing the lick- and cue-evoked firing rates, synchrony between the LFPs in both regions, and phase-locking of single units with task-evoked oscillations. These analyses should provide insight into the nature of sustained changes in reward seeking induced by chronic stress.

Disclosures: L.B. Johnston: None. **A.A. Amilcar:** None. **I.B. Bando:** None. **A. Harris:** None.

Poster

227. Neural Mechanisms of Relevance to Psychiatric Illness and Treatment

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 227.06

Topic: G.05. Mood Disorders

Support: NIH grant P01DA047233-02

Title: Striatal circRIMS2 regulates depression-like behavioral abnormalities

Authors: *V. LEHMANN[1], M. P. HEYER[2], O. ISSLER[4], A. RAMAKRISHNAN[5], E. J. NESTLER[3], P. J. KENNY[3];
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Abstract: Circular RNAs (circRNAs) are formed by back-splicing of pre-mRNA transcripts to generate a circularized product, many of which may have regulatory functions in the brain. Functional deficits in medium spiny neurons (MSNs) in the nucleus accumbens (NAc) may contribute to behavioral abnormalities in depression and other stress-related disorders. Here, we investigated the role of circRNAs in the NAc in regulating stress-induced behavioral deficits. We found that expression of circRIMS2, which is derived from exons 20-22 of the RIMS2 gene, was increased in postmortem NAc from male, but not female, patients who had suffered from major depressive disorder prior to death. Similar increases in cirRIMS2 were detected in the NAc of male mice subjected to social defeat stress (SDS). circRIMS2 overexpression in the NAc, accomplished using an AAVDJ-sEF1a-ZKSCAN1-circRIMS2-sYFP vector, decreased the expression of depression-like deficits in anxiety and reward-related behaviors in male mice subjected to SDS or restraint stress. Single-cell RNA sequencing (scRNAseq) was performed using 10X Chromium on NAc tissue from stressed and unstressed male mice. Stress induced profound alterations in the expression of genes relevant to mitochondrial-mediated bioenergetics including mitochondrial solute carriers, transcriptional plasticity that was reversed by circRIMS2 overexpression. Currently, we are exploring the role of circRIMS2-regulated mitochondrial genes with respect to stress-induced behavioral abnormalities.

Disclosures: V. Lehmann: None. **M.P. Heyer:** None. **O. Issler:** None. **A. Ramakrishnan:** None. **E.J. Nestler:** None. **P.J. Kenny:** None.

Poster

227. Neural Mechanisms of Relevance to Psychiatric Illness and Treatment

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 227.07

Title: WITHDRAWN

Poster

227. Neural Mechanisms of Relevance to Psychiatric Illness and Treatment

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 227.08

Topic: G.05. Mood Disorders

Title: Olfactory bulbectomy induces hyperlocomotion associated with increased density of astrocytes in male rats

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Abstract: Major depression disorder (MDD) induces a constellation of behavioral deficits including difficulties to adapt to novelty. There are several hypothesis including the neuroimmune hypothesis to explain MDD. The prefrontal cortex (PFC) is a brain region involved in the physiopathology of MDD. Olfactory bulbectomy (OBX) is a well-known preclinical model of depression-related behavior in rodents. In the present study, we tested the hypothesis that OBX induce alterations to adapt to a novel environment as a result of gliosis in the PFC in a time-dependent manner in the rat. To test a possible lack of adaptation to novel places, we measured horizontal and vertical behaviors in the open field test (OFT). To determine whether OBX modulated astrocytic and/or microglial expression, we measured the number and morphology of glial fibrillary acidic protein (GFAP) and ionized calcium binding adaptor molecule 1(IBA1) positive cells, markers of astrocytes and microglia, respectively, in the PFC. All these measurements were done in three independent cohorts at three critical times after surgery: one, four and fifteen weeks. OBX rats presented hyperlocomotion in the OFT at one and four weeks after surgery, interpreted as a failure of adaptation to novel environment. At four weeks after surgery, OBX rats displayed increased rearing and grooming, suggesting alterations in stress-coping behaviors. The number of astrocytes was increased in the PFC in OBX rats at one and four weeks after surgery. The data from this preclinical animal model supports the immunological theory of MDD. These results add further support to the validity and usefulness of the OBX rat as a model of depression.

Disclosures: J. Morales-Medina: None. **M. Bautista-Carro:** None. **G. Flores:** None.

Poster

227. Neural Mechanisms of Relevance to Psychiatric Illness and Treatment

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 227.09

Topic: G.05. Mood Disorders

Title: Proliferation of putative GABA/glutamate co-releasing synaptic terminals in the primate habenula

Authors: *L. RIOS[1], C. TAN[2], K. GLEASON[1], A. HAJEISSA[3], Y. SHARMA[1], V. NARLA[1], C. A. TAMMINGA[1], S. SHABEL[1];
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Abstract: The lateral habenula (LHb) responds to aversive stimuli, and its hyperactivity is hypothesized to contribute to depression. Recent studies in rodents have shown that inputs from the basal ganglia and ventral tegmental area to the lateral habenula co-release GABA with glutamate; the balance of GABA and glutamate at these synapses may regulate LHb responses to aversive stimuli and mood. In the present study, we investigated whether the magnitude and topography of co-release of GABA and glutamate in the LHb is conserved in primates. Our data indicate substantial co-labeling of GAD (the synthesizing enzyme for GABA) and VGLUT2 (vesicular glutamate transporter) in synaptic terminals in the monkey and human LHb, consistent with co-release of GABA and glutamate from individual terminals onto primate LHb neurons. Furthermore, our data shows that there are differences in the topography of co-release in the primate and rodent LHb. In the rodent LHb, there is more co-labeling of GAD and VGLUT2 in the ventral-lateral regions, whereas in the primate LHb there is more co-labeling in the dorsal region, perhaps due to expansion of the LHb in primates. We obtained similar results when labeling the vesicular GABA transporter, VGAT, instead of GAD. Thus, co-release of GABA with glutamate may be a conserved mechanism for regulation of LHb activity and mood in rodents and primates.

Disclosures: L. Rios: None. **C. Tan:** None. **K. Gleason:** None. **A. Hajeissa:** None. **Y. Sharma:** None. **V. Narla:** None. **C.A. Tamminga:** None. **S. Shabel:** None.

Poster

227. Neural Mechanisms of Relevance to Psychiatric Illness and Treatment

Location: SDCC Halls B-H

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Program #/Poster #: 227.10

Topic: G.05. Mood Disorders

Support: Brookhaven National Laboratory Directed Research and Development Program, funded by the US Department of Energy (LDRD-07-096 to FAH)
Quinnipiac University School of Health Sciences Faculty Scholarship Grants (to MMM)
NIH R21DA029245 (to DS)

Title: Molecular Basis for Antidepressant Effects of Deprenyl in Rodents with Congenital Learned Helplessness

Authors: *M. M. MIRRIONE[1,3], M. L. PRINA[1], A. A. MAAS[1], K. R. MARKS[2], F. A. HENN[3], D. SCHULZ[4,3];
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Abstract: The congenital learned helpless (cLH) rat model is thought to exhibit features of treatment-resistant depression. We hypothesized that increasing dopamine in the synaptic cleft with deprenyl, a monoamine oxidase-B (MAO-B) inhibitor, might mitigate treatment resistance in this animal model. After verifying congenital helpless behavior with the helplessness paradigm, adult male cLH rats were split into six treatment groups: saline (n=5), imipramine (10 mg/kg, n=5), three doses of deprenyl [2.5 (n=4), 5 (n=5), and 10 (n=5) mg/kg], and deprenyl 10 mg/kg + dopamine-1 receptor (D1) antagonist (n=4). After 14 days of drug treatment, subjects were re-tested for helplessness phenotype once per day over three consecutive days while continuing treatment. A repeated measures two-way ANOVA revealed significant increases in lever pressing turning off foot-shock (behavior indicative of resilience), for the 10 mg/kg deprenyl group on test 1 ($p<0.01$), 2 ($p<0.01$), and 3 ($p<0.001$), and for imipramine by test 3 ($p<0.01$). The highest dosage of deprenyl improved lever pressing most effectively by test 3 (11.0 +/- 2.8 lever presses, mean +/- SEM) compared to saline (0.4 +/- 0.2) or imipramine (6.4 +/- 1.5), which was blocked in rats treated with the D1 antagonist (0 +/- 0). These behavioral data suggest that increasing dopamine with deprenyl increases motivational aspects of depressive-like behavior and restores negative reinforcement learning, and that D1 neurocircuitry is necessary. We then hypothesized the improved behavior across testing days results from the synergism between learning and increased dopamine. Therefore, we quantified extent of MAO-A or -B inhibition as an indicator of dopamine activity, and measured molecules involved in synaptic plasticity (including CaMKII and syntaxin-1) using western blotting. Blinded to treatment group, tissue samples from the prefrontal cortex, striatum, hypothalamus, dorsal and ventral hippocampus were collected and evaluated. Deprenyl significantly inhibited both MAO-A and -B in all brain regions. The western blot data showed an upward trend of expression of CaMKII in the dorsal hippocampus, while syntaxin-1 expression was significantly increased in the dorsal and ventral hippocampus following deprenyl, but not imipramine (t-test, $p<0.01$, compared to saline). While we continue exploring the molecular mechanisms for resilient behavior, our data suggest that combining dopamine-boosting drugs with reinforcement learning may be effective for alleviating treatment-resistance, and that inhibiting both MAO-A and MAO-B enzymatic activity can play an important role in improving motivational dysfunctions in depression.

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Poster

227. Neural Mechanisms of Relevance to Psychiatric Illness and Treatment

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 227.11

Topic: G.05. Mood Disorders

Support: RO1 - MH090067
BX004693
BX004646

Title: Differential regulation of dopamine neuron activity during perimenopause and menopause

Authors: *S. M. PEREZ, D. J. LODGE;
UTHSCSA, San Antonio, TX

Abstract: The transition to menopause (or perimenopause) and menopausal stages of a woman's life, are periods in which a woman is vulnerable to developing a number of psychiatric disorders, such as depression, and anxiety, as well as an increased risk of developing a psychotic disorder. Despite the known changes in mental health associated with perimenopause, there has been minimal research into the effects of perimenopause and menopause on psychiatric symptoms and the brain circuits underlying them. Furthermore, traditional hormone replacement therapies (HRT), used to treat symptoms of menopause, remain controversial due to adverse side effects when given to older women and do not adequately treat psychiatric conditions. This is important to note because psychiatric symptoms can be debilitating and severely affect a woman's quality of life. Thus, developing a better understanding of the neurocircuitry that contributes to psychiatric alterations experienced by perimenopausal and menopausal women is necessary to develop targeted therapies that can drastically improve their quality of life. Of relevance, is the dopamine system and its regulation by the hippocampus because of the role these circuits play in regulating psychiatric symptoms. We have previously demonstrated that convergent inputs from the vHipp and paraventricular nucleus of the thalamus (PVT) to the nucleus accumbens (NAc) work in concert to regulate dopamine neuron activity in naturally cycling female rats. Interestingly, we now report that in the ovariectomized (OVX) rat model of menopause, that the vHipp specifically (and not the PVT) is no longer able to regulate dopamine system function. Further, we observed differences in behaviors associated with hippocampal and dopamine system function. These findings demonstrate that dopamine neuron activity, and its regulation by discreet neurocircuits (vHipp-NAc and PVT-NAc), is differentially regulated during perimenopause and menopause which may explain the vulnerability to developing a psychiatric disorder during these periods.

Disclosures: S.M. Perez: None. **D.J. Lodge:** None.

Poster

227. Neural Mechanisms of Relevance to Psychiatric Illness and Treatment

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 227.12

Topic: G.05. Mood Disorders

Support: NIH Grant MH070727
NIH Grant MH081060

Title: The role of ERK activation in ketamine-induced synaptic plasticity and antidepressant actions

Authors: *Z. Z. MA[1], P.-Y. LIN[2], J.-W. KIM[1], N. J. GUZIKOWSKI[1], R. M. ALTAMIRANO[1], E. T. KAVALALI[1], L. M. MONTEGGIA[3];
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Abstract: Major Depressive Disorder (MDD) is a prevalent mental disorderworldwide and has a devastating impact on individuals and society, highlightingthe need for rapid-acting antidepressants. A single low dose of ketamine, anon-competitive N-methyl-D-aspartate receptor (NMDAR) antagonist, produces bothrapid (within hours) and sustained (up to one week) antidepressant effects inMDD patients. In recent studies, we found that ketamine promotes thephosphorylation of extracellular signal-regulated kinase (ERK) in the mousehippocampus, which depends on tropomyosin receptor kinase B (TrkB) signaling.Here, we pharmacologically inhibit ERK activity in the hippocampus to explorethe ERK functions in the sustained antidepressant effects of ketamine. At 24hours post-treatment, the suppression of ERK activity blocks ketamineantidepressant-like effects assessed by the forced swim test, suggesting a keyrole of ERK activity in the sustained ketamine actions. Since multiple studieshave shown that the antidepressant-like behaviors of ketamine stronglycorrelate with the ketamine-induced synaptic plasticity in the hippocampus, wealso measured field excitatory postsynaptic potentials (fEPSPs) in hippocampalslices prepared from mice after 24 hours of treatment and discovered thatketamine-induced synaptic potentiation in CA1 is attenuated with alteration ofpostsynaptic AMPAR-mediated synaptic response rather than presynapticglutamatergic release probability at CA3-CA1 synapses. Now we are furtherexploring how ERK activity in the hippocampus impacts ketamine-induced CA1 synapticpotentiation and antidepressant-like effects with multiple behavioral paradigmsafter ERK activity is suppressed or enhanced via pharmacological approaches.Moreover, we are testing the cross-section of ERK activity and TrkB signalingin the excitatory neurons of the mouse forebrain. This study explores novelmeans to regulate ketamine-mediated synaptic plasticity and may help develop astrategy to facilitate ketamine antidepressant efficacy.

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Poster

227. Neural Mechanisms of Relevance to Psychiatric Illness and Treatment

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 227.13

Topic: G.05. Mood Disorders

Support: Brain Links Brain Tools

Title: Elucidation of spatial dissociation of neural activities associated with multimodal medial forebrain bundle stimulation of diverse midbrain pathways with variable stimulation parameters

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Abstract: Major depressive disorder is one of the most common mental disorders. Recent clinical trials indicate that deep brain stimulation of the superolateral medial forebrain bundle (mfb) can have rapid and long-term antidepressant effects in patients with treatment-resistant depression. However, the mechanisms of action are elusive. In this study, we selectively stimulate VTA dopaminergic (DA), VTA-NAc and VTA-PFC mfb pathways in rodents with optogenetics and compare neural activities with variable stimulation parameters (5 Hz, 20 Hz, 30 Hz burst). Anatomically, around 30% of the VTA-NAc and 7% of the VTA-PFC neurons are dopaminergic. VTA-NAc neurons located more posterior, medial and dorsal than the VTA-PFC neurons. Acute stimulation (30mins) with 30 Hz burst on VTA DA mfb pathways altered c-fos expression in PFC, NAc and VTA, while 20 Hz on VTA-NAc and VTA-PFC pathways changed c-fos expression in NAc and PFC, respectively. Electrophysiological recording showed transient alteration of neural activities in mfb downstream structures (NAc and PFC), while antidromic stimulation was not identified in the VTA. Chronic optogenetic mfb stimulation in rodent model of depression prevented depressive-like behavior and improved anxiolytic effect. The results elucidated the stimulation effects of varies bottom-up midbrain mfb pathways and highlight that acute stimulation on mfb pathways is sufficient to modify neural activities in VTA pathways, but chronic accumulative stimulation is essential for antidepressant and anxiolytic effect.

Disclosures: Y. Tong: None. **A. Wenzel:** None. **Z. Duan:** None. **V. Coenen:** None. **M. Dobrossy:** None.

Poster

227. Neural Mechanisms of Relevance to Psychiatric Illness and Treatment

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 227.14

Topic: G.05. Mood Disorders

Support: DFG Grant EXC 1086

Title: A photometric roadmap of dopamine dynamic profiles under the action of medial forebrain bundle deep brain stimulation in the nucleus accumbens

Authors: *L. MIGUEL TELEGA[1,2,3], D. ASHOURI VAJARI[4,5], T. STIEGLITZ[4,5,6], V. A. COENEN[1,3,5,7,8], M. D. DÖBRÖSSY[1,2,3,7];
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Abstract: New *in vivo* imaging technologies, like fiber photometry, have emerged powerfully in overcoming the long-standing methodological limitations and helped to offer a better understanding of neuronal population dynamics. This study aimed at employing this novel technique in combination with the state-of-the-art dopamine indicator ($GRAB_{DA2m}$, Sun et al., 2020) to detail a methodological roadmap and proof of concept for the proposed protocol in the investigation of the medial forebrain bundle (mfb) DBS impact on the dopamine release patterns in the upstream mesolimbic hub: Nucleus Accumbens (NAc). Long-Evans rats (female, male), underwent unilateral DBS electrode implantation in the mfb, viral injection of $GRAB_{DA2m}$ sensor, and optic fiber implantation in the NAc. The validation of the designed platform was done by performing daily measurements in a longitudinal fashion (8 weeks), investigating: 1) pulse width (PW) effects (100/250/350 µs) during 5s/20s of DBS (alternating weekly) using the clinically relevant 130 Hz frequency and, 2) frequency effects (5/20/30/130 Hz, one parameter per day) employing 80 µs PW during 20s DBS with 50s inter-stimulation time and 20 repetitions. All parameters were randomized within days and weeks. Our results suggest reliable readouts of dopamine transients over all recording sessions. We showed that mfb DBS is able to elicit an increased dopamine response during stimulation (5s and 20s DBS, mean area under the curve (AUC) z-scores for all weeks-PWs: 459 ± 49 (a.u.) and 1407 ± 203 (a.u.), respectively, mean ± SEM, n=4) compared to its baseline dopamine state, reaching its peak amplitude in 1.3 ± 0.1s, and then recovering back after stimulation. The effect of different DBS PWs also suggests a potential differential effect on this neurotransmitter response. Different frequencies (20, 30 and 130 Hz, but not 5 Hz) were also able to elicit a notably high dopamine response, in a frequency-dependent fashion. Dopamine plays a pivotal role in motivational and reward-related behavior and therefore potentially also in depression. Our current findings suggest that the platform developed was able to record chronically dopamine readouts and register its mediated mfb DBS effects. These findings will help to further explain the impact of different DBS parameters on the dopamine physiology. This line of investigation will generate more precise information about neurotransmitter release dynamics and potentially lead to a refinement of therapeutical DBS treatment strategies for depression.

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Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 228.01

Topic: G.05. Mood Disorders

Support: CoMRAD - 422467

Title: Lithium orotate is more effective and less toxic than lithium carbonate in a mouse model of mania

Authors: *A. G. PACHOLKO[1], L. K. BEKAR[2];
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Abstract: Background: Lithium carbonate (LiCO) is a mainstay treatment option for the prevention of mood-episode recurrences in bipolar disorder (BD). Unfortunately, LiCO displays a narrow therapeutic index associated with common thyroid and kidney complications that lead to high rates of non-adherence. Lithium orotate (LiOr) is an alternative compound suggested to possess superior uptake properties to LiCO which may manifest in reduced dosing requirements. As lithium-induced toxicities correlate with serum lithium content, lessened dosage requirements may mitigate the incidence of adverse effects and consequently improve treatment compliance.
Methods: Dose responses were assessed for a range of LiOr and LiCO concentrations in male and female C57BL/6 mice using an amphetamine-induced hyperlocomotion (AIH) murine model; AIH captures elements of the manic phase of BD and is sensitive to a dose-dependent lithium blockade. These studies were followed by an examination of the relative toxicities of LiOr and LiCO over 14 consecutive daily administrations.
Results:A partial block of AIH was maintained by LiCO at doses of 15 mg/kg or greater in males and 20 mg/kg or greater in females (67.18 ± 9.64% males; 82.4 ± 3.99% females). In contrast, a far more robust blockade was observed for LiOr at concentrations of just 1.5 mg/kg or greater in both males and females (75.46 ± 16.95% males; 97.45 ± 6.78% females), indicating improved efficacy and potency relative to LiCO. Prior-application of polyethylene glycol-400 (PEG-400) - which is a known inhibitor of the organic anion transporter 1A2 (OATP1A2) - completely blocked the effects of LiOr on AIH while sparing LiCO, suggesting differences in transport between the two compounds. The dissimilarity in the chemical nature of each drug was further supported by stark differences in dissociative behavior: LiCO-containing solutions displayed lesser resistivity to an applied current as well as increased inhibition of GSK3β *in vitro*, which implies that LiCO undergoes a greater degree of ionization than LiOr. Regarding toxicity, LiCO, but not LiOr, elicited polydipsia in both sexes, elevated serum creatinine levels in males, and robustly increased serum TSH expression in females at therapeutically relevant concentrations.
Conclusion: LiOr demonstrates superior efficacy, potency, and tolerability to LiCO in both male and female mice. When coupled with the contrasting dissociative properties and differential response to pharmacological agents, these differences in effect seem to disprove the long-held belief pertaining to lithium compounds that a “salt is a salt.”

Disclosures: **A.G. Pacholko:** None. **L.K. Bekar:** None.

Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 228.02

Topic: G.05. Mood Disorders

Support: NIH R01 MH111276

Title: Sex differences in responses to early morning bright light therapy in central orexin, BDNF, and neuroinflammatory markers in a diurnal rodent

Authors: ***A. COSTELLO**, C. VANDENBROOK, K. LINNING-DUFFY, J. S. LONSTEIN, L. YAN;
Psychology, Michigan State Univ., East Lansing, MI

Abstract: Bright light therapy (BLT) is the first-line treatment for seasonal affective disorder and has been shown to be effective for sleep disorders, non-seasonal depression, and for improving sleep and cognitive function in early-stage dementia. However, the neural mechanisms underlying the therapeutic effects of BLT are unclear. The objective of the present study was to understand the impact of BLT on corticolimbic regions involved in regulating affect and cognition using a diurnal rodent, Nile grass rats (*Arvicanthis niloticus*). Grass rats share similar behavioral responses to light as in humans -promoting wakefulness in both species - thus the results obtained are more readily translated to humans. We expected that BLT would activate the arousal-promoting orexin system. Orexin plays a key role in neuroplasticity and neuroinflammation, thus potential downstream effects in these domains were also evaluated, with the expectation that BLT would lead to enhanced expression of neurotrophic factors and reduced neuroinflammation. Male and female grass rats were housed under 12:12 hr light/dark cycle with dim lights (50 lux) on during the day from 0600-1800 hr. The experimental group received daily 1-hour early morning BLT from 0600-0700 using full-spectrum simulated daylight of 10,000 lux, while the control group received daily 1-hour monochromatic red light (680 nm). Following 4 weeks of treatment, brains were collected, and qPCR performed to examine the expression of orexin receptors OX1R, OX2R, the neurotrophic factor BDNF, microglia marker CD11b, and proinflammatory cytokines TNF-α and IL6. In males, the BLT group exhibited higher expression of OX1R and OX2R in the CA1 of the hippocampus, higher expression of BDNF in both the CA1 and BLA, and an unexpected upregulation of IL6 in the BLA, compared to the control animals receiving red light. In females, the BLT group showed higher expression of prepro-orexin in the hypothalamus accompanied by lower levels of OX1R and OX2R expression in the BLA, lower levels of BDNF expression in the CA1, and lower levels of TNF-α in the BLA, compared to the red-light exposed control group. Together, these results revealed sex differences in response to BLT in the central orexinergic system, as well as in neurotrophic factor BDNF and some neuroinflammatory markers potentially downstream of

orexin. These findings suggest distinct neural mechanisms between males and females underlying the therapeutic effects of BLT to improve sleep, mood, and cognition.

Disclosures: **A. Costello:** None. **C. Vandenbrook:** None. **K. Linning-Duffy:** None. **J.S. Lonstein:** None. **L. Yan:** None.

Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 228.03

Topic: G.05. Mood Disorders

Title: Sex differences in the gut microbiome of mice after chronic antidepressant treatment

Authors: *N. MASSA, K. A. HAUG, J. D. FRYER;
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Abstract: Depression is a debilitating mental disorder that affects ~15% of the population and disproportionately affects women. Although the pathophysiology of depression is not entirely understood, several neurological pathways are negatively impacted, including serotonin signaling. Selective serotonin reuptake inhibitors (SSRIs), which restore the levels of this neurotransmitter in the brain, are the first-line treatment for depression. Pharmacologically, roughly 6 weeks of continuous SSRI treatment is required. However, SSRI effectiveness is still highly variable. More than 50% of patients have incompletely resolved symptoms of depression, and 30–40% do not experience substantial improvement from using this medication. Therefore, there are discrepancies regarding SSRI pharmacology and beneficial clinical outcomes. One area that has emerged to potentially explain these discrepancies is the potential impact of SSRIs on the gut microbial community. Patients diagnosed with psychiatric disorders, such as depression also exhibit gut dysbiosis. Therefore, the impact of SSRIs on gut microbiota is likely underappreciated and warrants further investigation. Furthermore, despite women being predominantly afflicted with depression, most rodent models used to investigate the SSRI-gut microbiome interactions are done in male rodents. This invalidates the sex/hormonal interactions that may augment SSRI treatment outcomes. Recent evidence suggests that SSRIs have antimicrobial properties, which may have an underappreciated impact on gut microbial biodiversity. In support of this, preliminary data from our lab showed that commonly prescribed SSRIs such as fluoxetine and citalopram significantly increased Bacteroidetes while decreasing Firmicutes in mice. Our data is similar to other groups that have found relative changes in abundance in several levels of taxonomy, though the specifics are slightly different in our cohorts. We are currently exploring changes in gut microbiota composition in male and female BALB/c mice after chronic treatment with fluoxetine or citalopram followed by microbiome profiling using 16s rRNA sequencing from fecal sample, including analysis of alpha (within community diversity) and beta (between communities) diversity across three-time points: baseline, midpoint, and after 28 days of SSRI administration to then compare between groups.

The results of these experiments may help shed light on the possible mechanism of action of these SSRIs in the gut microbiome and the potential differences between males and females in therapy outcomes.

Disclosures: **N. Massa:** None. **K.A. Haug:** None. **J.D. Fryer:** None.

Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 228.04

Topic: G.05. Mood Disorders

Support: NSERC CGS-D

Title: Reelin and ketamine increase hippocampal long-term potentiation in a parallel manner after chronic corticosterone administration

Authors: ***J. N. JOHNSTON**[1], J. T. ALLEN[3], C. LIRIA SANCHEZ-LAFUENTE[1], B. S. REIVE[2], K. SCHEIL[2], S. LIANG[4], B. R. CHRISTIE[1], L. E. KALYNCHUK[2], H. J. CARUNCHO[1];
[1]Div. of Med. Sci., [2]Univ. of Victoria, Victoria, BC, Canada; [3]Univ. of Melbourne, Melbourne, Australia; [4]Psychology, San Diego State Univ., San Diego, CA

Abstract: Major Depressive Disorder is the leading cause of disability worldwide, with a lifetime prevalence rate around 16%. Despite this ubiquity, traditional antidepressants have a substantial therapeutic time delay and low efficacy rates. Ketamine has been discovered to have rapid and robust antidepressant effects, mediated through an elevation in glutamatergic signaling and increases in long-term potentiation (LTP). Our lab and others have shown that Reelin, an endogenous glycoprotein, parallels the behavioral and biological effects of ketamine, however the impact of Reelin on LTP after chronic stress has not been studied. Male Long Evans rats (n=30) underwent a chronic stress paradigm of daily corticosterone (CORT) or vehicle injections (40mg/kg s.c. for 21 days). On the 21st day, rats were administered an acute dose of reelin (3µg, i.v.) ketamine (10mg/kg i.p), or vehicle. 24 hours after treatment, hippocampal synaptic plasticity was evaluated *in vivo* using field electrophysiological recordings in anesthetized rats (urethane, 1.5g/kg, intraperitoneally) in the medial perforant path. A theta-burst stimulation protocol was used to stimulate LTP (4 x 10 bursts, 5 pulses at 400Hz). Input/output (I/O) function was assessed for baseline excitatory synaptic transmission and normalized fEPSP (field excitatory post-synaptic potential) slopes were analyzed and compared between experimental groups for post-tetanic potentiation (1 min post stimulation) and long-term potentiation (55-60 min post stimulation). No significant differences in I/O function were found between experimental groups. Chronically stressed rats had significantly lower post-tetanic potentiation in comparison to vehicle-treated rats ([$F(3,12) = 5.793$, $p = 0.011$]; $p = 0.0013$), though this was not impacted by Reelin or ketamine. Significant differences in long-term potentiation were found across all

groups [F(3,12) = 4.473, p = 0.025], where chronic stress decreased LTP from vehicle levels (p = 0.0045), but the decrease was significantly rescued with both Reelin (p = 0.034) and ketamine (p = 0.04). This study demonstrates that a chronic stress model using CORT show deficits of LTP in the hippocampus. The parallel effects of Reelin and ketamine on LTP provide further support for Reelin-based therapeutics, adding to previous molecular research. *In vivo* electrophysiological analyses of Reelin also help confirm the importance of Reelin for recovery of synaptic plasticity in chronic stress models. This study is a forward step towards the elucidation of mechanisms underlying leading-edge therapeutics that can target treatment-resistant depression populations.

Disclosures: J.N. Johnston: None. **J.T. Allen:** None. **C. Liria Sanchez-Lafuente:** None. **B.S. Reive:** None. **K. Scheil:** None. **S. Liang:** None. **B.R. Christie:** None. **L.E. Kalynchuk:** None. **H.J. Caruncho:** None.

Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 228.05

Topic: G.05. Mood Disorders

Title: Evaluation of glutamatergic receptors as targets for anxiolytic and/or antidepressant effects in an avian model of treatment-resistant depression

Authors: *S. W. WHITE[1], G. D. SQUIRES[1], S. J. SMITH[1], G. M. WRIGHT[1], K. J. SUFKA[2], J. M. RIMOLDI[3], R. S. GADEPALLI[3];
[1]Psychology & Philosophy, Sam Houston State Univ., Huntsville, TX; [2]Psychology, [3]BioMolecular Sci., Univ. of Mississippi, Oxford, MS

Abstract: Evaluation of Glutamatergic Targets for Anxiolytic and/or Antidepressant Effects in an Avian Model of Treatment-Resistant Depression
Stephen W. White[1], Kenneth J. Sufka[2], Sequioa J. Smith[1], Gwendolyn Squires[1], Gwendolyn M. Wright[1,] John M. Rimoldi[3], Rama S. Gadepalli[31]Department of Psychology & Philosophy, Sam Houston State University[2]Department of Psychology, University of Mississippi[3] Department of Biomolecular Science, University of Mississippi
We have characterized a stress-vulnerable, treatment-resistant, ketamine-sensitive avian genetic line. Rodent models of anxiety and depression have demonstrated modulation of glutamate receptors can provide anxiolytic and/or antidepressant effects. Separate dose response studies (n=12-18) were conducted for the following drugs: the AMPA positive allosteric modulator LY392098 (2.5mg, 5.0mg,10.0mg), the mGluR 5 antagonist MPEP (3.0mg, 10.0mg, 30.0mg), the mGluR 2/3 agonist LY404039 (1.0mg, 3.0mg, 10.0mg), and the mGluR 7 agonist AMN082 (1.0mg, 3.0mg. 10.0mg). Dose response studies for the NMDA antagonist ketamine and the norepinephrine α2 agonist clonidine were included as control comparison for antidepressant and anxiolytic effects, respectively. One-day old male Black Australorp chicks were group housed

with access to food and water. Testing occurred on days 5-7 post-hatch. All drugs were administered intraperitoneally (i.p.)15 or 30 minutes prior to exposure to a 90-minute isolation stressor in which distress vocalizations were recorded as the behavioral measure. A one-way ANOVA was conducted to determine drug effects and post-hoc analysis using Fisher's LSD was used to determine specific group differences. Ketamine 10mg/kg significantly elevated DVoc rates during the depression phase (i.e. antidepressant effects) and clonidine doses of 0.15mg and 0.20mg significantly reduced DVoc rates in the first 3 minutes of isolation (i.e. anxiolytic effect). The compounds LY392098 2.5 mg/kg, AMN082 1.0 mg/kg, significantly elevated DVoc rates in the later hour of isolation, representative of antidepressant effects similar to that of ketamine. Doses of LY404039 3.0 and 10.0 mg/kg decreased DVoc rates during the first 3 minutes of isolation indicative of anxiolytic effects similar to that of clonidine. The dose response study for MPEP is currently underway and preliminary analyses suggest the 3.0mg and 10.0mg doses significantly elevate DVoc rates in the depression phase (i.e., produced antidepressant effects). Collectively, these studies suggest that modulation of glutamatergic targets may be clinically useful in providing symptom relief for TRD sufferers.

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Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 228.06

Topic: G.05. Mood Disorders

Support: M.J. Murdock Charitable Trust NS-201914142
CCCU/SCIO Supporting Structures Grant to Whitworth University

Title: Modulation of the endocannabinoid system through various treatments in a rodent model of epilepsy and depression comorbidity

Authors: D. GANU[1], T. GEE[2], K. GILL[3], H. A. KHAN[4], C. KOULIBALI[1], A. L. BEST[3], G. D. PEDERSEN[1], ***S. A. EPPS**[1];
[1]Psychology, [2]Chem., [3]Hlth. Sci., [4]Biol., Whitworth Univ., Spokane, WA

Abstract: A bi-directional comorbidity of depression and epilepsy is present in the clinical population, suggesting a shared neurobiology. The SwLo (Swim Low-Active) rat is a well-characterized animal model of comorbid depression and epilepsy. Through selective breeding for low activity in the Porsolt Swim Test (PST), these rats reflect increased depression-related behaviors in multiple paradigms. When exposed to chemical and electrical-induced seizures and pilocarpine-induced epileptogenesis, this model also exhibits enhanced susceptibility. Given the resistance this comorbidity often has to current pharmacological solutions, this study sought to explore a non-pharmacological treatment path through diet, as it can decrease symptoms in the

two illnesses alone. A calorie-restricted diet for male SwLo rats was hypothesized to have an antidepressant and anticonvulsant effect in the PST and pilocarpine seizure tests, respectively. Calorie-restricted SwLo rats showed increases in PST struggling behavior compared to ad lib rats, $F(1,13) = 7.399$, $p = 0.018$. Similarly, SwLo rats fed a calorie-restricted diet were found to have significant increases in latency to seize, $F(1,12) = 8.798$, $p = 0.012$. Previous genetic analyses revealed elevated levels of *Faah*, which codes for the fatty acid amide hydrolase that breaks down endocannabinoids, in SwLo rats. To test the hypothesis that caloric restriction exerted its effects through modulation of the endocannabinoid system, CBr1 blocker SR141716 was administered alongside a calorically restricted or ad lib diet to evaluate if the benefits of calorie-restriction would be inhibited when the endocannabinoid system was blocked. The data showed findings suggestive of a U-shaped curve. Rats in the ad lib + vehicle group showed PST struggling consistent with baseline SwLo rats. Antithetically, rats in the caloric restriction + vehicle group also struggled for an increased time. However, rats in the caloric restriction + SR141716 group struggled for a time consistent with a baseline SwLo rat, $F(1,28) = 5.934$, $p = 0.021$. Both the ad lib and calorie-restricted groups were found to have significant decreases in latency to seizure when treated with SR141716, $F(1,28) = 5.228$, $p = 0.030$. To further examine the comorbid relationship and the endocannabinoid system, the pharmaceutical URB597 is currently being used to inhibit *Faah* in the SwLo rats. We hypothesize that SwLo rats given URB597 will exhibit increased antidepressant and anticonvulsant effects. These findings suggest that the endocannabinoid system is a route for new pharmacological and non-pharmacological treatment of comorbid epilepsy and depression.

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Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 228.07

Topic: G.05. Mood Disorders

Title: Differential network activity of benzodiazepines and neuroactive steroids observed with cortical EEG in rat.

Authors: *R. S. HAMMOND, A. L. ALTHAUS, B. J. FARLEY, A. C. SMITH, S. M. LOTARSKI, A. C. KORALEK, C. M. MACIAG, M. C. QUIRK, A. J. ROBICHAUD;
Sage Therapeut., Cambridge, MA

Abstract: Type A γ-amino butyric acid receptors ($GABA_A$Rs) are the primary inhibitory neurotransmitter receptors in the mammalian central nervous system (CNS). They are chloride-conducting ion channels made of five subunits: two α, two β, and, typically, one γ_2 or δ (Olsen & Sieghart 2009). γ subunit containing $GABA_A$Rs primarily localize at synapses and mediate phasic inhibitory currents; δ subunit-containing $GABA_A$Rs are extrasynaptic and mediate tonic

inhibitory currents (Belelli 2009). Benzodiazepines are positive allosteric modulators (PAMs) of γ subunit containing $GABA_A$Rs, while neuroactive steroid (NAS) $GABA_A$R PAMs modulate both γ and δ subunit containing $GABA_A$Rs. Recent FDA approval of NAS $GABA_A$R PAMs brexanolone and ganaxolone for postpartum depression and CDKL5 deficiency disorder, respectively, indicate the broad therapeutic potential of this compound class. $GABA_A$R PAMs modulate brain network oscillations, as measured by cortical electroencephalogram (EEG) recordings. Benzodiazepines and NAS $GABA_A$R PAMs both increase β-band power in rat EEG studies, but recent studies demonstrate differential modulation of θ-band EEG power in rats (Antonoudiou 2022). At exposures that elicit a 100% increase in β-band power, NAS $GABA_A$R PAMs zuranolone and SGE-516 increase θ-band power, while the benzodiazepine diazepam reduces θ-band power (Antonoudiou 2022). Increased θ-band power was observed clinically with zuranolone (Antonoudiou 2022), whereas reduced θ-band power was reported in humans with lorazepam (Gilles 2002). To better understand the effects of different classes of GABAergic modulators on EEG power across frequencies, we examined NAS $GABA_A$R PAMs (SGE-516 and SGE-872), a benzodiazepine (diazepam), a non-benzodiazepine "Z-drug" (alpidem), and a benzodiazepine receptor partial agonist (imepitoin) in awake rat pharmacodynamic EEG studies. Experiments were performed as described in Althaus et al., 2020. Compounds were administered to adult male Sprague Dawley rats (n = 6 - 10/dose group) implanted with epidural screw electrodes. EEG and electromyogram activity were recorded for up to 6 hours after dosing. Changes in EEG power were analyzed across δ, θ, α, β, γ_1 and γ_2 frequency bands. SGE-516 and SGE-872 robustly increased θ-band power in rat EEG recordings whereas the other compounds exhibited minimal θ-band modulation. All compounds increased β-band power. The differential effects on θ-band power in rat EEG recordings between NAS $GABA_A$R PAMs and the other GABAergic compounds tested may reflect the differential modulation of $GABA_A$R subpopulations, since only the NAS $GABA_A$R PAMs in this study modulate both γ and δ subunit-containing $GABA_A$Rs.

Disclosures: **R.S. Hammond:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **A.L. Althaus:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **B.J. Farley:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **A.C. Smith:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **S.M. Lotarski:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **A.C. Koralek:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **C.M. Maciag:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **M.C. Quirk:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual

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Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 228.08

Topic: G.05. Mood Disorders

Title: Automated analysis of head twitch response with deep neural networks: A new approach to an old assay

Authors: A. SUHONEN, R. DE FEO, J. KUOSMANEN, U. DATTA, ***S. BÄCK**, T. BRAGGE;
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Abstract: The head twitch response (HTR), a rapid rotational side-to-side head movement, is a 5-HT2A receptor-dependent behavior observed in laboratory rodents following the administration of serotonergic hallucinogens. With the recent growth in psychedelic research, the HTR has become a routine behavioral functional assay to screen for 5-HT2A receptor activation. Additionally, HTR assay appears to have translational relevance for predicting subjective potency of hallucinogens in humans. In mice, the responses happen in characteristic rhythmic pattern and high frequency (order of about hundred Hertz), with less than 10 individual side-to-side rotations per event and it becomes readily obvious that the observation and description of these events are preferably done utilizing high-speed video recordings together with automated video-analyses. Here we approached the evaluation of HTR events utilizing AI-driven automated video analysis by DeepLabCut (DLC, Mathis et al. Nature Neurosci 2018 (21): 1281-1289). Following an i.p. injection of vehicle (day 1) or 5-hydroxy-L-tryptophan, a precursor of 5-HT (5-HTP, 100 mg/kg, day 2), the movements of the C57Bl/6J mice (n=5, CRL Germany) were captured with an overhead high-speed camera for 30 min. Ear and tail base landmarks were automatically detected using DLC. The position and velocity of these landmarks were combined with visual features from each video frame for a markerless semi-automated detection of HTR. Following the 5-HTP injection, we observed increased number of HTR events, (56 ± 7) / 30 min, in comparison to vehicle injection, (10.3 ± 1.4) HTRs / 30 min. Our study demonstrates that DLC based automated high-speed video analysis is applicable to assess HTR events in pharmacological preclinical studies. In addition, this work demonstrate a proof of concept for development of deep neural networks-driven automated analysis methods in various video recordings e.g. behavioral tests, animal scoring, decreasing manual labor and improving reliability and throughput.

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Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 228.09

Topic: G.05. Mood Disorders

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Title: Reinforcement sensitivity phenotype interacts with the effects of chronic stress and chronic escitalopram treatment in rats

Authors: *K. NOWORYTA[1], A. CIEśLIK[1], P. SUROWKA[2], R. RYGULA[3];
[1]Affective Cognitive Neurosci. Lab., [2]Maj Inst. of Pharmacol. Polish Acad. of Sci., Krakow, Poland; [3]Affective Cognitive Neurosci. Lab., Inst. of Pharmacol. Polish Acad. of Scienc, Krakow, Poland

Abstract: Gray's reinforcement sensitivity theory presupposes that differences in sensitivity to negative (NR) and positive reinforcement (PR) may underlie the personality dimensions that are associated with affective disorders. Recently it has been proposed that reinforcement sensitivity might also determine the effectiveness of antidepressant drugs. In the present study, we investigated in an animal model how various phenotypes of reinforcement sensitivity differ in susceptibility to chronic stress and antidepressant treatment. For this, using the preclinical version of the probabilistic reversal learning (PRL) test, we differentiated 4 phenotypes of reinforcement sensitivity in rats, and named them after the affective disorders they might be associated with: pro-depressive (sensitive to NR and insensitive to PR), pro-manic (sensitive to NR and insensitive to PR), irritable (sensitive to NR and PR), and dysthymic (insensitive to NR and PR). Subsequently, we subjected the rats to 4 weeks of chronic restraint stress and 2 weeks of treatment with the antidepressant drug escitalopram (Escit, 3 mg/kg/day). The treatment started after the second week of stress and was parallel to the stress procedure. The interphenotypic differences in the effects of stress and Escit treatment on anxiety, appetitive motivation, cognitive flexibility, and reinforcement sensitivity were investigated using the light-dark box, progressive ratio schedule of reinforcement, and PRL tests, respectively. Our experiments revealed that following chronic stress, the rats representing pro-manic and irritable phenotypes significantly increased the latency to make a decision in the PRL test, compared to their not stressed conspecifics; an effect suggesting increased anxiety or reward sensitivity deficit. The effect of stress was not observed in animals classified as dysthymic or pro-depressive. In turn, the latter two phenotypes turned out to be more sensitive to antidepressant treatment. Following chronic Escit administration, the rats classified as dysthymic became more anxious, while those classified as pro-depressive became less anxious than their vehicle-treated controls. These effects of Escit treatment were not observed in animals classified as pro-manic or irritable. We observed no statistically significant interaction between the phenotype of

reinforcement sensitivity and the effects of stress or antidepressant treatment on appetitive motivation. Our results demonstrate for the first time that the phenotype of sensitivity to reinforcement could have important implications for vulnerability to affective disorders and their treatment.

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Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

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Program #/Poster #: 228.10

Topic: G.05. Mood Disorders

Support: MOST 110-2320-B-A49A-503
MOST 110-2320-B-A49A-504
MOST 111-2811-B-A49A-504

Title: Mechanism of continuous theta-burst stimulation in synaptic function in antidepressant-resistant rat model

Authors: *C.-W. LEE[1], M. CHU[2], C.-H. CHANG[2], T.-J. YANG[2], T.-N. PENG[2], H. CHI[2], Y.-C. LIN[2], H.-C. LIN[2,3];
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Abstract: Treatment-resistant depression (TRD) which is defined as major depression with poor or unsatisfactory response to two different classes of antidepressants. Previous studies demonstrated that traumatic stress plays a critical role in the development of TRD. Moreover, dysregulated synaptic plasticity, including long-term potentiation (LTP) and long-term potentiation (LTD) in the prefrontal cortex (PFC) also been reported in previous studies. Recent studies have demonstrated two forms of repetitive transcranial magnetic stimulation, intermittent and continuous theta-burst stimulation (iTBS and cTBS) which produce LTP-like and LTD-like plasticity. The clinical study has indicated that iTBS treatment may effective in treating TRD. However, the treatment effect and mechanism of cTBS in treating TRD still unclear. Hence, the aim of study tries to identify the treatment effect of cTBS in synaptic plasticity. Here we applied the footshock stress as traumatic events to develop an antidepressant-resistant depression rat model. First, results that the footshock-induced depressive-like behaviors were not reversed by chronic imipramine and venlafaxine. Next, footshock-induced depressive-like behaviors were improved by 7 days of cTBS treatment. The footshock-induced aberrant paired-pulse facilitation was improved by 7 days of cTBS treatment in PFC. The footshock-induced aberrant low-frequency induced LTD was improved by 7 days of cTBS treatment in PFC. Moreover, the footshock-induced aberrant chemical LTD was also improved by 7 days of cTBS treatment in

PFC. However, the footshock-induced impaired LTP was not improved by 7 days of cTBS treatment in PFC. Present study suggests that the synaptic function was improved by cTBS treatment after footshock and the treatment of cTBS in synaptic plasticity preform the inhibitory synaptic effects in the PFC.

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Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 228.11

Topic: G.05. Mood Disorders

Support: GRF 15100018
NFSC 81801346

Title: Activating adiponergic signaling by AdipoRon treatment elicits rapid antidepressant effect independent to changes in hippocampal synaptic plasticity

Authors: *S. YAU[1], D. FORMOLO[2], H. LEE[3];
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Abstract: Depression is a devastating mental illness with a high lifetime prevalence globally. Currently available antidepressants have a delayed therapeutic onset and poor efficacy, while the recently approved rapid-acting antidepressant Esketamine has psychomimetic side effects for chronic treatment. Therefore, there is an urgent need for better rapid antidepressant treatment options. We have previously shown that adiponectin, an adipocyte-secreted hormone mediates the antidepressant effects of physical exercise via enhancing hippocampal cell proliferation, and that activating adiponectin signaling by its receptor agonist AdipoRon mimics the pro-cognitive effects of physical exercise in diabetic animals. Here we investigated the potential antidepressant effects of sub-chronic (7-day) treatment with AdipoRon (20 mg/kg, i.p), and its underlying mechanisms. Depression/anxiety-like behaviors were assessed using behavioral tests including forced swim test, sucrose preference test, open field test, novelty suppressed feeding and light/dark box test. Our results showed that AdipoRon treatment elicits significant antidepressant and anxiolytic effects in concurrent with an increase in hippocampal cell proliferation. Interestingly, sub-chronic treatment decreased immediate neuronal activation in the ventral hippocampus as indicated by decreased in c-fos immunostaining positive cells, and impaired synaptic plasticity as shown by reduced long-term potentiation formation and BDNF protein expression in the hippocampus. Our current data indicated that AdipoRon treatment elicits antidepressant and anxiolytic effects in association with increased hippocampus cell proliferation, but independent to changes in hippocampal synaptic plasticity. The results have

suggested that increase in hippocampal cell proliferation could be important for antidepressant action modulated by adiponergic system. Potential involvement of other brain areas that express adiponectin receptors (e.g. mPFC and dorsal raphe nucleus) warrants further investigation.

Disclosures: S. Yau: None. **D. Formolo:** None. **H. Lee:** None.

Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 228.12

Topic: G.05. Mood Disorders

Support: Hope for Depression Research Foundation

Title: Revealing the effect of subcallosal ACC deep brain stimulation on brain-wide networks in non-human primates

Authors: *S. H. FUJIMOTO[1], A. FUJIMOTO[1], C. ELORETTE[1], D. FOLLONI[1], L. FLEYSHER[3], K. CHOI[2], B. E. RUSS[4], H. S. MAYBERG[2], P. H. RUDEBECK[5]; [1]Neurosci., [2]Nash Family Ctr. for Advanced Circuit Therapeut., Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]BioMedical Engin. and Imaging Inst., Icahn Sch. of Mount Sinai at Mount Sinai, New York, NY; [4]NIMH/NIH, New City, NY; [5]Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Deep brain stimulation targeting subcallosal anterior cingulate cortex and adjacent white matter (scACC-DBS) is a promising therapy for treatment resistant depression (TRD). However, the neural mechanisms through which scACC-DBS facilitates recovery from depression are not fully characterized, making it difficult to optimize treatment for all patients. It remains unclear how DBS alters brain-wide circuits in healthy brains, an essential first step in determining the mechanisms in the depressed brain. Thus, the aim of this study was to establish how scACC-DBS works in healthy brains, focusing on determining the brain-wide network-level functional effects of stimulation. Modeling the approach used to successfully treat TRD patients, we implanted scACC-DBS electrodes in two rhesus macaques. We estimated the confluence of the cingulum bundle (CB), forceps minor (FM), and uncinate fasciculus (UF) using diffusion tractography imaging (DTI). We then implanted a DBS lead unilaterally in this location, the other hemisphere serving as a control. One month after electrode implantation, stimulation (5mA, 130Hz, 90μsec) began and was maintained for 6 weeks. Whole brain resting-state functional MRIs (rs-fMRIs) were acquired before electrode implantation and following 6 weeks of scACC-DBS stimulation. Functional data were analyzed using a seed-based comparative-connectome approach where scACC-DBS stimulation induced changes in functional connectivity (FC) were determined. After 6 weeks of scACC-DBS stimulation, scACC (Area 25) FC decreased with the medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC), dorsolateral prefrontal cortex (DLPFC), superior temporal lobe and hippocampus. By contrast,

FC with dorsal insular cortex and thalamus was increased. PCC and mPFC are main components of the default mode network, which is heavily implicated in the pathophysiology of depression. Further, we confirmed that DBS-induced increases or decreases in FC were spatially consistent across animals. While the mPFC, PCC, insula and hippocampus all have direct structural connections to the scACC via the FM, CB, and UF, respectively, DLPFC and superior temporal lobe do not, and thus changes in these regions suggest secondary or trans-synaptic effects. Our findings reveal the putative mechanisms of action of DBS therapy, information that may inform further optimization of scACC-DBS for TRD patients.

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Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 228.13

Topic: G.05. Mood Disorders

Title: Novel non-stimulants rescue hyperactive phenotype in an adrgl3.1 mutant zebrafish model of ADHD

Authors: H. S. SVEINSDOTTIR[1], C. CHRISTIAN[1], T. HARALDUR[1], P. LAVALOU[2], M. O. PARKER[3], A. SHKUMATAVA[2], W. H. J. NORTON[4], E. ANDRIAMBELOSON[5], S. WAGNER[5], ***K. Æ. KARLSSON**[6];
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Abstract: ADHD is a highly prevalent neurodevelopmental disorder. The first-line therapeutic for ADHD, methylphenidate, suffers serious side effects including weight loss, insomnia, risk of abuse and hypertension. Therefore, the development of non-stimulant based therapeutics has been prioritized. However, many of these also result in side effects, most notably somnolence, and they tend to lack efficacy. Here, we have used a uniquely powerful genetic model and unbiased drug screen to identify novel ADHD non-stimulant therapeutics. We first found that *adgrl3.1* null zebrafish larvae showed a robust hyperactive phenotype. Although the hyperactivity was rescued by three ADHD non-stimulant therapeutics, all interfered significantly with sleep. Second, we used wild-type zebrafish larvae to characterize a simple behavioral phenotype generated by atomoxetine and screened the 1200 compound Prestwick Chemical Library for a matching behavioral profile resulting in 67 hits. These hits were re-assayed in the *adgrl3.1* null. Using the previously identified non-stimulants as a positive control, we identified

four compounds from the 67-hit selection which matched the effect of the non-stimulants: aceclofenac, amlodipine, doxazosin and moxonidine. We next validated moxonidine in mice using a T-maze spontaneous alternation task and rescued scopolamine induced cognitive impairment. Moxonidine, is a partial α2 adrenergic agonist but has higher affinity for imidazoline 1 receptors. In an attempt to tease apart the effects of these receptor systems we assayed a pure imidazoline 1 agonist, LNP599, which generated an effect closely matching other non-stimulant ADHD therapeutics suggesting a role for this receptor system in ADHD etiology. In summary, we introduce a novel genetic model of ADHD in zebrafish and identify five putative novel therapeutics. The findings offer a novel tool for understanding the neural circuits of ADHD, suggest a novel mechanism for its etiology and identify novel therapeutics.

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Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 228.14

Topic: G.05. Mood Disorders

Support:
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•EU Horizon 2020 Research and Innovation Program No. 739593 – HCEMM
•Japan Society for the Promotion of Science grant 18KK0236

Title: Reinstating olfactory bulb derived limbic gamma oscillations alleviates depression-like behavioral deficits in rodents

Authors: *Q. LI[1], Y. TAKEUCHI[3], J. WANG[2], L. BARCSAI[2,4,5], L. PEDRAZA[2], G. KOZAK[1], S. NAKAI[6], S. KATO[7], K. KOBAYASHI[8], M. OHSAWA[9], M. LőRINCZ[2,10,11], O. DEVINSKY[12], G. BUZSAKI[13], A. BERENYI[1,14,4,5];
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Abstract: Although the etiology of major depressive disorder remains poorly understood, impairment of gamma oscillations is an emerging biomarker. We set out to investigate the functional role of gamma rhythms of olfactory origin in the development and treatment of depression. By the means of chemogenetics, suppressing olfactory bulb neuronal activity decreased gamma oscillation power in multiple brain areas and induced anxiety-like behavior in open field test (OFT) in mice. Then, temporally suppressing the synaptic transmission of OB to pyriform by optogenetics significant lower performance in sucrose preference test (SPT) in intact Long-Evans rats, which was positively correlated with reduction of gamma power in the pyriform. Moreover, we established a phase-dependent closed loop neuromodulation of cortical areas. Naïve animals with AntiPhase gamma electrical stimulation (E-Stim) showed dramatically decreased sucrose preference (anhedonia), and it lasted several days after termination of E-Stim. Meantime, the effects of AntiPhase E-Stim decreased gamma power in the pyriform, while InPhase increased gamma activity power. Importantly, there were no side effects either in spontaneous movement in the home cage, nor in the frequency distribution of native gamma and the incidences of gamma events after both AntiPhase and InPhase E-Stim. Furthermore, in animals with lipopolysaccharide induced depression, pacing the pyriform cortex by the native OB gamma oscillations effectively alleviated their ahedonic behaviors in the SPT and anxiety-like behaviors in both OFT and elevated plus maze test. These results suggests that restoring gamma oscillations is a promising way to improve depression-related symptoms.

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Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 228.15

Topic: G.05. Mood Disorders

Support: Danish Medical Research Council
Karen Elise Jensen Foundation
Doctor Eilif Trier Hansen and Wife Ane Trier Hansen Foundation

Title: Anatomy and connectivity of the Göttingen minipig subgenual cortex (Brodmann area 25 homolog)

Authors: A. N. GLUD[1], H. ZAER[1], ***D. ORLOWSKI**[2], M. S. NIELSEN[1], J. C. H. SØRENSEN[1], C. R. BJARKAM[3];
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Abstract: Background: The subgenual gyrus is a prospect target for treating major depression using deep brain stimulation (DBS). However, there is a lack of translational, large animal models to test this treatment modality.
Aim: To describe the anatomy and connections of the Göttingen minipig subgenual cortical area (sgC) as a possible non-primate, large animal model for this therapy.
Materials and methods: Thirteen female Göttingen minipigs (age 6-12 months, weight 20-25 kg) were used. The frontal pole of five minipigs was cryosectioned into 40 µm coronal and horizontal sections and stained with Nissl and NeuN-antibody to visualize cytoarchitecture and cortical lamination. The remaining eight animals received a unilateral stereotaxic injection of the anterograde (BDA) and retrograde (FluroGold) neuronal tracers into the sgC to reveal the connectivity pattern of this brain structure.
Results: The Göttingen minipig sgC has a dimension of 4 x 3 x 1 mm and is located ventral to the rostral tip of the corpus callosum, medial to the anterior part of the lateral ventricle, and anterior to the septum. Homologically to human nomenclature (Brodmann 1909), it can be subdivided into three distinct areas: area 25 (BA25), area 33 (BA33), and indusium griseum (IG). BA25 is a narrow agranular cortex, approximately 1 mm thick. Laminar differentiation in the deeper layers is poor due to a similar appearance of layer III and V neurons. Perpendicular to the surface, columns of white matter stretch deep into layers II and III, thereby segregating small groups of closely arranged neurons in the superficial layers. BA33 is less differentiated than BA25. The cortex is narrower and lacks laminar differentiation due to diffusely arranged small, lightly stained neurons. It abuts the indusium griseum which is a neuron-dense band of heavily stained small neurons separating BA33 directly from the corpus callosum and the posteriorly located septum. The sgC receives prominent afferent connections from adjoining prefrontal and cingulate cortices, insula, perirhinal cortex, amygdala, and hippocampal formation. Furthermore, afferent connections were seen from the anterior and mediodorsal thalamic nuclei, hypothalamus, ventral tegmental area, and PAG. Efferent connectivity was seen to adjoining cortical areas, hippocampus, thalamus, and hypothalamus.
Conclusion: The minipig sgC displays a cytoarchitectonic pattern and connectivity similar to the human, which makes this animal suitable for further translational studies on BA25-DBS treatment against depression.

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Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 228.16

Topic: G.05. Mood Disorders

Title: Acute tetrabenazine (TBZ) challenge as a translational approach to study novel antidepressants

Authors: *G. A. HIGGINS, L. B. SILENIEKS, M. BROWN, C. MACMILLAN, D. FENG, L. MAGOMEDOVA, I. A. M. DE LANNOY;
InterVivo Solutions Inc, Fergus, ON, Canada

Abstract: Tetrabenazine (Xenazine®; TBZ) is an FDA approved drug treatment for hyperkinetic movement disorders such as Huntington's Chorea. As a reversible inhibitor of the VMAT2 protein, TBZ produces a temporary and reversible depletion of the monoamines 5-HT, DA and NA in forebrain and limbic CNS regions (Frontal cortex, hippocampus, striatum), through a disruption of vesicular storage. As a likely consequence, depression is a recognised side-effect of TBZ in humans, as well as the behavioural changes reported in rodents characteristic of depression relevant endophenotypes such as anergia, amotivation, inattention, negative bias and fatigue (e.g. Nunes et al (2013) J.Neurosci. 33:19120-19130; Stuart et al (2017) BJP 174: 3200-3210). In the present studies we have characterized the effect of TBZ (0.3-3mg/kg) on measures of monoamine neurochemistry, motivation (progressive ratio (PR), food choice tests), attention/vigilance (5-choice serial reaction time task (5-CSRTT)), and locomotor endurance (running wheel) in adult, male, Long-Evans rats. TBZ doses were established that produced robust, yet reversible declines in forebrain monoamine levels and concomitant behavioural changes, allowing for repeated measures study designs to study drug interactions. TBZ 0.3-1mg/kg doses resulted in plasma levels consistent with therapeutic exposures in humans (i.e. 3-8ng/ml). For example, TBZ (0.3-3 mg/kg) reduced break points for food maintained responding in rats trained to a PR task (e.g. Veh: 13.4$\pm$0.8; TBZ(1): 11.2$\pm$0.9; $P<0.01$), and an early decline in hit rate in rats trained to an extended trials 5-CSRTT (% hit collapsed over 250 trials 0.3s SD: Veh: 54.7$\pm$4.7%; TBZ(1): 25.3$\pm$5.1%; $P<0.01$). Pretreatment with the NA/DA reuptake inhibitor bupropion (10-20 mg/kg), but not the SSRI citalopram (1-10 mg/kg), attenuated these TBZ(0.75mg/kg)-induced deficits (e.g. 5-CSRTT: total % hit: Veh/Veh: 41.8$\pm$4.8%; TBZ/Veh: 32.2$\pm$4.0%; TBZ/Bup(20): 42.2$\pm$4.1%; $P<0.05$ TBZ/Bup vs. TBZ/Veh). It is proposed that a TBZ challenge model may represent a useful translational approach to study novel anti-depressant drugs both in rodents and humans. Based on the present and prior preclinical studies, TBZ is reversible, dose titratable, and influences multiple endophenotypes associated with clinical depression that can be assessed using cross-species tests.

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Poster

228. Animal Models of Relevance to Psychiatric Illness and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 228.17

Topic: G.05. Mood Disorders

Title: Downregulation of connexin43 in primary cultured astrocytes potentiates the amitriptyline-induced brain-derived neurotrophic factor through lysophosphatidic receptor$_{1/3}$, Src, and extracellular signal-regulated kinase

Authors: *N. TOKUNAGA, Y. NAKAMURA, K. HISAOKA-NAKASHIMA, N. MORIOKA; Pharmacol., Hiroshima Univ., Hiroshima, Japan

Abstract: Connexin43 (Cx43) is highly expressed in astrocytes, which is the most abundant glia cells in the brain, and contributes to not only intracellular molecular transportation but also intracellular signaling. It is demonstrated that Cx43 expression is reduced in the prefrontal cortex and hippocampus of depressive patients, suggesting that Cx43 may be involved in the pathology of depression. In general, antidepressants affect through the increase of monoamine concentration at synaptic cleft. Although antidepressants upregulate monoamine level immediately after administration, several months are required for the manifestation of antidepressant effect in patients of depression, suggesting the other mechanism which is independent with monoamine. Our previous study showed that Cx43 expression in hippocampus was negatively correlated with antidepressant effect of amitriptyline (AMI) in the depressive model mice. In addition, we demonstrated that AMI increases brain-derived neurotrophic factor (BDNF) expression in primary cultured astrocytes, and this response was potentiated in Cx43-downregulated astrocytes. The current study examined this potentiation mechanism through the downregulation of Cx43. Primary cultured astrocytes were prepared from the cerebral cortex of neonatal Wistar rats. The expression of Cx43 was downregulated by RNA interference. The expression of mRNA and protein were measured by real-time PCR and western blotting, respectively. Downregulation of Cx43 potentiated the AMI-induced BDNF expression in cultured astrocytes. This potentiation was significantly suppressed by blockade of $LPA_{1/3}$ receptors, Src tyrosine kinase, or extracellular signal-regulated kinase (ERK). The current study revealed that reduced Cx43 expression potentiates the AMI-induced BDNF expression in cortical astrocytes. In addition, $LPA_{1/3}$-mediated Src-ERK signaling might be essential in the potentiation of AMI-induced BDNF expression in Cx43-downregulated astrocytes. These results indicate the downregulation of Cx43 observed in the depressive patients might contribute to therapeutic effect of antidepressants.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 229.01

Topic: G.09. Drugs of Abuse and Addiction

Support: Swedish Research Council VR # 2018-02320 (to Eric Augier)

Title: Sex differences in pathological choice of oxycodone over healthy rewards

Authors: *S. ERIKSSON SOLANDER[1], V. SCHWABL[2], M. ATUDOREI[3], G. AUGIER[3], E. AUGIER[4];
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Abstract: Opioid misuse has become a major concern and a rapidly evolving public health crisis that requires innovative scientific approaches. Opioid addiction leads to a progressively increased choice of drugs over healthy rewards. However, the availability of alternative non-drug rewards has so far been largely overlooked in preclinical models of opioid use disorder (OUD). We recently used a procedure in which about 15% of outbred rats choose alcohol over an alternative high-value reward, a rate similar to human addiction, and identified that decreased expression of the GABA transporter GAT-3 within central amygdala (CeA) was causal for alcohol choice behavior and translated to humans. Whether this mechanism also operates for OUD and choice of alcohol over a sweet reward translates to oxycodone is currently unknown. In the present study, we therefore evaluated choice between oral oxycodone and an alternative reward (sweet or social) in both male and females Wistar rats. Using operant conditioning, we first trained male and female rats (n=32 per sex) to self-administer oxycodone orally. We found that males earned more oxycodone reinforcers and showed a higher motivation for oxycodone. Blood oxycodone metabolites concentrations were in addition strongly correlated with oxycodone reinforcers earned during self-administration. We then used an exclusive choice-based method to identify both male and female rats that continue to self-administer oxycodone at the expense of a high-value natural reward, a sweet solution and found that, in agreement with our previous work with alcohol, only a minority of outbred rats (10% or less) choose oxycodone over an alternative high-value reward. Finally, we investigated whether GAT-3 also mediates pathological choice of oxycodone over healthy rewards by injecting an independent group of animals with an AAV-shRNAi targeting GAT-3, or a scrambled control vector, into the CeA Together, our results indicate profound sex differences in oxycodone-related behaviors. They also confirm and extend to oxycodone previous data obtained with preclinical choice models.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 229.02

Topic: G.09. Drugs of Abuse and Addiction

Support: Swedish Research Council, VR project number 2018-02320 (to Eric Augier)

Title: Wistar rats choose alcohol over social interaction in a discrete-choice model

Authors: *G. AUGIER[1], V. SCHWABL[2], A. LGUENSAT[3], M. ATUDOREI[3], E. AUGIER[4]; [1]BKV, Linköping, Sweden; [2]Clin. and Exptl. Med., [3]BKV, Linköping Univ., Linköping, Sweden; [4]Ctr. for Social and Affective Neurosci., Dept. of Clin. and Exptl. Med., Linköping, Sweden

Abstract: Animal models of substance use disorders have been criticized for their limited translation. One important factor behind seeking and taking that has so far been largely overlooked is the availability of alternative non-drug rewards. We recently reported that only about 15% of outbred Wistar rats will choose alcohol over a sweet solution of saccharin. In parallel, it was shown using a novel operant model of choice of drugs over social rewards that social interaction consistently attenuates self-administration and craving for stimulants and opioids. Whether this is also true for alcohol and choice of alcohol over a sweet reward translates to social rewards is currently unknown. We therefore evaluated choice between alcohol and a social reward in different experimental settings in both male and females Wistar rats. We found, in marked contrast to prior work that employed discrete choice of drugs versus social reward, that rats almost exclusively prefer alcohol over social interaction, irrespective of the nature of the social partner (cagemate *vs.* novel rat), the length of interaction, housing conditions and sex. Alcohol choice was reduced when the response requirement for alcohol was increased. However, rats persisted in choosing alcohol, even when the effort required to obtain it was 10 to 16 times higher (for females and males respectively) than the one for the social reward. Finally, we found that the $GABA_B$ PAM ADX 71 441 but not naltrexone reduces alcohol choice, although this was not accompanied by a shift towards the social reward. Altogether, these results indicate that the social choice model may not generalize to alcohol, pointing to the possibility that specific interactions between alcohol and social reward, not seen when a sweet solution is used as an alternative to the drug, may play a crucial role in alcohol vs social choice experiments.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 229.03

Topic: G.09. Drugs of Abuse and Addiction

Support: Swedish Research Council VR # 2018-02320 (to Eric Augier)

Title: Sex differences in pathological choice of alcohol over a healthy reward

Authors: G. AUGIER[1], O. CONSOLER LYERE[1], *E. AUGIER[2];
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Abstract: Addiction leads to a progressively increased choice of drugs over healthy rewards. However, the availability of alternative non-drug rewards has so far been largely overlooked in preclinical models of alcohol use disorder (AUD). To address this issue, we recently used a procedure in which about 15% of outbred rats choose alcohol over an alternative high-value reward, a rate similar to human AUD, and identified that decreased expression of the GABA transporter GAT-3 within central amygdala (CeA) was causal for alcohol choice behavior and translated to humans.
These results were however only obtained in male rats and an important remaining question is whether this mechanism also operates in females. We therefore aimed at investigating whether, similarly to males, only a minority of female rats would choose alcohol over an alternative high-value reward. We trained an equal number of male and female Wistar rats (n=32 per group) to self-administer a solution of 20% alcohol. Once stabilized, they were offered daily sessions of mutually exclusive choice between alcohol and 0.2% saccharin. We found that, although females tend to acquire 20% alcohol self-administration quicker than males during the first sessions of operant conditioning, males stabilized at higher levels of self-administration. Males were also more motivated for alcohol as shown by higher breakpoints and chose more alcohol over saccharin compared to females. In addition, alcohol choosing rats showed other traits reminiscent of clinical addiction, namely high motivation to obtain alcohol, and pursuit of alcohol despite adverse consequences.
Finally, we investigated whether GAT-3 also mediates pathological choice of alcohol over the sweet reward in females. We injected an independent group of animals with an AAV-shRNAi targeting GAT-3, or a scrambled control vector, into the CeA and found that GAT-3 KD potently promoted pathological choice of alcohol over saccharin, at an even higher extend in females.
All together, these results support a higher prevalence to develop addiction-like behaviors in male compared to female rats, which aligns with human epidemiological data. Furthermore, they indicate that decreased expression of GAT-3 within CeA is causal for choice behavior in both sexes and that rescuing impaired GABA clearance due to suppressed GAT-3 expression might be a successful therapeutic mechanism in AUD.

Disclosures: G. Augier: None. **O. Consoler Lyere:** None. **E. Augier:** None.

Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 229.04

Topic: G.09. Drugs of Abuse and Addiction

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Title: Effects of classical and dissociative psychedelics on neuronal activity in the cortex-basal ganglia system

Authors: I. BRYS[1,2], S. A. BARRIENTOS[3], J. E. WARD[3], J. WALLANDER[3], P. PETERSSON[3,2], ***P. HALJE**[3];
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Abstract: The neurophysiological mechanisms behind the profound perceptual changes induced by psychedelic drugs are not well understood. To identify neuronal activity specific to the psychedelic state, we here investigated the effects of classical psychedelics (5-HT2A agonists; LSD, DOI) and dissociative psychedelics (NMDA antagonists; ketamine, PCP) on neuronal firing rates and local field potentials. We recorded simultaneously from cortex, basal ganglia and thalamus using 128-channel microelectrode arrays in freely moving rats.
Spontaneous neuronal firing rates from 365 units were compared before and after drug administration. 5-HT2A agonists had a net inhibitory effect on putative interneurons and principal cells in all recorded regions. NMDA antagonists had a similar inhibitory effect on principal cells, but an opposite excitatory effect on interneurons in most regions. However, the inhibitory effect on principal cells were not specific to the psychedelic state, as the same effect was observed with a positive non-psychedelic control (amphetamine).
In local field potentials, psychedelics dramatically increased the prevalence of high-frequency oscillations (HFOs) around 150 Hz, especially in the prefrontal cortex, the ventral striatum and the olfactory cortex. Both classical and dissociative psychedelics induced a very similar pattern of HFOs - despite their different pharmacological mechanisms - while the non-psychedelic control did not induce any HFOs. Oscillation frequency varied over time and condition but were remarkably similar between brain structures at any given time. Phase analysis revealed strong phase locking between structures. Phase differences were consistent but very small, corresponding to temporal delays of less than 1 ms. This suggests that the HFOs are generated locally in multiple regions and obtain phase synchrony via weak coupling.
In summary, the most specific neurophysiological correlate of psychedelic drug action was clearly the enhancement of widespread phase-coupled HFOs at 150 Hz. The similarities in HFO features across brain structures with different cytoarchitecture, together with the emergence of a

common oscillation phase, implies that important aspects of this phenomenon can only be understood on the systems level. We suggest a conceptual model where local oscillators couple weakly via gap junctions or via the extracellular field to form synchronized HFOs across prefrontal and limbic brain regions. The effect of widespread phase-coupled HFOs on cognition is unknown, but it is likely that their effect on spike-time dependent information processing is severe.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 229.05

Topic: G.09. Drugs of Abuse and Addiction

Title: Investigating altered consciousness and the role of serotonin receptors in the therapeutic effects and mechanisms of actions of psilocybin

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Abstract: The search for rapid and long-lasting antidepressants is critical in the fight against mental illness. Current clinical trials are showing exciting evidence and strong potential for treatment of PTSD, depression, anxiety, and addiction with hallucinogens. Psilocybin, the active psychedelic compound in “magic mushrooms”, has shown therapeutic actions in humans in the clinical treatment of depression and anxiety. Psilocin, the active metabolite of psilocybin, binds many serotonin subtypes, however, its mechanism of action remains unknown. Recent studies have suggested that these therapeutic effects could be independent of altered consciousness (hallucinations) caused by serotonin 2A receptor activation. Investigating the role of other serotonin receptors in the antianhedonic actions of psilocybin without altering consciousness would therefore benefit clinical applications of this controversial drug treatment for patients suffering from depression and others. Moreover, previous work has shown that psilocybin strengthens hippocampal TA-CA1 synapses after chronic stress. Coincidently, serotonin potentiates excitatory synapses formed by hippocampal TA-CA1 pathway via activation of 5-HT1B receptors. Given that 5-HT1B receptors contribute to synaptic plasticity and behavioral antidepressant-like responses, we hypothesized that psilocybin may influence depressive-like behaviors via activation of 5-HT1BRs. In this study, I first investigate the effects of psilocybin on anxiety, reward, and anhedonia as measured via multiple behavioral paradigms in mice. I then explore the necessity of the subjective conscious experience of psilocybin on the anti-depressant effects by injecting psilocybin under anesthesia. Finally, I aim to test the role of the serotonin 1B receptor as a potential mechanism via which psilocybin operates using transgenic knock-out mice lacking the 1B receptor gene. The results presented in this study show that psilocybin

reduces anxiety-like behaviors, increases reward motivation and value, and decreases anhedonia. The data also suggest that the subjective conscious experience of psilocybin is not required to observe these antianhedonic and anti-depressive effects.

Disclosures: S. Fleury: None. **K.M. Nautiyal:** None.

Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 229.06

Topic: G.09. Drugs of Abuse and Addiction

Support: Wellcome Trust Grant 220050/Z/19/Z

Title: Structural and behavioral signatures of enhanced plasticity resulting from a single dose of the psychedelic drug DOI

Authors: *M. ŠABANOVIC[1], A. LAZARI[2], M. BLANCO-POZO[1], T. AKAM[1], V. VYAZOVSKIY[3], J. P. LERCH[2], D. M. BANNERMAN[1], M. E. WALTON[1];
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Abstract: Psychedelics have the potential to treat various mental disorders, possibly by increasing brain plasticity. While high cell plasticity was found for weeks after treatment, it is unclear if these changes can be integrated across higher levels of analysis - from brain structure to behavior. We asked if a single dose of the psychedelic drug DOI leads to a lasting state of comprehensive high plasticity in male young adult C57BL/6 mice. Following a top-down approach, we investigated behavior, cognition, and brain structure. On a behavioral level, a highly plastic state should be highly environmentally sensitive, so we compared quadratic Poisson fits of dose-response curves to the saline vehicle, 0.5, 1, and 2mg/kg DOI in a familiar and novel context (between-subjects n_{group}=8). Novelty increased sensitivity to both head twitch (P=0.002) and ear scratch (P<0.001) hallucination-like effects. On a cognitive level, high plasticity can mean high adaptability and new strategies. To test these, we trained mice on a two-step decision making task to choose between two left/right options to access up/down ports for probabilistic water reward. Reward probabilities were reversed periodically within sessions to test reward learning adaptation. Transitions between steps also followed a probabilistic schedule that was fixed during training but reversed once after drug treatment to test task structure adaptation. Adaptability to a transition reversal (TR) 24h after 2mg/kg DOI injection was comparable to the controls (n_{group}=14-15; RM ANOVA drug P=0.708). In contrast, when we gave the TR one week after injection, giving time for cell plasticity to develop, DOI-treated mice learned the new structure faster than the controls (n_{group}=13; drug P=0.035, η_p^2=0.17). Also, their strategy shifted to incorporate not only rewards but reward omissions too (drug P=0.027, η_p^2=0.19). Moreover, they were quicker to adapt to reward reversals in the first post-drug week,

before the TR (drug X trial P=0.006, η_p^2=0.08). On a structural level, enhanced cell plasticity in the form of new dendritic branches and spines could result in gray matter volume (GBV) changes. Using *ex vivo* magnetic resonance imaging, we showed that GBV of several sensory areas was greater 24h after 2mg/kg DOI compared to the vehicle (q<0.2; n_{group}=8). The primary visual cortex and temporal association area survived the stricter q<0.05. Yet, brains imaged three weeks after injection showed no significant GBV differences (n_{group}=12). To conclude, we found signs of DOI-induced higher brain plasticity at several levels of analysis that do not necessarily coincide as structural plasticity preceded the cognitive-level changes.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 229.07

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant DA047976
BBRF Grant 28897

Title: A socio-sensory mechanism buffering drug choice

Authors: K. PAPASTRAT, A. C. PUCHE, ***M. VENNIRO**;
Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Volitional social interactions with peers are highly rewarding and can be used as a buffer against abused drugs. Organisms across all species require sensory systems to capture emotions and communicate during social interactions. Here, using rats, we study the role of olfactory systems in mediating volitional social interaction and social preference-induced drug abstinence. First, we tested the role of olfactory system in either acquisition or maintenance of volitional social interaction. Using our social-choice self-administration model, we trained male and female rats for either food (2-h/d, 5-d) or social (2-h/d, 10/12-d) self-administration. We removed the olfactory bulbs (bulbectomy or sham) before (experiment 1) or after (experiment 2) acquisition of social behavior. In experiment 3, we tested the role of the olfactory system in social choice-induced inhibition of drug self-administration. After training bulbectomy or sham rats for social and cocaine (6-h/d, 12-d) self-administration, we tested the rats' preference between social reward and cocaine. In experiment 1, bulbectomy selectively prevented acquisition of volitional social interaction while maintaining reliable food self-administration. In experiment 2, bulbectomy selectively prevented maintenance of volitional social interaction while maintaining reliable food self-administration in both male and female rats. In experiment 3, bulbectomy selectively prevented acquisition of social interaction while maintaining reliable cocaine self-administration in both male and female rats. Rats with intact olfactory system

showed strong social preference whereas cocaine preference resumed in rats deprived of the olfactory system.We identified the olfactory system as a new socio-sensory communication mechanism mediating volitional social interaction and the protective effect of social reward on drug choice. From a translational perspective, these findings highlight the need to identify critical sensory cues during peers' communication for implementation of social-based addiction treatments.

Disclosures: K. Papastrat: None. **A.C. Puche:** None. **M. Venniro:** None.

Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 229.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R00 DA038110

Title: Effects of cocaine exposure and estrous cycle fluctuations on excitatory synaptic transmission in the basolateral amygdala

Authors: *C. M. CORBETT[1], E. N. D. MILLER[1], B. D. ROOD[1], D. J. CHANDLER[1], J. A. LOWETH[2];
[1]Cell Biol. and Neurosci., [2]Cell Biol. & Neurosci., Rowan Univ. Sch. of Osteo. Med., Stratford, NJ

Abstract: Fluctuations of ovarian hormones across the reproductive cycle are known to influence cocaine use disorder and relapse vulnerability. In rodent models in which cue-induced cocaine seeking increases or "incubates" during withdrawal from extended-access cocaine self-administration, females in the estrus stage of the estrous cycle (Estrus Females) show an increase in the magnitude of incubated craving compared to both Males and females in all other stages of the cycle (Non-Estrus Females). However, little is known regarding the cellular and synaptic mechanisms contributing to estrous cycle effects in females following cocaine-exposure and withdrawal. One region known to play a critical role in the expression of incubated cocaine craving is the basolateral amygdala (BLA). We have previously reported an increase in the spontaneous firing rate of glutamatergic pyramidal neurons in the BLA in male rats during early withdrawal (2-3 weeks) from extended-access cocaine self-administration. Here we assess effects of cocaine exposure and estrous cycle fluctuations on BLA excitatory synaptic transmission in female rats during a similar withdrawal period. *Ex vivo* whole-cell patch clamp recordings from BLA pyramidal neurons were conducted following 2-4 weeks of withdrawal from extended-access cocaine self-administration or drug-naive conditions in adult female rats. We observed an overall increase in excitatory synaptic transmission in cocaine-exposed females compared to drug-naïve females. While estrous cycle fluctuations influenced excitatory synaptic transmission in drug-naïve females, we observed the most robust increase in excitatory synaptic

transmission in cocaine-exposed Estrus Females compared to cocaine-exposed Non-Estrus Females. In addition, we observed an increase in intrinsic membrane excitability of BLA pyramidal neurons in cocaine-exposed Estrus Females compared to both cocaine-exposed Non-Estrus Females and drug-naïve controls. Together our data identify an interaction between cocaine exposure and estrous cycle fluctuations on neuronal excitability in the BLA, which may contribute to observed estrous cycle-dependent changes in incubated cue-induced cocaine craving.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 229.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R00045758

Title: Cocaine-seeking on extinction day 1 induces sex-specific transcripts in the dorsal hippocampus

Authors: *S. KELSEN[1], J. ZHAO[3], M. M. BERRY[1], A. S. KOHTZ[1,2];
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Abstract: The inability to maintain abstinence is a trademark of addiction, yet effective maintenance therapiesremain elusive. Craving during initial abstinence can predict long-term relapse outcomes in humans.Furthermore, promoting abstinence success may be particularly complex in women as psychological andbiological responses to drugs of abuse differ in women compared to men. Several measures of cocainedependence are greater in women, and can be paralleled in female rodents, yet the biological mechanismsfor these sex differences remain unclear. Thus, understanding circuit and molecular signatures that driveincreased cocaine seeking among females is critical to the development of effective SUD therapies.Extinction day 1 (ED1) marks the initiation of abstinence where the expected drug is not available, and isa particularly stressful time point where drug cravings may increase. Since cravings during the initiationof abstinence in humans and rodents predicts later relapse in both species, ED1 may be a critical timepoint for targeting treatment in addiction. We have previously shown that the dorsal hippocampus playsa significant role in driving sex-specific engagement in cocaine-seeking behavior on ED1. Here, we usedwhole-transcriptome sequencing (RNA-Seq) analysis to identify sex-specific gene expression patternselicited by exposure to the cocaine self-administration context on ED1 that correlate with cocaine seekingbehavior. Current RNA-Seq

analysis of gene expression in sex differences remains sparse, lacks depth, andhas not been linked to specific high-risk behavioral outputs, e.g. cocaine-seeking. Fresh-frozen wholedorsal hippocampus from male and female Sprague-Dawley rats were sacrificed as naïve, in 24hrwithdrawal from cocaine (WD1), or immediately following ED1 testing. First, we identified 101 transcriptsin females and 121 transcripts in males that had fold-change differences on WD1 compared to naïve rats.We also identified 22 transcripts in females and 149 transcripts in males that had fold-change differenceson ED1 compared to WD1 controls. Interestingly, only three targets overlapped between the sexes.Furthermore, five genes identified in females, and one in males, significantly correlated to cocaine-seekingbehavior on ED1 with R^2 values > 0.70. We propose these transcripts may play a crucial role in sex-specificsignaling driving cocaine seeking persistence and can be targeted to promote successful maintenance ofabstinence. This work was supported by R00045758 to ASK.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 229.10

Topic: G.09. Drugs of Abuse and Addiction

Support: 1ZIADA000599

Title: An exploration of sex differences in serotonin 5-HT_2 receptor function in rat orbitofrontal cortex parvalbumin neurons

Authors: K. M. MOONEY[1], A. F. HOFFMAN[1], *C. R. LUPICA[2];
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Abstract: The orbitofrontal cortex (OFC) is involved in higher-order decision-making and flexible behavioral adaptations during changes in environmental contingencies. Behavioral rodent studies show that OFC control over decision-making and goal-oriented behavior are disrupted for months after withdrawal from cocaine self-administration (CSA), and our prior studies show that serotonin (5-HT) modulation of OFC neurons is also disrupted after CSA. Specifically, our previous studies reported that $5HT_{2A}$ receptor-mediated effects were reduced in OFC pyramidal neurons (PyNs), and that baseline glutamatergic synaptic transmission and membrane properties of OFC GABAergic parvalbumin neurons (OFC^{PV}) differed in a sex-dependent manner after CSA (Wright et al., 2017, Cerebral Cortex, 27: 5463; Wright et al., eNeuro, 2021, Jul 8;8(4):ENEURO.0017-21.2021). In the latter study, the 5-$HT_{2A/C}$ antagonist ketanserin, blocked the depolarizing effect of 5-HT in female, but not male OFC^{PV} neurons. We hypothesize that the differential sensitivity to ketanserin may indicate distinct expression levels of 5-HT_2 receptor subtypes in male and female OFC^{PV} neurons and conducted studies to evaluate

this using *in vitro* electrophysiology and pharmacology. Fluorescent proteins were expressed in parvalbumin-*cre* rats (RGD ID# 10412329) using viral vectors injected into OFC, and this permitted identification of OFC^{PV} neurons for whole-cell patch clamp recordings with fluorescence-aided microscopy in brain slices. We measured the effects of the non-selective, 5-HT_2 agonist, R-(−)-2,5-Dimethoxy-4-iodoamphetamine (DOI), on membrane currents in OFC^{PV} neurons in the presence of selective 5-HT_{2A} (MDL 11,939) and 5-HT_{2C} (SB 242084) antagonists. Thus far, our data show that DOI activates similar inward currents in both male and female OFC^{PV} neurons, and that SB 242084 does not block this effect in female cells. A complete dataset for both male and female OFC^{PV} neurons and 5-HT_{2A} and 5-HT_{2C} effects will be presented.
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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 229.11

Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA046403-04
UF1 NS107659-01

Title: The effects of social housing on social behavior and stimulated DA release after methamphetamine exposure

Authors: *I. GONZALEZ[1], N. LEONARDO[2], B. D. LUMA[2], P. R. PATEL[3], D. C. JAKLIC[2], C. A. CHESTEK[4], J. B. BECKER[5];
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Abstract: Methamphetamine (METH) is an addictive stimulant and there has been in increase in usage among people in the United States. Factors like social isolation can contribute to the vulnerability in addiction to METH. Previous studies using cocaine self-administration have shown that housing pairs of rats of the same sex attenuated motivation for cocaine in females but did not affect males' motivation for cocaine. However, there were individual differences in motivation among socially housed females. To understand these individual differences, as well as the sex differences, in how social behavior attenuates motivation for cocaine and METH the quality of the relationship between socially housed cage mates was examined. Rats have a social hierarchy, so animals were screened for social dominance using two weeks of 10-minute social behavioral recordings where behavior such as social investigation and social play were

examined. For social play, the behaviors within play such naping, pinning and supine were recorded and used to determine which animal was dominant. A carbon-fiber multiarray electrode was lowered into the nucleus accumbens (NAc) and a stimulating electrode into the ventral tegmental area. Stimulating pulses were triggered to release dopamine (DA) in the NAc and the peak DA was measured. Each test session, the animal was given 3 i.p. injections of 0.5mg/kg METH for a total dose of 1.5mg/kg. During the test session, baseline was collected and then a set of 3 stimulations was given (30Hz 15p, 60Hz 30p & 60Hz 60p). After each METH injection, the same procedure of baseline and stimulation were collected. Social behavior of paired rats was analyzed after exposure to METH. Results showed that in some pairs social hierarchy remained the same for males and females, while in other pairs dominance changed or was unstable; social play decreased for both sexes after METH. These results show that the quality of the relationships between males and females could play a potential role in motivation for drugs. Data were also collected investigating DA release in the NAc core and shell of socially housed and individually housed rats using fast scan cyclic voltammetry. **NAc Core:** There was a significant effect of social housing on the effect of electrical stimulation after METH in both males and females. There was also a significant interaction between sex and housing at baseline. **NAc Shell:** There was a significant effect of social housing on the effect of electrical stimulation in both males and females for all conditions. These data will help to better understand the DA response in the NAc core and shell and how it is affected by METH, social housing conditions and sex differences.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

Location: SDCC Halls B-H

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Program #/Poster #: 229.12

Topic: G.09. Drugs of Abuse and Addiction

Support: R01 - DA – 049795; UF1 NS107659-01

Title: Activation of g-protein coupled estradiol receptor-1 modulates stimulated dopamine release in the dorsal striatum of male rats

Authors: *C. A. TURNER[1], B. D. LUMA[2], N. B. LEONARDO[2], P. R. PATEL[3], C. A. CHESTEK[3], J. B. BECKER[1,2];
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Abstract: Women start using drugs and enter treatment earlier with more severe symptoms than men. The neurochemical changes underlying these sex differences are largely unknown. Estradiol (E2) receptor activity has been shown to play a sex-specific role in modulating the

brain's reward pathway. Specifically, it has been demonstrated that E2 enhances the rewarding properties of cocaine in females. There are three E2 receptors: ERa, ERß, and G-Protein Coupled Estradiol Receptor 1 (GPER-1), that may be mediating these effects. Research from our lab has shown that G1, a GPER-1 receptor agonist, attenuates the development of a preference for rewarding substances like cocaine and saccharin in male but not female rats. In the brain, the dorsal lateral striatum (DLS) is thought to be responsible for enhanced cue reactivity and compulsive drug taking after repeated drug use. E2 has been shown to modulate dopamine (DA) signaling in the DLS, enhancing both stimulated and drug-induced DA release in females but not in males. As such, it may be that GPER1 receptor activation differentially modulates DA signaling in the DLS of males and females and drives the sex-specific effects we see on the formation of a preference for saccharin or cocaine. In our current study, gonadectomized male and female rats received three electrode implants: a 16-channel carbon fiber working electrode targeting the DLS, a bipolar stimulating electrode targeting the medial forebrain bundle, and a guide cannula for a reference electrode in the contralateral cortex. Animals underwent weekly test sessions with a total of four sessions. First, a session to test the electrode, followed by three treatment sessions. Each treatment session consisted of three sub-cutaneous injections of the designated treatment: Peanut Oil (Vehicle), G1, or E2, each 30 mins apart. Recording occurred 10 mins after each injection. For G1, animals received three treatments of 10 µg/kg G1 for a cumulative dose of 30 µg/kg. For E2, animals received one injection of 16.67 µg/kg and then oil injections for the following two treatments. After each treatment, three different electrical stimulations were applied to the bipolar stimulating electrode: 60 Hz 30 pulses; 60 Hz 60 pulses; and 60 Hz 120 pulses. At the conclusion of the session, animals received a treatment with the D_2 receptor antagonist raclopride to verify that the release was DA. We report that administration of G1 attenuates stimulated DA release in the DLS of male, but not female rats, and confirm that E2 enhanced stimulated DA release in females. These findings highlight GPER-1 as a potential therapeutic target and systemic G1 administration as a potential treatment for addiction in males.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 229.13

Topic: G.09. Drugs of Abuse and Addiction

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UF1 NS107659-01

Title: Cellular localization of fast-scan cyclic voltammetry multielectrode array tips

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Abstract: Changes in dopamine (DA) release during acquisition and escalation of drug taking have been difficult to study due to the lack of methods to measure DA release quantitatively and reliably over time. Fast-scan cyclic voltammetry (FSCV) is an electrochemical method which allows for in vivo quantification of electrically active molecules with high chemical sensitivity and temporal resolution to study the role of DA in drug use and abuse. DA is commonly studied using FSCV in the dorsal striatum (DS) or nucleus accumbens (NAc) using acutely or chronically implanted silicon electrodes with carbon fiber tips. There is often a large immune response for these electrodes which makes examining the neural environment at the recording site after chronic implantation in the brain difficult. Thus, understanding the role of changes in DA release with FSCV, during or as a consequence of drug taking behavior, has been technically challenging. Using a novel biocompatible 16 channel multielectrode array (Yu Huan et al 2021 J. Neural Eng. 18 066033) we can attain high spatial resolution with low immune system response. The linearly aligned carbon fibers are implanted chronically and provide unique in vivo monitoring of neural responses with very little damage to the neural extracellular environment at the tips. Electrodes are left attached to the headcap and whole skulls are extracted from the animal post-mortem. After decalcification of the skull, brains are sectioned with the recording fibers left in place to provide placements for the electrode in situ. Sections are then stained with tyrosine hydroxylase (TH) to identify DA rich regions (Abcam AB76442) and NeuN to identify neurons (Millipore MAB377), plus the NAc is stained for calbindin to determine core vs. shell (Swant CB-38a) and the DS is stained for μ-opioid receptor to identify patch vs. matrix (Immunostar 24216). Using confocal microscopy to examine 300 μm sections we can determine the placement of all 16 fibers on the electrode used for simultaneous recordings. We can thus determine where recording sites are in core vs. shell subregions in the NAc or other adjacent DA-rich regions, and patch-matrix or medial-lateral subregions of the DS. FSCV can describe different aspects of molecular release and our histological technique can take advantage of this added data resolution by informing us more deeply about the neural environment surrounding the electrode recording sites.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

Location: SDCC Halls B-H

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA R01DA053328

Title: Sex differences in the biochemical correlates of incubated sucrose craving within medial PFC subregions

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Abstract: As observed in animal models of drug-taking, the capacity of discrete cues associated with the presentation of palatable food to elicit conditioned responding incubates with the passage of time. The similar temporal profile of incubated food- vs. drug-seeking has led to the hypothesis that these two behavioral phenomena may involve common, time-dependent, biochemical adaptions within neural circuits governing motivated behavior. However, we reported previously that incubated cocaine-seeking reflects changes in extracellular glutamate within ventromedial PFC that are opposite those observed in sucrose-seeking rats. Herein, we extend this prior work by immunoblotting for protein changes within the prelimbic (PL) and infralimbic (IL) cortices of male and female Sprague-Dawley rats exhibiting incubated sucrose-seeking. Rats were trained to lever-press for delivery of 45 mg banana-flavored sucrose pellets, paired with a light + tone compound stimulus, during daily 6-h sessions over 10 consecutive days. One or 30 days following the last operant-conditioning session, rats were subjected to a 2-h test to measure cue-elicited responding (Cue Test) and PL and IL tissue collected immediately following the session. Rats of both sexes exhibited incubated sucrose-seeking, although the magnitude of the incubated response was larger in females vs. males. In contrast to our prior immunoblotting data for incubated cocaine-craving, we detected only 1 protein change in the PL and this was a female-selective reduction in p-Akt expression. Also distinct from cocaine, we detected time-dependent increases in IL expression of mGluR5, p-Akt and p-mTOR. Taken together, the present results, coupled with those previous for cocaine-incubated animals, suggest that both the biochemical mechanisms and brain regions involved in the incubation of sucrose-craving are distinct from those mediating the incubation of cocaine-craving. The present results also indicate a sex difference in the neurobiology of incubated sucrose-craving, with sucrose-craving associated with increased glutamate-related signaling within the IL of male, but not female, rats.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA grant R01DA053328

Title: Glutamate receptor correlates of incubated cocaine-craving under short-access self-administration procedures

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Abstract: The incubation of craving is phenomenon where cue-elicited craving intensifies during protracted withdrawal. Previously, our laboratory demonstrated an increase in extracellular glutamate within the ventromedial prefrontal cortex (vmPFC) in rats that exhibited incubated cocaine-seeking, which likely activates AMPA and NMDA ionotropic glutamate receptors (iGluRs). Indeed, incubated cocaine-seeking involves increased expression of GluA2-lacking AMPA receptors within the nucleus accumbens and elevated GluN2A and GluN2B-containing NMDA receptor expression in dorsomedial PFC. To the best of our knowledge, few studies have examined the relationship between incubated cocaine-craving and iGluR changes within vmPFC. In the present study, rats were trained to self-administer IV cocaine for either 2 h/day for 10 days or for 6 h on day 1, followed by 2 h/day for 9 days. Each cocaine delivery was paired with a light-tone stimulus complex. Then, at 3 or 30 days withdrawal, rats underwent a 2-h cue test for cue-reinforced responding and then tissue from the prelimbic cortex (PL) and infralimbic cortex (IL) were immunoblotted for AMPA and NMDA receptor subunits, as well as other proteins previously implicated in incubated cocaine-craving under more standard 6-h self-administration procedures. Both self-administration procedures induced an incubation of cocaine-craving, the magnitude of which was larger under the "6-h/2-h" model. However, we detected no changes in AMPA or NMDA subunit expression in either subregion. Both paradigms increased AKT activity and Homer2a/b expression within the PL in animals tested during protracted withdrawal. These results demonstrate that short-access cocaine self-administration paradigms are sufficient to elicit incubated cocaine-seeking and certain time-dependent increases in protein expression implicated in incubated cocaine-seeking following longer-access procedures. However, our short-access procedures are not sufficient to alter AMPA and NMDA subunit expression within the vmPFC during either early or protracted withdrawal.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

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Topic: G.09. Drugs of Abuse and Addiction

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Title: Investigating the role of PFC-NAC activity in methamphetamine reinforcement

Authors: C. N. BROWN[1], J. M. BELTRAN[2], E. K. FULTZ[3], T. T. TRAN[1], B. BARGER[1], N. WILLIAMS[1], W. YEN[1], A. PARK[1], T. E. KIPPIN[1], K. K. SZUMLINSKI[1];

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Abstract: Methamphetamine (MA) is a highly addictive stimulant that poses a difficult challenge to society and contributes more to violent crime than any other drug of abuse in the United States. Developing safe and effective therapies for MA use disorder requires understanding the neurobiological adaptations underlying MA abuse. Though much of the research on substance abuse has focused on dopaminergic signaling, particularly in the nucleus accumbens (NAC), the glutamate system is also critical for the neuroplasticity involved in long-term MA use. In particular, one key glutamatergic input to the NAC originates in the prefrontal cortex (PFC). Activity in sub-circuits within this projection was bidirectionally manipulated using a dual-virus chemogenetic strategy prior to operant-conditioning procedures. The results from this study argue that while traditional theories of the functional neuroanatomy of addiction tend to view relationships between the NAC core and shell or PFC prelimbic and infralimbic sub-regions as dichotomous, the regulation between these sub-circuits is more complex and may depend on the behavioral paradigm studied. Other inputs upstream from these regions of interest may be able to compensate for our manipulations via neurobiological redundancy. This work emphasizes the importance of targeting functional networks rather than single sites as research technologies advance in order to elucidate the neurobiological mechanisms contributing to multifaceted behaviors like substance abuse.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

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Support: BBRF NARSAD Young Investigator Award
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Title: Region-specific changes in excitatory synapse composition during development

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Abstract: The transition from adolescence to adulthood is marked by extensive changes in synapses across the brain. In contrast to the rapid synapse formation observed in early stages of development, this phase involves widespread synaptic pruning across multiple brain regions. Such synapse loss is thought to be essential for the full maturation of regions like the prefrontal

cortex (PFC) and critical for the development of cognitive functions relying on these regions. An understanding of the machinery enabling the manipulation and elimination of synapses and the timeline of its contributions to circuit refinement is thus vital for our understanding of brain development. Additionally, certain developmental stages are associated with higher risks for a variety of psychiatric disorders and substance abuse. For example, binge alcohol consumption patterns vary by both sex and age, and the propensity to binge-drink is believed to be due in part due specific excitatory synaptic proteins; thus, understanding how such proteins change through age will help determine the underpinnings of vulnerability to substance abuse. The ubiquitin-proteasome system functions as a major protein degradation system in eukaryotic cells and has been previously shown to dynamically regulate multiple aspects of excitatory synaptic function and architecture. Thus, the enzymes responsible for degradation-related signaling may be critical in the age-dependent synaptic changes observed throughout the brain. In this project, we collected brain tissue from mice at several key ages, obtaining samples from multiple regions including the PFC, hippocampus, bed nucleus of the stria terminalis (BNST), and the nucleus accumbens. Tissue lysates were processed using Western blotting techniques to evaluate changes in total protein levels in each region across time-points. Intriguingly, we observed age-dependent changes in a number of excitatory synaptic proteins as well as changes in two key degradation-related enzymes known to regulate the abundance of such proteins: the ubiquitin ligase Nedd4-1 and the deubiquitinating enzyme USP8. The time-course and direction of these changes varied based on brain region, with the PFC and hippocampus displaying contrasting changes. These results suggest that excitatory synaptic components and the enzymes controlling their abundance vary substantially throughout development in a region-specific manner. Future studies will explore these changes in greater detail to better appreciate the time-course of the gain and loss of synaptic proteins and will determine the functional relevance of the observed alterations in degradation-related enzymes.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

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Program #/Poster #: 229.18

Topic: G.09. Drugs of Abuse and Addiction

Title: A HISTORY OF BINGE-DRINKING INDUCES AGE RELATED DIFFERENCES IN NEUROCOGNITIVE ABILITIES DURING EARLY AND PROTRACTED WITHDRAWAL IN MALE AND FEMALE C57BL6/J MICE

Authors: *C. L. JIMENEZ CHAVEZ, G. SCHELDRUP, S. KHORSANDI, A. GARCIA, M. CASTRO, J. TORRES-GONZALES, J. HERBERT, K. SZUMLINSKI;
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Abstract: Excessive consumption of alcohol is one of the leading risk factors for early-onset Alzheimer's disease and other related cognitive impairments. We have previously shown that mature female mice when compared to their male counterparts, exhibit higher signs of cognitive impairment in 1-day alcohol withdrawal following a 30-day history of binge-drinking. These findings are consistent with clinical studies that demonstrate that females with a history of alcohol abuse are more susceptible to earlier and more severe cognitive impairments compared to males. To better identify these age and sex differences in cognitive abilities, we examined how the age of drinking onset and biological sex interact to alter cognitive processing during early and prolonged withdrawal from binge drinking. Adolescent (PND 28/29) and adult mice (PND 56-57) from both sexes underwent 14-days of drinking using the multi-bottle Drinking-in-the-Dark (DID) paradigm. Upon cessation of drinking, mice were then subjected to several behavioral assays to measure negative affect and cognitive functioning at either 1-day or 30-days after their last drinking session. Following a 1-day behavioral battery to measure negative affect, we examined cognitive abilities using a 3-week behavioral test battery consisting of the Morris Water Maze (6 days) and the Radial Arm Water Maze (14 days). Our results for the Radial Arm Water Maze indicated that adolescent females with a history of binge-drinking in early withdrawal demonstrated a significantly higher number of reference memory errors than their adolescent female water-drinking counterparts. Moreover, both adolescent and adult mice of both sexes showed a significant increase in the number of working memory incorrect errors when compared to their adolescent water drinking control counterparts in 1-day withdrawal. Female binge-drinkers also engaged in more chaining behaviors than the female water controls and more than the male mice of either alcohol history. In the Morris Water Maze, we also observed a trend showing that alcohol-drinking mice took longer to locate the flagged platform than their water drinking controls in protracted withdrawal. These preliminary results align with the results of previous research on working memory, which emphasized working memory impairments in spatial memory tasks in binge-drinking mice. Taken together, these data confirm that a history of binge-drinking produces a female-selective acceleration of cognitive decline in mice, which is independent of perturbations in emotionality.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

Location: SDCC Halls B-H

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Title: Chronic ketamine exposure affects brain structure and cognition in entering adulthood ketamine users

Authors: *Y.-H. LIU[1,2], C.-C. HUNG[3,4], C.-C. HUANG[6], P.-L. LEE[1], S. YIP[2], M. POTENZA[2], C.-S. R. LI[2], N. G. MUGGLETON[7], T.-H. LEE[5,4], C.-P. LIN[1];
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Abstract: *Ketamine* is a drug used in development to treat mental diseases recently and as a club drug of abuse. Many young people use ketamine during their adolescence to young adulthood, and this period is considered the critical and varying time point of brain development. This study investigated the pure effects of ketamine exposure on cortical and subcortical brain area changes and neurocognitive performance. 161 subjects were recruited, 73 ketamine users, 58 smokers (controls), and 30 healthy participants (brain image controls). High resolution T1-weighted structural magnetic resonance image scans were acquired using a 3.0-T Siemens MRI scanner to compare differences in gray matter volume (GMV) and thickness between groups using the standard full processing and the volume-based (subcortical) streams of FreeSurfer. The N-back task, requiring a stimulus to be compared to one previously presented, was used to measure working memory performance. Clinical and psychological scales were used as self-report measures for evaluating the use of drugs, and psychological and psychiatric performance, including the addiction severity index (ASI), the study of symptom checklist 90-revised (SCL-90), the Barratt impulsiveness scale-11 (BIS-11). The Buss-Perry aggression questionnaire (BPAQ) and the sensitivity to punishment and reward questionnaire (SPSRQ). We adapt the analysis of covariance (ANCOVA) statistic to compare the differences among groups. Imaging results showed that, compared to controls, ketamine users had significantly decreased GMV/thickness in cortical brain regions and also decreased GMV in subcortical regions, including the amygdala, hippocampus, and thalamus. The ketamine participants showed a more significant decreased pattern of accuracy with increased difficulty on the N-back task, and higher scores on the sub-item (non-planning) of BIS-11. A significant negative correlation was found between some brain cortical regions, which were revealed as ketamine users' reduced regions compared with controls, and frequency of ketamine use. Thalamus region volumes were also found to be positively correlated with the accuracy of the N-back task in ketamine users. This study carefully recruited young adulthood, pure ketamine users without poly-drug use to investigate the psychological, psychiatric, and brain structural effects of ketamine use. Results suggest chronic ketamine exposure results in reduced working memory ability and significant structural changes in the cortical and subcortical brain regions. The differences in affected brain regions showed correlations with working memory performance and the frequency of ketamine use.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

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Title: Neuronal activity and neurovascular coupling are altered by hallucinogenic and non-hallucinogenic 5-HT2A receptor agonists.

Authors: *J. A. PADAWER-CURRY[1], J. S. SIEGEL[2], G. E. NICOL[2], J. G. MCCALL[3], A. Q. BAUER[1];
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Abstract: Psychedelics are an appealing potential therapeutics for neuropsychiatric conditions because of their rapid, sustained results. The effects of these drugs appear to be mediated by serotonin (5-hydroxytryptamine, 5-HT) receptor agonism, especially the 5-HT2A receptor (5-HT2AR). Serotonin is a potent vasoconstrictor and 5-HT2AR plays several other physiological roles including regulating neuronal plasticity and arousal. Recent human functional neuroimaging studies evaluating acute effects of psychedelics have shown dramatic changes in functional network organization, which were mitigated after administration of a 5-HT2AR antagonist. However, these studies have not accounted for acute neurovascular effects of 5-HT2AR activation, nor how non-hallucinogenic 5-HT2AR agonists affect brain function. We aimed to determine whether a psychedelic -- 2,5-Dimethoxy-4-iodoamphetamine (DOI) -- alone and a psychedelic in combination with a 5-HT2AR antagonist -- MDL100,907 (MDL) -- differentially affect cortical neuronal and hemodynamic activity, functional connectivity (FC), and neurovascular coupling (NVC). We hypothesized that 5-HT2AR agonism alters neuronal activity and NVC to cause apparent downstream affects such as changes in cortical hemodynamic activity and functional network organization. We test this hypothesis using a non-hallucinogenic 5-HT2AR agonist (Lisuride). We used eight mice expressing a genetically encoded calcium indicator (jRGECO1a) under a Thy1 promoter to image for 60-minutes under awake, resting-state conditions using wide-field optical neuroimaging. Brain function was evaluated before and after compound administration through measures of power spectral density,

FC, and NVC. DOI reduced neuronal infraslow (ISA, p=0.002) and delta-band activity (p=0.059) but not hemodynamic activity. Lisuride significantly reduced neuronal ISA activity (p=0.005) and delta-band activity and hemodynamic ISA activity (p=0.034). Delayed NVC was observed under DOI (p=0.019) and Lisuride (p=0.006). Further, Lisuride altered interhemispheric, homotopic FC in a region- and frequency-band-specific manner, with global reduction in neuronal delta-band FC strength (p=0.036). Results suggest that the hallucinogenic 5-HT2AR agonist DOI differentially alters NVC, and that non-hallucinogenic 5-HT2AR agonism alters hemodynamic activity, FC, and NVC. Future work will evaluate acute and sustained effects of other commonly used psychedelics (e.g., psilocybin) on brain function, and differentiate hallucinatory vs. non-hallucinatory 5-HT2AR activation on persistent anti-depressant effects.

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Poster

229. Addictive Drugs and Psychedelics: Social Interactions and Sex Difference

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Title: Psychedelics alter neurovascular coupling- new evidence from mouse and human brain imaging

Authors: *J. S. SIEGEL[1], D. PERRY[2], G. NICOL[2], N. DOSENBACH[6], A. Z. SNYDER[7], S. SUBRAMANIAN[3], J. PADAWER-CURRY[8], A. Q. BAUER[9], J. G. MCCALL[4], R. CHACKO[10], T. RENEAU[5];
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Abstract: BACKGROUND: Classic psychedelics have become a topic of great interest in psychiatry because of large antidepressant effects. Functional MRI (fMRI) studies of acute effects of psychedelics have observed dramatic changes (primarily decreases) in evoked response and functional connectivity (FC). However, 5-HT2A receptors (and DA receptors) are expressed on neurovasculature and known to have vasoactive effects. Thus, it is not clear to what extent FC

changes reflect changes at the neuronal, neurovascular, or vascular level.**METHODS**: We test the hypothesis that changes in neurovascular coupling and hemodynamic response are influencing changes in resting and evoked BOLD fluctuations caused by psychedelics. 1) We measure resting blood-oxygenation-level-dependent (BOLD) spectral power and functional connectivity in multiple extant human psychedelics datasets. 2) In humans, we acquired fMRI data during rest, stimulus-evoked response, and respiratory response exposed to psilocybin. 3) In mice, we used wide-field optical imaging to simultaneously measure cortical neuronal (calcium) and hemodynamic activity following exposure to various drugs: DOI [5HT2A psychedelic], lisuride [non-psychedelic 5HT agonist], MDL [5HT2A antagonist], DOI+MDL. Mouse data were used to map the impulse response function between neuronal and hemodynamic signals over the cortex. **RESULTS**: We observed that decreases in functional connectivity by psychedelics correspond closely to decreases in spectral power of the BOLD signal ($f < 0.1hz$, corresponding to neuronally-driven BOLD fluctuations). 2) This translated into a decrease in the amplitude of task-evoked and respiratory-evoked BOLD response in multiple cortical areas despite no change in performance. 3) Mouse data showed reduced amplitude and increased latency of hemodynamic responses to neuronal activity after psychedelics. Similar, but smaller effects, were observed in lisuride and DOI+MDL conditions.**CONCLUSION**: These results suggest that 5HT2A psychedelics (and other 5HT-modulating drugs) transiently alter the relationship of neural activity to hemodynamic response. An implication of this is that non-BOLD-dependent imaging modalities may offer better insight in to acute effects of psychedelics on neural activity and brain networks.

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Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

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Title: Systemic des-acyl ghrelin attenuates acute and chronic alcohol-mediated effects in rodents

Authors: *S. WITLEY, J. VESTLUND, C. ARANÄS, M. TUFVESSON-ALM, C. EDVARSSON, E. JERLHAG;
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Abstract: On a global perspective harmful use of alcohol is one of the main risk factors of population health. The underlying mechanisms of alcohol use disorder (AUD) involves dysfunction of the mesolimbic dopamine system, where gut-brain peptides play a crucial role as modulators. The orexigenic peptide ghrelin increases both the acute and chronic effects of alcohol whereas suppression of ghrelin receptors attenuates these behaviours. In plasma ghrelin exists mainly as the non-octanoylated form of ghrelin, des-acyl ghrelin (DAG). Initially considered as an inactive form of ghrelin, the role of DAG in AUD is unknown. Since new evidence suggest that DAG decreases ghrelin-induced food intake, we hypothesise that DAG attenuates alcohol mediated behaviours. Acute and repeated treatment with DAG decrease alcohol intake in female and male rats in a dose-dependent manner. Repeated treatment with DAG was associated with increased dopamine metabolites in the ventral tegmental area (VTA) in male rats, a key area for reward. In consistency with DAG modulating dopamine in the VTA, DAG attenuated alcohol mediated behaviours in mice. In male mice DAG inhibits alcohol-induced locomotor activity. In conditioned placed preference (CPP) paradigm DAG blocks expression but not acquisition of alcohol related CPP. This is supported by increased dopamine metabolites in the VTA but unaltered neurotransmission in hippocampus. Collectively, our data show that systemic DAG attenuated alcohol mediated behaviours in rodents, an opposite effect to that of acyl-ghrelin.

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Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 230.02

Topic: G.09. Drugs of Abuse and Addiction

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Title: Omega-3 supplementation positively impacts on the endocannabinoid system impaired in the adult brain after binge drinking during adolescence

Authors: *M. SERRANO[1,2], L. LEKUNBERRI[1,2], I. ANAUT-LUSAR[1,2], A. MIMENZA[1,2], M. SAUMELL-ESNAOLA[3,4], G. GARCÍA-DEL CAÑO[4,5], J. SALLÉS[3,4], N. PUENTE[1,2], I. ELEZGARAI[1,2], I. RICO-BARRIO[1,2], P. GRANDES[1,2];

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Abstract: Alcohol is the most consumed psychoactive substance worldwide. The misuse of alcohol causes long-term mitochondrial dysfunction as well as neuronal and glial loss that prompt memory impairment. Compelling evidences have shown that omega-3 fatty acids restore long-term deficits in hippocampal synaptic plasticity and prevent oxidative damage caused by prenatal ethanol exposure. However, the contribution of omega-3 to the recovery of the long-term damage caused by binge drinking during adolescence remains unknown. For this purpose, 4-week-old C57BL/6J male mice were exposed to a drinking in the dark procedure (DID) from postnatal development (PND) 32 to 59 (H2O and EtOH). During withdrawal (PND 60-73), half had access to omega-3 enriched diet (O3-H2O and O3-EtOH). Last days of withdrawal, memory was evaluated by the novel object recognition test (NORT) and Barnes maze test. In addition, light immunohistochemistry and western blotting were carried out to determine the expression of several components of the endocannabinoid system. Finally, field excitatory postsynaptic potentials at the medial perforant path (MPP) synapses were recorded. In NORT, recognition memory was improved by omega-3. Thus, discrimination index was significantly higher in both omega-3 treated groups relative to those in standard diet. In the Barnes maze, EtOH mice used random strategy more often than H2O and O3-EtOH, suggesting that omega-3 decreases cognitive impairment induced by binge drinking. While there were not CB1 receptor optical density differences in dentate gyrus, CB1 receptor expression was significantly higher in hippocampal synaptosomes in EtOH and O3-EtOH than in H2O. Moreover, Crip-1a significantly increased after alcohol exposure and in omega-3 diet. Conversely, DGLα was reduced in both omega-3 conditions relative to H2O. Functionally, CB1 receptor-mediated long-term depression at MPP synapses impaired by binge drinking during adolescence (Peñasco et al., 2020), was seen to recover by omega-3 intake. Furthermore, the excitatory long-term potentiation triggered in O3-H2O and O3-EtOH was mediated by different endocannabinoid mechanisms involving CB1 and TRPV1 receptors, respectively. In conclusion, omega-3 can recover the long-term impaired cannabinoid-dependent synaptic plasticity and memory caused by abusive alcohol consumption during adolescence.

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Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

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Topic: G.09. Drugs of Abuse and Addiction

Support: PAPIIT grant IA202120
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Title: The relationship between macro and microstructural changes in chronic ethanol consumption in Wistar rats

Authors: *J. MAYA-ARTEAGA[1], D. ANGELES-VALDEZ[2], C. CARRANZA[2], J. RASGADO[2], A. LOPEZ[2], D. ORTUZAR[2], E. GARZA-VILLARREAL[1];
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Abstract: Alcohol use disorder (AUD) is an affliction which impairs motivational circuits, involving several brain regions such as the prelimbic cortex, infralimbic cortex, caudate-putamen, globus pallidus, anterior amygdala, ventral tegmental area, hippocampus, and cerebellum. Magnetic resonance imaging (MRI) through whole-brain volumetric analysis has identified changes in gray matter volume (GMV) compromised by AUD in humans and animal models. However, the cellular components related to volume changes in AUD remained unclear. Here, we used an intermittent access 2-bottle choice model at 20% ethanol concentration which consists of 20 sessions of alcohol (45 days), 10 days of withdrawal, and 10 sessions of alcohol relapse (23 days) with male and female Wistar rats (n=90). After the alcohol sessions, we used deformation-based morphometry (DBM) in ex-vivo MRI whole brain images to compare local volume between ethanol and control groups. Then, we use immunofluorescence to measure density and volume alterations in cells of 8 regions of interest (ROI): neurons (NeuN), astrocytes (GFAP), and microglia (Iba1). Lastly, we ran a multiple regression model of macro and microstructural measures to find their relationship. Our preliminary results showed lower DBM local volume mainly in the left thalamus, left caudate-putamen, and left dentate gyrus. We also found a region-specific pattern of a reduced number of NeuN-positive neurons with a lower volume, an increased number of astrocytes with a higher volume, and an increased number of microglia with a higher volume. Finally, the multiple regression model will explain a great percentage of the GMV variance.

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Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

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Program #/Poster #: 230.04

Topic: G.09. Drugs of Abuse and Addiction

Support: ERAB Grant EA1714

Title: Administration of the novel positive allosteric modulator of the $GABA_B$ receptor, COR659, effectively prevents binge-like alcohol drinking in rodents

Authors: *I. LORRAI[1,2,4], C. SHANKULA[1], J. MARQUEZ GAYTAN[1], T. KAWAMURA[1], P. MACCIONI[4], C. MUGNAINI[5], F. CORELLI[5], G. GESSA[4,3], P. SANNA[1], G. COLOMBO[4]; [1]Dept. of Immunol. and Microbiology, Scripps Res. Inst., San Diego, CA; [2]Dept. of Biomed. Sci., Univ. of Cagliari, Cagliari, Italy; [3]Dept. of Biomed. Sci., Univ. of Cagliari, Monserrato, Italy; [4]Neurosci. Institute, Section of Cagliari, Natl. Res. Council of Italy, Monserrato, Italy; [5]Dept. of Biotechnology, Chemistry, and Pharm., Univ. of Siena, Siena, Italy

Abstract: Binge drinking (BD) is a pattern of drinking characterized by consuming intoxicating amounts of alcohol in a short time frame, associated to a higher vulnerability to developing alcohol use disorder (AUD). Recent studies demonstrated that COR659 [methyl 2-(4-chlorophenylcarboxamido)-4-ethyl-5-methylthiophene-3-carboxylate)], a novel $GABA_B$ PAM, effectively reduces a) excessive alcohol drinking under the 2-bottle choice regimen, b) operant oral alcohol self-administration under both fixed and progressive ratio schedule of reinforcement and c) cue-induced reinstatement of alcohol seeking in Sardinian alcohol-preferring (sP) rats. The present study was aimed at further investigating the *in vivo* pharmacological profile of COR659, specifically evaluating whether the *anti-alcohol* properties of COR659 extend to binge-like drinking in rodents. To this end, male C57BL/6J mice (n= 48) and male sP rats (n= 64) were exposed to the "drinking in the dark" (DID) protocol and the recently developed 4-bottle "alcohol [10%, 20%, 30% (v/v) versus water"] choice regimen with limited and unpredictable access to alcohol, respectively. Vehicle-treated C57BL/6J mice and sP rats consumed an intoxicating amount of alcohol (> 2 g/kg) which resulted in the achievement of psychopharmacological relevant blood alcohol levels (BALs >80 mg/dl). COR659 administered intraperitoneally at the *non-sedative* doses of 10, 20, and 40 mg/kg, effectively prevented the intake of the excessive amount of alcohol promoted by exposing rodents to the abovementioned procedures. Overall, these data extend the *anti-alcohol* properties of COR659 to binge-like drinking in rodents and contribute to supporting the use of molecules belonging to the class of $GABA_B$ PAMs as a potential pharmacotherapy for AUD, including BD.

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Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

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Program #/Poster #: 230.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01AA026820
NIH R03AA029013

Title: Design and characterization of a highly potent, metabolically stable, and brain-penetrant GPR88 agonist RTI-122

Authors: M. RAHMAN[1], A. M. DECKER[1], S. BEN HAMIDA[3], K. VAN VOORHIES[4], E. DARCQ[5], B. L. KIEFFER[5], J. BESHEER[4], ***C. JIN**[2];
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Abstract: GPR88 is an orphan G protein-coupled receptor (GPCR) and has a high expression in both dorsal and ventral areas of the striatum. Emerging evidence suggests that GPR88 plays an important role in the regulation of striatal functions and is implicated in a variety of striatal-associated disorders, including Parkinson's disease, schizophrenia, and drug addiction. Both GPR88 knockout mouse studies and pharmacological studies using the first-generation agonist RTI-13951-33 demonstrate therapeutic potential for GPR88 agonism to treat alcohol addiction. In the pharmacokinetic (PK) study following an intraperitoneal injection, RTI-13951-33 has short half-life and moderate brain bioavailability which need further optimization. Herein, we present the design, synthesis, and characterization of the next-generation agonist RTI-122. RTI-122 has an EC_{50} of ~10 nM in functional cAMP accumulation and GTPγS binding assays. In the mouse PK study, RTI-122 has good metabolic stability with a half-life of ~6 h and good brain-penetration with a brain/plasma ratio of >1. Finally, RTI-122 is active in vivo and significantly attenuated alcohol drinking and reinforcement in mice and rats. This work is supported by NIH R01AA026820.

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Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

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Program #/Poster #: 230.06

Topic: G.09. Drugs of Abuse and Addiction

Support: National Institutes of Health (NIH) NINDS R01NS084975
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Title: A spike timing dependent plasticity-based approach for treating alcohol use disorder

Authors: *A. ASP[1], S.-Y. CHANG[2,3], S. B. DE SOUZA[3], J. LUJAN[2,3];
[1]Mayo Clin. Grad. Sch. of Biomed. Sci., [2]Physiol. and Biomed. Engin., [3]Dept. of Neurologic Surgery, Mayo Clin., Rochester, MN

Abstract: Introduction: Alcohol use disorder (AUD) costs the U.S. approximately $249 billion annually and causes significant societal and personal burdens. Emerging data suggest that deep brain stimulation (DBS) of subcortical brain structures such as the dorsal medial striatum (DMS) may offer an alternative approach treating AUD. The DMS is responsible for mediating goal-

directed behavior and consists of GPi-projecting dopamine receptor-1 (D1)-containing medium spiny neurons (MSN) and GPe-projecting dopamine receptor-2 (D2)-containing MSNs. Chronic alcohol consumption is associated with DMS D1-MSN hyperactivity and D2-MSN hypoactivity, resulting from altered glutamatergic efferents such as the anterior cingulate cortex (ACC). Reducing the D1/D2 MSN imbalance decreases alcohol consumption. Here, we propose an electrical stimulation approach using ultra-low (≤1Hz) frequency spike-timing dependent plasticity (ULF-STDP) to reduce DMS D1/D2 MSN imbalances by repeatedly stimulating D1-MSN afferents into the GPi before the ACC glutamatergic projections to DMS.
Methods: Alcohol consumption was monitored in the limited access alcohol self-administration paradigm and the two-bottle choice paradigm. The ULF-STDP protocol consists of 10 minutes of 1 Hz stimulation with GPi stimulation occurring 18 ms prior to ACC stimulation. Evoked field excitatory post synaptic potentials, local field potentials, single units, and whole-cell excitatory postsynaptic current (mEPSC) amplitude and frequency were recorded from DMS MSNs.
Results: Our ULF-STDP D1-MSN longt-term depression (LTD) protocol reduces alcohol consumption in high alcohol-preferring animals without altering general motivated behavior ($n=7\text{-}15$, $Alpha=0.05$, $p<0.05$). Conversely, ULF-STDP D1-MSN long-term potentiation (LTP) protocol increases alcohol consumption in low alcohol preferring-animals ($n=5$, $Alpha=0.05$, $p<0.05$). Behavioral data are supported by electrophysiological evidence of changes in synaptic function showing ULF-STDP decreases mean DMS MSN firing rate, the amplitude of multiunit potentials, and mEPSC current ($n=3\text{-}5$, $p<0.05$, $Alpha=0.05$).
Conclusion: Here, we demonstrate a novel ULF-STDP electrical stimulation protocol capable of selectively modulating maladaptive plasticity associated with AUD. Specifically, ULF-STDP selectively decreases alcohol seeking and DMS D1-MSN hyperactivity in mouse models of AUD. Achieving cell-specific control of synaptic plasticity with a conventional deep brain stimulation approach has significant potential to enable the treatment of neurological conditions with historically poor treatment outcomes.

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Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01AA024109

Title: Follow your heart: the relation of autonomic nervous system function, measured through heart rate variability, in promoting alcohol drinking and anxiety-like behavior

Authors: *R. M. FRASIER, T. DE OLIVEIRA SERGIO, F. W. HOPF;
Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Alcohol Use Disorder (AUD) remains a serious public health issue, costing more than $250 billion annually in the US alone. A hallmark of AUD is compulsive alcohol drinking, defined as drinking despite predictable medical, financial, relational, and legal problems to the individual. Anxiety and stress are also intimately linked to alcohol drinking, at least in some individuals. Mounting evidence from both human and rodent literature posits that dysfunctions in autonomic nervous system (ANS) balance between the sympathetic and parasympathetic branches are likely to play a role in the expression of various psychiatric conditions, including AUD and its common comorbidities such as anxiety disorders. Heart Rate Variability (HRV), defined as the variations in time between successive heartbeats, can be assessed with a series of measures considered to be robust metrics for indicating the balance of the opposing ANS branches. In particular, higher HRV is thought to reflect parasympathetic branch dominance, while a lower HRV signifies sympathetic branch dominance. Studies herein measured the HRV in male and female rats that were either alcohol-naive or chronic alcohol drinkers (>3 months intermittent access to 20% alcohol), and tested during compulsive-like alcohol drinking (CLAD) and various paradigms of anxiety-like behavior (including Novelty Suppression of Feeding and Light-Dark Box, methods as in De Oliveira Sergio et al., 2021, *Psychopharmacology* 238:2775-2787). To date, approximately 30 rats (adult Wistar male and female) were surgically implanted with a TSE Stellar Telemetry device that provides real-time cardiovascular physiology data in awake, behaving rats. HRV measurements were taken at various baselines throughout the day, during alcohol drinking (alcohol-only and CLAD), and during anxiety-like behavior. Results thus far suggest that HRV decreases during anxiety-like testing relative to baseline, and is lower in females compared with males regardless of alcohol drinking history. Together, our studies would provide valuable insight into sex and drinking-history differences in autonomic regulation (expressed through HRV) that can impact the expression of alcohol drinking and anxiety-like behavior.

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Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

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Title: Effects of systemic treatment with glycine and leucine-glycine on accumbal glycine and dopamine levels and alcohol intake in the male Wistar rat

Authors: *Y. OLSSON, H. LIDÖ, K. DANIELSSON, M. ERICSON, B. SÖDERPALM; Inst. of Neurosci. and Physiology, Univ. of Gothenburg, Gothenburg, Sweden

Abstract: Objectives Improved pharmacotherapy for Alcohol Use Disorder (AUD), aimed at the mechanisms by which alcohol interferes with the dopamine (DA) reward pathway, is warranted. Alcohol targets glycine receptors (GlyR) in the nucleus Accumbens (nAc), among other receptors. Local (into the nAc) and systemic treatment with glycine-transporter-1-inhibitors that modulate glycine levels and local perfusion with glycine itself, elevate basal and attenuate alcohol-induced nAc DA release, pivotal for negative and positive reinforcement to drink respectively, and thereby reduce alcohol intake in the rat. Glycine treatment protocols in man require large doses and demonstrate variable brain glycine levels, probably due to impeded blood brain barrier (BBB) passage. Leucine-glycine (Leu-Gly), where glycine is anchored onto leucine that more readily passes the BBB, elevates whole brain tissue levels of DA in mice, measured *ex vivo*. In this study, the concept of elevating central glycine levels in order to reduce alcohol intake was further explored.
Materials and methods The effects of glycine (200, 400, 800 mg/kg) or Leu-Gly (1, 10, 100, 1000) i.p. on nAc glycine and DA levels were examined using *in vivo* microdialysis in Wistar rats. The effects of the intermediate dose of glycine on voluntary alcohol intake and preference were examined in a limited access two-bottle alcohol/water model in the rat.
Results Systemic glycine treatment increased nAc glycine levels in a dose-related manner whereas nAc DA levels were elevated in a subpopulation of animals, defined as DA responders. Alcohol intake and preference decreased after systemic glycine treatment. In contrast, Leu-Gly did not significantly alter nAc glycine or DA levels.
Conclusions The results from the present study give further support to the concept of elevating central glycine levels to reduce alcohol intake and indicate that targeting the glycinergic system may represent a pharmacologic treatment principle for AUD.

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Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

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Transdisciplinary Program in Translational Medicine and Therapeutics

Title: Elevated serum ferritin levels covary with gray matter iron in alcohol use disorder: a quantitative susceptibility mapping study

Authors: *A. ADAMS[1], J. BYANYIMA[1], X. LI[1], T. D. NGUYEN[4], D. D. LANGLEBEN[1], T. POND[1], Y. WANG[4], B. MOON[2], E. SWEENEY[3], H. R. KRANZLER[1], W. R. WITSCHEY[2], Z. SHI[1], C. E. WIERS[1];
[1]Psychiatry, [2]Radiology, [3]Biostatistics, Epidemiology, and Informatics, Univ. of Pennsylvania, Philadelphia, PA; [4]Radiology, Weill Cornell Med., New York, NY

Abstract: Aims: Chronic alcohol use elicits neurotoxic effects and increases cognitive decline and risk of early-onset dementia. Iron has high oxidative potential and may contribute to the pathological effects of chronic alcohol use. Elevated peripheral iron levels have been reported in individuals with alcohol use disorder (AUD), but its association with brain iron loading has not been explored. We aimed to (1) compare serum and brain iron loading in individuals with AUD and non-dependent healthy controls, and (2) examine the association between serum and brain iron loading.
Methods: Participants included individuals with AUD (n=4, 2 female; mean age=36.3±14.8SD years old, drinking > 15 drinks per week) and matched non-dependent healthy volunteers (n=4, 2 female; mean age=30.5±11.0 years old). Participants completed a serum iron panel (Labcorp) following an overnight fast and a 3T MRI scan with quantitative susceptibility mapping (QSM) to non-invasively quantify brain iron concentrations. QSM analysis was performed using the morphology enabled dipole inversion (MEDI) algorithm. QSM values were extracted from the brain gray matter as well as from four striatal regions of interest (caudate, nucleus accumbens, globus pallidus, putamen). No outliers (>2SD from the mean) were detected.
Results: As hypothesized, serum ferritin levels were higher in the AUD group than in controls ($mean_{AUD}$=149.8±78.9ng/mL, $mean_{control}$=57.5±50.1ng/mL, t_6=2.0, p=0.048). A group comparison of gray matter brain iron levels did not reach significance ($mean_{AUD}$=-14.9±6.4, $mean_{control}$=-19.1±6.6, t_6=0.9, p=0.20). Upon examination of striatal regions of interest, iron levels in the caudate were higher in the AUD group than in controls ($mean_{AUD}$=44.0±19.2, $mean_{control}$=25.3±10.9, t_6=1.7, p=0.071). There were no significant group differences in iron levels for the nucleus accumbens, globus pallidus, or putamen (all p>0.1). When the 8 subjects' serum ferritin values were pooled, they correlated positively with gray matter iron levels (r=0.550, p=0.079). Neither serum ferritin nor gray matter iron levels correlated with age (all p>0.5).
Conclusion: This is the first study to analyze both blood and brain iron loading in individuals with AUD. Despite the small sample size, substantially elevated serum ferritin levels and QSM-MRI measures of caudate iron levels were found in the AUD group compared to controls. Moreover, ferritin levels correlated positively with gray matter brain iron levels. Definite studies in larger samples are needed to examine the effects of alcohol use on iron loading and its associations with cognition and neurodegeneration.

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Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01 DA041808

Title: The ethanol metabolite acetic acid activates accumbens shell neurons via an NMDAR-dependent mechanism that is more robust in female C57BL/6J mice

Authors: *A. CHAPP[1], C. NWAKAMA[2], M. THOMAS[3], P. G. MERMELSTEIN[4]; [1]Neurosci., Minneapolis, MN; [2]Neurosci. Dept., [3]Univ. of Minnesota, Minneapolis, MN; [4]Univ. of Minnesta, Minneapolis, MN

Abstract: The role of acetic acid in alcohol use disorder (AUD) is not well-understood. Furthermore, sex-specific differences in acetic acid production and neurophysiological responses following acute ethanol exposure have not been extensively explored. In this study, we investigated sex-specific metabolism of ethanol (2g/kg) and production of acetic acid *in vivo* in C57BL/6J mice to guide our electrophysiology experiments in the accumbens shell (NAcSh), a key node in the mammalian reward circuit. We found a sex-dependent difference in ethanol metabolism and production of acetic acid in serum following acute ethanol exposure, males>females at 5 min (3.77 ± 0.23 mM vs 2.99 ± 0.25 mM) post injection, quantified via ion chromatography. *Ex vivo* electrophysiology recordings in brain slices containing the NAcSh demonstrated that acetic acid (2 mM and 4 mM) increased NAcSh neuronal excitability in both sexes. The magnitude of change from baseline excitability was greater in females compared to males at the higher concentration of acetic acid (4 mM). Furthermore, NMDAR inward currents in the presence of acetic acid (4 mM) were greater in females compared to males (-54.7 ± 16 pA vs -17.4 ± 2.8 pA). NMDAR antagonism with memantine (30 μM) significantly attenuated the effects of acetic acid (4 mM) on NAcSh neuronal excitability and NMDAR inward currents.

These findings suggest a novel NMDAR-dependent mechanism by which acetic acid may underlie the behavioral, neurophysiological and sex-dependent effects of ethanol.

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Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 230.11

Topic: G.09. Drugs of Abuse and Addiction

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Title: Ethanol-induced suppression of GIRK-dependent signaling in the basal amygdala

Authors: *E. MARRON[1], M. E. TIPPS[1], B. HAIDER[1], T. R. ROSE[1], B. N. VO[1], M. C. DEBAKER[2], K. WICKMAN[1];
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Abstract: Ethanol exposure fosters neuroadaptations that regulate mood, learning, and goal-directed behavior. The basolateral amygdala (BLA) regulates mood and associative learning and has been linked to the development and persistence of alcohol use disorder (AUD). The GABAB receptor (GABABR) is a promising therapeutic target for AUD, and previous work suggests that exposure to ethanol and other drugs can alter neuronal GABABR-dependent signaling. The impact of ethanol on GABABR-dependent signaling in the BLA was examined using slice electrophysiology following repeated ethanol injection and/or chronic intermittent exposure to ethanol vapor in mice. The relevance of ethanol-induced plasticity in the BLA was explored using tests of mood-related behavior and associative learning, combined with neuron-specific, viral genetic manipulations. The inhibitory effect of GABABR activation on principal neurons in the basal (BA) but not lateral (LA) sub-region of the mouse BLA was diminished following repeated ethanol exposure ($t_{58}=5.072$, ****$P<0.001$; N=30/group; unpaired Student's t-test). This adaptation was attributable to the suppression of the G protein-gated inwardly rectifying K+ (GIRK) channel activity. While GIRK1 and GIRK2 subunits are critical for GIRK channel formation in BA principal neurons, GIRK3 was necessary for the ethanol-induced plasticity as the adaptation was not present in GIRK3KO mice exposed to ethanol ($t_{42}=0.3170$, $P=0.7528$, N=22/group; unpaired Student's t test). In wild type mice, exposure to ethanol altered several

mood-related behaviors. In the light-dark box test ethanol-exposed animals spent less time on the light side (t22=3.208, **P=0.0041, N=12/group; unpaired Student's t-test). In the marble burying test ethanol-exposed animals buried more marbles (t22=3.174, **P=0.0044, N=12/group; unpaired Student's t-test). In the bottle-brush test ethanol-exposed animals had an increased irritability (t22=12.19, ****P=<0.0001, N=12/group; unpaired Student's t test). Importantly, all these behavioral effects were absent in ethanol exposed GIRK3KO mice: light-dark box (t16=1.719, P=0.1048, N=8-10/group; unpaired Student's t test); marble burying (t16=0.2327, P=0.8189, N=8-10/group; unpaired Student's t test); bottle-brush (t16=0.8220, P=0.4232, N=8-10/group; unpaired Student's t test). Finally, some, but not all, of these ethanol exposure-dependent behavioral effects could be mimicked in ethanol naive mice by the selective suppression of GIRK channels in BA principal neurons. Therapeutic approaches designed to prevent this plasticity and/or enhance GIRK channel activity may prove useful for treatment of AUD.

Disclosures: E. Marron: None. **M.E. Tipps:** None. **B. Haider:** None. **T.R. Rose:** None. **B.N. Vo:** None. **M.C. DeBaker:** None. **K. Wickman:** None.

Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

Location: SDCC Halls B-H

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SPARC Grant SPARC/2018- 2019/P435/SL
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Title: Dendrimer Stabilized Albumin Nanoparticles of Asiatic Acid (AA-DSANPs) Attenuates Chronic Alcohol Consumption in IA2BC Paradigm

Authors: *D. CHOUHAN[1], A. UNIYAL[2], A. .[3], V. TIWARI[4];
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Abstract: Dendrimer Stabilized Albumin Nanoparticles of Asiatic Acid (AA-DSANPs) Attenuates Chronic Alcohol Consumption in IA2BC Paradigm
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Abstract; Alcohol addiction is a serious neurological disorder which leads to significant mortality and morbidity worldwide. The currently available therapeutics for the treatment of alcohol addiction lacks proper efficacy and represent multiple side effects. The present study was designed with an aim to investigate the cellular and molecular intricacies associated with alcohol addiction and the therapeutic targeting of the same. Here, we developed the dendrimer (3G) stabilized albumin nanoparticles of asiatic acid (AA-DSANP) and evaluated the effect of the same in rat model of chronic alcohol abuse. Intermittent access to 20 % alcohol in two bottle choice (IA2BC) paradigm was used for developing alcohol addiction in male Wistar rats. We examined the role of proinflammatory cytokines and mitochondrial bioenergetics in IA2BC rats. Interestingly we found that AA (10, 20 & 40 mg/kg) and the AA-DSANPs (10 mg/kg) treatment significantly reduced the alcohol consumption as well as its preference in Wistar rats. Further, we observed that AA (40 mg/kg) and AA-DSANPs (10 mg/kg) significantly attenuated the mRNA expression of proinflammatory cytokines (IL1β, IL6, TNFα, NFKB, Caspase 3) and mitochondrial proteins (MFN1 and MFN2). Moreover, we did not observe any effect of AA and AA-DSANPs on the levels of DNM1L. Overall our behavioral and molecular findings confirmed that AA-DSANPs administration inhibited the alcohol consumption in rats. Thus, AA can be used as therapeutic alternative for the treatment of alcohol addiction.
Keywords: Alcohol Addiction; IA2BC; Asiatic Acid; Dendrimer, Mitochondrial bioenergetics; Translational Research

Disclosures: **D. Chouhan:** None. **A. Uniyal:** None. **A. .:** None. **V. Tiwari:** None.

Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

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Pearson Center for Alcoholism and Addiction Research
Drug was donated by Amgen

Title: The effects of the phosphodiesterase 10A inhibitor AMG579 on alcohol intake, locomotor activity, and place conditioning in mice

Authors: *S. RANJAN[1,3], A. G. GHOOGASIAN[1,4], L. B. BERTOTTO[1], G. MACEDO[1], A. E. PIMENTEL[1], A. J. ROBERTS[2], C. CATES-GATTO[2], E. P. ZORRILLA[1];
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Abstract: Alcohol use disorder (AUD) affects millions of Americans every year, and new therapeutic targets are needed for this chronic disorder. Alcohol use increases availability of phosphodiesterase 10A (PDE10A), a striatal enzyme that inactivates cAMP/cGMP in medium spiny neurons, implicating a possible role in alcohol intake. We tested the hypothesis that AMG579, a PDE10A inhibitor, altered alcohol (EtOH) intake. For this, C57BL/6J mice (*N*=10/sex) received 2-bottle choice (2BC) EtOH (20% *v/v*) and water access in 2-hr sessions after pretreatment (-1 hr) with AMG579 (i.p., 0 (HPßC), 55.5, 111, 222, 444 nmol/kg) in a within-subject design.To determine whether doses in this range affected locomotion, ambulation and rearing were measured for 2 hours in separate mice via a between-subject design. After AMG579 injection (i.p., 0, 10, 31.8, 100, 318, 1000, 1769 nmol/kg; *N*=8/dose, *N*=16 vehicle) mice were placed in polycarbonate cages with 2 photocell beams at the bottom of the cage at different heights, and activity was compared after a 1-hr pretreatment interval. To see if AMG579 has intrinsic aversive or rewarding properties, a place conditioning test was conducted using an unbiased 2-chamber procedure;HPßC vehicle was used as a negative control, cocaine (i.p. 10 mg/kg [~33 umol/kg]) as a positive control. Split equally across sex, mice received HPßC (*N*=8), cocaine (*N*=8), or AMG579 (10, 31.8,100 nmol/kg, *N*=16/dose). Each mouse experienced 3 pairings of drug with one of the chamber sides, and 3 pairings with vehicle in the other. AMG579 reduced EtOH intake most effectively at 111 nmol/kg (*M*+SEM: 2.71+0.45 vs. 1.57+0.35 g/kg, $p<0.05$). Suppression diminished slightly at higher doses. AMG579 reduced horizontal ambulation at 318, 1000, and 1769 nmol/kg doses. AMG579 biphasically affected rearing; lower doses (10, 31.8 nmol/kg) increased and higher doses (318, 1000, 1769 nmol/kg) decreased rearing. In contrast to cocaine, no significant place preference (or aversion) was seen at any AMG579 dose. In conclusion, AMG579 showed pharmacological activity across the entire dose range. Based on the present results we are further studying the ability of AMG579 at doses under 300 nmol/kg to reduce alcohol intake without nonspecific locomotor suppression or place conditioning effects.

Disclosures: S. Ranjan: None. **A.G. Ghoogasian:** None. **L.B. Bertotto:** None. **G. Macedo:** None. **A.E. Pimentel:** None. **A.J. Roberts:** None. **C. Cates-Gatto:** None. **E.P. Zorrilla:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Drug donated by Amgen.

Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA AA006420
NIAAA AA026999
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NIAAA T32 AA007456

Title: Blockade of orexin receptors in the infralimbic cortex prevents stress-induced reinstatement of alcohol-seeking behavior in rats with a history of alcohol dependence.

Authors: *F. J. FLORES RAMIREZ, J. M. ILLENBERGER, F. DI OTTAVIO, R. MARTIN-FARDON;
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Abstract: Alcohol use disorder (AUD) remains a treatment-elusive medical condition. A major problem in the management of AUD is the high vulnerability to relapse, even after long periods of abstinence. Chronic alcohol use dysregulates responsivity to stress, and with repeated cycles of alcohol use, stress systems become hyporesponsive, rendering individuals vulnerable to relapse. Orexin (Orx; also called hypocretin) plays a well-established role in regulating diverse physiological processes, including stress. Though exclusively produced in the hypothalamus, Orx neurons project to the infralimbic cortex (IL). The present study assesses the hypothesis that Orx transmission in the IL plays a significant role in stress-induced reinstatement of alcohol-seeking behavior in male and female rats with a history of alcohol dependence. To achieve this, animals were trained to self-administer 10% alcohol (30 min/day for 3 weeks) and were either made dependent via chronic intermittent alcohol vapor exposure (14h ON, 10h OFF) for 8 weeks or exposed to air (nondependent). Starting on week 7, the rats were submitted to extinction training (5 days/week) at 8h abstinence (or the corresponding time for the nondependent group) for 10 sessions. Following extinction training, rats received an intra-IL microinfusion of the dual Orx receptor antagonist TCS1102 (15 µg/0.5 µl/side) and were tested for footshock stress-induced reinstatement of alcohol-seeking behavior. Interestingly, TCS1102 prevented reinstatement in dependent animals, only. Furthermore, qPCR analysis showed that *Hcrtr* mRNA expression in the hypothalamus and *Hcrtr1/2* mRNA expression in the IL were increased in alcohol-dependent animals at the time of testing. Overall, these results implicate Orx transmission in the IL during stress-induced reinstatement of alcohol-seeking behavior in subjects with a history of alcohol dependence and suggest maladaptive recruitment of Orx transmission in the IL by alcohol dependence.

Disclosures: F.J. Flores Ramirez: None. **J.M. Illenberger:** None. **F. Di Ottavio:** None. **R. Martin-Fardon:** None.

Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

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Program #/Poster #: 230.15

Topic: G.09. Drugs of Abuse and Addiction

Support: Indiana Academy of Sciences
Butler University Holcomb Awards

Title: Low, but not moderate, caffeine increases alcohol intake in male and female C57 mice

Authors: M. J. EVANS, ***J. N. BERRY**;
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Abstract: Caffeine and alcohol are two of the most popular and legal drugs around the world. In America, both drugs are easily accessible, and this has led to the two drugs being used in frequent combination with one another. Previous studies have revealed much about the mechanisms of each drug, but the effects of co-consumption are not yet fully understood. The two drugs appear to have opposing effects, as caffeine is a stimulant and alcohol is a depressant, but more research is needed on their effects when combined. One main concern is that caffeine could reduce how intoxicated a person feels, leading to increased alcohol consumption or other risky behaviors. Mice have previously been used to study alcohol, caffeine, and their combination. This previous research is not conclusive, but does suggest the potential for dose-dependent effects of caffeine when combined with alcohol. This experiment intended to compare the consumption rates of caffeine and alcohol and to observe the withdrawal effects of these two drugs. Specifically, varying levels of caffeine and alcohol were examined to see if the effects of the combination is dependent upon dose. C57BL/6J mice (*n*=48) were provided with caffeine (0.015% or 0.03%) and/or alcohol (3-20%) in a two-bottle intermittent access voluntary paradigm. Fluid consumption was recorded daily for five weeks and mice were then individually tested in an elevated plus maze (EPM) to assess anxiety-like behaviors during withdrawal. Although small amounts of caffeine alone were consumed throughout the study, the amount of alcohol consumed steadily increased until the highest concentration (20%) was presented. Both sexes consumed the most alcohol when it was paired with the lower dose of caffeine (0.015%) compared to either alcohol alone or alcohol in combination with the higher dose of caffeine (0.03%) and consumed more caffeine at each concentration when it was paired with alcohol. Overall, less consistent consumption patterns were observed in female mice. The results from the EPM revealed no significant difference between experimental groups and no differences between male and female mice. These results suggest that a specific amount of caffeine may be necessary to impact alcohol consumption in mice. More research is necessary to determine the psychological effects of withdrawal from these drugs.

Disclosures: M.J. Evans: None. **J.N. Berry:** None.

Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA Grant P50AA022537
NIAAA Grant R01AA027581

Title: Preclinical assessment of tideglusib, a selective GSK3B inhibitor, as a treatment for alcohol use disorder

Authors: *S. GOTTLIEB[1], A. VAN DER VAART[2], D. BLEDSOE[2], A. MORGAN[2], J. MALTMAN[2], J. WOLSTENHOLME[2], M. MILES[2];
[1]Virginia Commonwealth Univ. Neurosci. Grad. Program, Richmond, VA; [2]Virginia Commonwealth Univ., Richmond, VA

Abstract: Background: Prior studies from our laboratory show changes in glycogen synthase kinase 3 beta (GSK3B) abundance or activity regulate ethanol (EtOH) consumption, suggesting potential as a therapeutic target in alcohol use disorder (AUD). Here we report actions of the highly selective GSK3B inhibitor, tideglusib, on EtOH consumption, basal behaviors, and modulation of GSK3B targets. Since tideglusib has been approved for clinical trials on several neurological disorders, these studies have implications for treatment of AUD. **Methods:** C57BL/6J males and females given i.g. 200mg/kg tideglusib, except EtOH pharmacokinetics (males only) and drinking-in-the-dark (males;100mg/kg i.p.). *Intermittent ethanol access (IEA)*: Mice underwent IEA x 6wks. Every other day, one of two water bottles replaced with 20% EtOH for 24hr. 2hr and daily EtOH consumption and preference were calculated. Tideglusib or vehicle began week 6 and drinking behaviors compared to baseline were assessed. *Drinking-in-the-dark (DID)*: Mice given 20% EtOH 4hrs, 4d/wk x 3wks and then i.p. tideglusib or vehicle x 4 d in a Latin Square design with EtOH consumption measured daily. *Light/Dark Box*: Mice gavaged with tideglusib or vehicle and i.p. injected with 1.8g/kg EtOH or saline then tested for 10min. *Taste Preference*: Mice received tideglusib x 6d and then tested daily for saccharin or quinine taste preference. *Pharmacokinetics*: Mice treated with tideglusib or vehicle and 30min later i.p. injected with 2.0g/kg EtOH. Blood EtOH content measured 10, 30, 60, and 90min post-EtOH. *Western Blots*: Mice given tideglusib or vehicle 3x/wk x 2wks and mPFC assayed for phosphorylated and total GSK3B, Dynamin1, and PSD-95. **Results:** Tideglusib decreased binge IEA and DID consumption, transiently increased locomotion, and had no effect on anxiety-like behaviors, taste preference or EtOH pharmacokinetics. Females showed tideglusib-induced increased Dynamin1 and decreased pDynamin1/total Dynamin1. **Conclusion:** Tideglusib is a promising AUD therapeutic, decreasing EtOH consumption in a model of progressive alcohol consumption and binge-drinking model. Tideglusib is likely not reducing consumption by altering taste, anxiety-like behaviors, or EtOH metabolism. Dynamin1 is integral in activity-dependent bulk endocytosis and requires GSK3B for rephosphorylation. Tideglusib increased Dynamin1 levels may represent a compensatory response to decreased GSK3B activity, providing insight to tideglusib's mechanism in EtOH behaviors.

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Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

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Program #/Poster #: 230.17

Topic: G.09. Drugs of Abuse and Addiction

Title: A combination of amylin and GLP-1 receptor agonists synergistically reduce alcohol intake in male and female rats

Authors: *C. ARANÄS, C. E. EDVARDSSON, J. VESTLUND, L. ZENTVELD, E. JERLHAG;
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Abstract: Alcohol use disorder (AUD) is a severe neuropsychiatric disorder with high mortality and morbidity rates. Existing pharmacotherapies are often insufficient and patients readily relapse, thus novel treatment options are urgently needed. Our research suggests gut-brain peptides as such candidate. Specifically, the anorexigenic gut-brain peptides amylin and glucagon-like peptide-1 (GLP-1), independently reduces alcohol intake. Recent advances show that a combination of amylin receptor (AMYR) and GLP-1 receptor (GLP-1R) agonists synergistically decreases food intake, however the effect on alcohol intake is unknown. The present study therefor explores the hypothesis that the combination of the AMYR and GLP1R agonists, salmon calcitonin (sCT) and dulaglutide reduces alcohol consumption in rats. By means of the intermittent alcohol model the ability of sCT and dulaglutide, acute or repeatedly, to reduce alcohol intake was explored. Firstly, we showed that an acute combination treatment decreases alcohol intake in male, but not female rats. Secondly, repeated combination treatment administrated in a stepwise paradigm decline alcohol intake in both sexes. Thirdly, the combination of doses without an effect *per se* when administrated as monotherapies, decreases alcohol intake synergistically in male rats. Altogether, sCT and dulaglutide works synergistically on alcohol intake, we therefor suggest that the combination therapy should be evaluated as a potential treatment option for obese AUD patients.

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Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

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Program #/Poster #: 230.18

Topic: G.09. Drugs of Abuse and Addiction

Support: PAPIIT IN301717

Title: Oral ethanol self-administration in rats is modulated by intra-accumbal administration of M1 muscarinic acetylcholine receptors ligands

Authors: *J. C. JIMÉNEZ[1], R. RUIZ-GARCIA[2], D. E. HERNANDEZ-CASTILLO[2], F. CORTES-SALAZAR[3], A. BARRIENTOS-NORIEGA[3], F. MIRANDA[3];
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Abstract: INTRODUCTION. The mesocorticolimbic dopamine (DA) system plays a key role in mediating addictive effects of ethanol (EtOH). This system is comprised of the DAergic neurons in the ventral tegmental area (VTA) that project their axons to nucleus accumbens (nAcc), prefrontal cortex, amygdala and other limbic structures. Although mesocorticolimbic DA system is the main neurochemical substrate for regulating the addictive effects of EtOH, other neurotransmitter systems modulate dopaminergic function in nAcc such as acetylcholine. Acetylcholine actions are induced through binding to nicotinic and muscarinic receptors, and the M1 muscarinic acetylcholine receptor is expressed in various regions of the forebrain including the nAcc and could play a role in the behavioral effects of drug of abuse. For example, some studies have been reported that administration of a M1 acetylcholine receptor antagonist reduced rewarding properties of cocaine and morphine. The present study was designed to assess the effects of intra-accumbal administration of the M1 muscarinic acetylcholine receptor ligands on the oral self-administration of EtOH in rats. METHOD. Male Wistar rats (250-300 g) were used. Rats were water deprived for 24 h, and then trained a lever-press for water reinforcement on a FR1 schedule by 3 days. Then, rats were trained to lever-press for EtOH (0.01 ml of EtOH in water at 12%) on a FR1 schedule by 3 days. After this training, the reinforcement contingency was changed to FR3 for EtOH access until response rate remained stable at 80%. After this behavioral training, rats received intra-accumbal injection of M1 muscarinic acetylcholine receptor agonist carbacol (1.25, 2.5 y 5.0 μg) or M1 muscarinic acetylcholine receptor antagonist pirenzepine (8.75, 17.5 y 35.0 μg) or the combination of carbacol (5.0 μg) + pirenzepina (8.75, 17.5 y 35.0 μg). Each dose per session test, cannulae were implanted at nAcc shell (AP +2.0 mm of Bregma, ML ± 0.8 mm, DV -4.5 mm). RESULTS. The data showed that intra-accumbal injections of carbacol increased oral self-administration of EtOH, while pirenzepine reduced the oral self-administration of EtOH. These findings suggest that M1 muscarinic acetylcholine receptor ligands modulate the oral self-administration of EtOH in rats.This study was supported by PAPIIT IN301717 (UNAM, Mexico).

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Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

Location: SDCC Halls B-H

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Program #/Poster #: 230.19

Topic: G.09. Drugs of Abuse and Addiction

Title: Pharmacological Evidence for a Functional Role of The Ghrelin System in Binge-like Alcohol Drinking

Authors: *R. RICHARDSON[1,2,3,4], G. F. KOOB[4], L. VENDRUSCOLO[4], L. LEGGIO[1,5,6,7,8]; [1]Clin. Psychoneuroendocrinology and Neuropsychopharm. Section, Translational Addiction Med. Branch, Natl. Inst. on Drug Abuse Intramural Res. Program and Natl. Inst. on Alcohol Abuse and Alcoholism, Baltimore, MD; [2]Univ. of North Carolina MD/PhD Program, Chapel

Hill, NC; [3]Univ. of North Carolina Dept. of Cell Biol. and Physiol., Chapel Hill, NC; [4]Neurobio. of Addiction Section, Natl. Inst. on Drug Abuse Intramural Res. Program, Baltimore, MD; [5]Brown Univ. Dept. of Behavioral and Social Sciences, Ctr. for Alcohol and Addiction Studies, Providence, RI; [6]Medication Develop. Program, Mol. Targets and Medications Discovery Branch, Intramural Res. Program, Natl. Inst. on Drug Abuse, Baltimore, MD; [7]Johns Hopkins Univ. Sch. of Medicine, Div. of Addiction Medicine, Dept. of Med., Baltimore, MD; [8]Georgetown Univ. Med. Center, Dept. of Neurosci., Washington, DC

Abstract: Alcohol use disorder (AUD) is a chronic and relapsing neuropsychiatric disease characterized by impaired ability to stop or control unhealthy alcohol use. It is a highly prevalent public health issue and excessive alcohol use costs the US about $250 billion a year. Binge drinking is very common, harmful, and is an important step in the AUD spectrum. Yet, there are only a few Food and Drug Administration (FDA)-approved medications for the treatment of AUD. It is, therefore, important to develop and identify new medications to treat AUD. Towards that end, we investigated the effects of novel compounds that target the ghrelin system on binge-like drinking in a mouse model of AUD. Ghrelin is a stomach-derived peptide hormone with known roles in regulating appetite and food intake. Ghrelin receptors (GHSR-1a) are expressed both in the brain and the periphery. Recent studies show that the ghrelin system is also implicated in alcohol-related behaviors. For example, ghrelin administration increases alcohol intake, whereas blockade of GHSR-1a with specific antagonists such as JMV 2959 decreases alcohol intake. Most of these studies utilized male subjects only and did not measure binge-like drinking. “Drinking in the Dark” (DID) is a model of binge drinking that has been validated in C57Bl6 mice. Mice are given access to alcohol for a short time each day, and this has been shown to lead to pharmacologically significant levels of blood alcohol concentration during a discrete time period. In the present study, we measured the effects of the prototype GHSR-1a antagonist JMV 2959, of four additional novel ghrelin receptor blockers (PF-5190457, PF-6870961, HM-04, YIL-781) and of the endogenous GHSR-1a antagonist LEAP-2, for their ability to reduce binge drinking in female and male mice. We hypothesized that pharmacological blockade of GHSR-1a would decrease binge-like alcohol intake in mice of both sexes. We used 8 male and 8 female C57Bl6 mice of 11 weeks of age in all of the behavioral experiments. A within-subjects, Latin-square design was also employed. Alcohol intake was measured in g/kg of body weight and results were analyzed by two-way ANOVA. Our results showed that systemic administration of JMV 2959, PF-5190457, PF-6870961, and HM-04 reduced alcohol intake in mice of both sexes ($p < 0.05$). YIL-781 reduced intake in male but not female mice. LEAP-2 had no effect on alcohol intake. Our observations provide novel information that supports the role of the ghrelin system in binge drinking and identify that system as a potential target for pharmacological interventions against AUD. Future clinical studies will test the potential relevance of our observations in patients with AUD.

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Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

Location: SDCC Halls B-H

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Program #/Poster #: 230.20

Topic: G.09. Drugs of Abuse and Addiction

Title: Effects of delta-8 THC on ethanol consumption in adolescent mice

Authors: E. ANDERSON, E. KIELSA, A. LUND, C. MUELLER, ***S. DICKINSON**;
St Olaf Col., Northfield, MN

Abstract: The shifting legal status of marijuana across the United States has been accompanied by a surge of interest in delta-8 tetrahydrocannabinol (delta-8 THC, or simply delta-8), a psychoactive substance produced naturally in low amounts in the cannabis plant. Concentrated amounts of delta-8 are typically manufactured from hemp-derived cannabidiol (CBD). While the legal status of delta-8 varies from state to state, it seems to be readily available in vape "juice" and in a variety of edibles. Livingston et al. (2022) found that internet searches including the words 'delta-8 THC' grew sevenfold from 2020 to 2021, and unpublished surveys of students on multiple college campuses report high levels of recreational use, often in combination with alcoholic beverages. Although a substantial number of studies in the past 15 years have examined interactions between marijuana and ethanol, the literature on delta-8 dates primarily from the 1970s.
To investigate the effect of delta-8 exposure on ethanol intake, three groups of adolescent female Swiss Webster mice were individually housed on a 12 h L:D cycle. Thirty minutes prior to lights out, mice were fed a quantity of peanut butter infused with delta-8 THC calculated to achieve doses of 10 mg/kg or 25 mg/kg, or regular peanut butter as a control. Three hours into the dark cycle a drinking in the dark (DID) protocol was implemented. During multiple 4-day runs of the DID protocol, water bottles were replaced with sipper tubes containing 20% ethanol or ethanol plus saccharin. As expected, ethanol consumption was generally low in these Swiss Webster mice. Adding saccharine and/or increasing the duration of sipper tube exposure increased ethanol consumption in all mice. In general, mice who received 25 mg/kg delta-8 drank less ethanol than did control mice, although group differences did not reach statistical significance.

Disclosures: E. Anderson: None. **E. Kielsa:** None. **A. Lund:** None. **C. Mueller:** None. **S. Dickinson:** None.

Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 230.21

Topic: G.09. Drugs of Abuse and Addiction

Support: Center for Medicinal Cannabis Research P64-01-004 (GdG)

Title: Longitudinal assessment of the effects of cannabidiol over the different stages of addiction in rat models of alcohol use disorder

Authors: *S. DIRIK, A. MARTINEZ, C. CROOK, R. QIAO, P. SCHWEITZER, M. KALLUPI, G. DE GUGLIELMO;
UC San Diego, La Jolla, CA

Abstract: Cannabidiol (CBD), a non-psychoactive constituent of the cannabis plant, has received attention for its potential to decrease drug and alcohol use given its anti-inflammatory, antioxidant, and neuroprotective effects. Here we used a multidisciplinary approach, combining state-of-the-art behavioral models, immunohistochemistry, and electrophysiology to evaluate the effects of chronic (60 mg/kg/day) CBD treatment in several alcohol-related behaviors and on the alcohol-induced neurodegeneration in alcohol dependent rats. We used two different animal models to induce alcohol dependence in rats: the chronic intermittent ethanol vapor exposure (CIE) model and the recently developed ethanol vapor self-administration model (EVSA). The new EVSA model highlights the volitional aspects of alcohol dependence. We found that chronic CBD treatment prevents the development of alcohol dependence in the EVSA model, by reducing alcohol-induced neurodegeneration in the nucleus accumbens shell (NAcSh) and the dorsomedial striatum (DMS). In animals treated after the establishment of alcohol dependence (CIE model), CBD reduces alcohol drinking, somatic and emotional signs of withdrawal. Finally, the treatment was also effective in reducing alcohol seeking and stress-induced reinstatement, possibly by reverting the reduction of neuronal excitability induced by alcohol in the basolateral amygdala (BLA). These results extend to the current literature and indicate a profile of potential benefits of CBD for the treatment of alcohol dependence.

Disclosures: S. Dirik: None. **A. Martinez:** None. **C. Crook:** None. **R. Qiao:** None. **P. Schweitzer:** None. **M. Kallupi:** None. **G. De Guglielmo:** None.

Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 230.22

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA041808

Title: Investigation of Sex Differences in Ethanol Metabolism and Acetic Acid Production in C57BL/6J Mice

Authors: *C. A. NWAKAMA[1,2], A. D. CHAPP[1,2], P. G. MERMELSTEIN[1,2], M. J. THOMAS[1,2];
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Abstract: Ethanol is a widely used and abused substance throughout the world. Known for its effects of euphoria, stress reduction, and the lowering of inhibitions, uncontrolled use and overconsumption of ethanol can develop into Alcohol Use Disorder. Upon consumption, ethanol is metabolized first to acetaldehyde and then to acetic acid. Previous work out of our lab suggests rapid, excitatory modulation of accumbens shell neurons from acetic acid, raising the intriguing possibility that acetic acid, not ethanol may drive alcohol misuse. The rapid conversion of ethanol to acetic acid could correspond to an increased abuse potential. The literature alludes to a propensity for females to display a higher sensitivity to ethanol and a tendency to metabolize ethanol at an appreciated rate compared to males. Thus, we were motivated to compare changes in ethanol-induced acetic acid concentrations between the sexes. Male and female C57BL/6J mice received a single intraperitoneal injection of ethanol (2g/kg) or saline and were sacrificed at varying time points (5 minutes, 15 minutes, 30 minutes, 1 hour, and 4 hours). Blood serum, in addition to brain tissue from the nucleus accumbens (NAc) and ventral tegmental area (VTA) were collected and analyzed for acetic acid concentrations using ion chromatography. Blood serum analysis revealed distinct time points to peak acetic acid (1 hour in males vs 5 minutes in females), 3.88 ± 0.38 mM vs 2.99 ± 0.25 mM, with a magnitude of change from baseline to peak concentrations of 2.25 mM and 1.96 mM respectively. Sex-dependent variations in acetic acid concentrations were also apparent in the NAc and VTA. The magnitude of change from baseline to peak concentrations in the NAc was 1.65 µmole/g tissue in males and 1.19 µmole/g tissue in females. The VTA magnitude of change from baseline to peak concentrations was 0.40 µmole/g tissue in males and 2.92 µmole/g tissue in females. We did not detect any differences in ethanol-induced acetic acid levels across the estrous cycle. This hints at the possibility of a different, non-hormonal-based mechanism
mediating the sex difference in ethanol-induced acetic acid production that will need to be explored in the future. Overall, our findings suggest that sex differences in ethanol-induced behaviors and pathologies may depend on the rate of acetic acid production rather than its absolute concentration.

Disclosures: C.A. Nwakama: None. **A.D. Chapp:** None. **P.G. Mermelstein:** None. **M.J. Thomas:** None.

Poster

230. Cellular Mechanisms and Pharmacological Treatment of Alcohol Use

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 230.23

Topic: G.09. Drugs of Abuse and Addiction

Support: VA Merit Grant I01 BX005367-01A2
NIH Grant 1R01AA024526-01A1

Title: Therapeutic mechanisms of CDPPB on neurocognitive deficits in a rodent model of PTSD/AUD comorbidity

Authors: *L. J. WILLS[1], B. SCHWARTZ[2], B. MCGUFFIN[3], J. T. GASS[4];
[2]Biomed. Sci. Dept., [3]Dept. of Biomed. Sci., [4]Biomed. Sci., [1]Quillen Col. of Med., Johnson City, TN

Abstract: Alcohol use disorder (AUD) is the leading cause of substance use disorders among Veterans and 55 to 75% of the population that are diagnosed with PTSD also receive a comorbid diagnosis of AUD. The co-diagnosis of PTSD/AUD is associated with neurocognitive changes such as increased impulsivity and risk-taking behavior, especially among individuals with combat-related trauma. Furthermore, increased neuroinflammation in subregions of the prefrontal cortex (PFC) are suggested to contribute to these neurocognitive changes. To better understand the cognitive deficits associated with co-occurring PTSD/AUD we incorporated a probabilistic discounting task (PDT) to model risk-based decision-making in male and female Wistar rats that were exposed to restraint stress (RS) and chronic intermittent ethanol exposure (CIE). Following RS and CIE, rats underwent lever press training through a series of different training phases, in which one lever delivered a small reward 100% of the time, and the other a large reward, delivered with descending probability each trial block. Pressing the large-reward lever during the final two trial blocks when it is disadvantageous to do so is considered "risky" behavior. A week prior to PDT, rats were treated prophylactically with CDPPB, a positive allosteric modulator of the metabotropic glutamate type 5 (mGlu5) receptor, to determine if the cognitive deficits caused by stress and alcohol exposure could be prevented. Additionally, to determine if our model mimicked the neuroinflammatory mechanism seen in the human condition and the therapeutic effects of CDPPB, we assessed TNF-α protein expression in a subset of rats. Our results indicated that male rats exposed to RS and CIE had significantly greater responding during the 3rd, 4th, and 5th risk blocks compared to all other groups. However, the administration of CDPPB reversed this effect. Females exposed to RS and CIE only displayed increased risky behavior at the highest risk block and this was also blocked with the administration of CDPPB. We also determined that RS and CIE significantly increased TNF-α levels in the IfL cortex compared to either RS or CIE alone and the prophylactic administration of CDPPB reduced TNF-α protein expression to control animal levels. In the present study, we demonstrate that exposure to stress and chronic alcohol leads to significant neurocognitive deficits resulting in increased risky decision-making, but these deficits can be attenuated through modulation of the mGlu5 receptor prior to behavioral testing. Additionally, these deficits could be due to deleterious neuroinflammation in subregions of the PFC.

Disclosures: L.J. Wills: None. **B. Schwartz:** None. **B. McGuffin:** None. **J.T. Gass:** None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 231.01

Topic: G.09. Drugs of Abuse and Addiction

Support: Stillman Drake funding to P.J.C.

Title: Mesolimbic peptidergic signaling in alcohol reward

Authors: *A. M. BRIGANDE[1,2], J. GUSS DARWICH[2], P. J. CURRIE[2];
[1]Neurobio., Duke Univ., Durham, NC; [2]Psychology, Reed Col., Portland, OR

Abstract: Emerging evidence suggests that brain- and gut-brain-derived peptides not only play a coordinated role in metabolic and feeding-homeostatic mechanisms but also impact reward and reward salience as part of their prescribed roles in mesolimbic functioning. We investigated three key neuropeptides of interest due to their known action on ventral tegmental area (VTA) dopamine neurons: ghrelin, neuropeptide Y (NPY), and glucagon-like peptide-1 (GLP-1). Rats were habituated to a 6% alcohol solution using a two-bottle limited access paradigm with alcohol provided during the nocturnal cycle. Guide cannulae were surgically implanted to target the VTA. In an initial series of experiments, the GLP-1 receptor agonist Exendin-4 (Ex-4) was pre-administered to rats receiving either ghrelin or NPY. Control rats received VTA injections of saline. We examined the effects of both threshold and subthreshold peptide dosing and found that Ex-4 reliably attenuated the effect of a threshold dose of ghrelin or NPY and, in addition, suppressed the effect of combined subthreshold doses of ghrelin and NPY. Finally, to extend results of these peptidergic interactions beyond reward salience for alcohol, to include palatability, separate groups of rats received the same pretreatments after training on a progressive ratio 3 (PR3) schedule to operantly respond for sucrose pellets. These data were consistent with our alcohol findings, suggesting that ghrelin, NPY, and GLP-1 may interact physiologically within the VTA in the control of alcohol and food reward.

Disclosures: A.M. Brigande: None. **J. Guss Darwich:** None. **P.J. Currie:** None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 231.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH RO1 AA026598 (AML);
NIH/NIDA T32 DA007097

Title: Regulation of alcohol reward by cholinergic midbrain nuclei

Authors: *S. MULLOY[1], E. M. ABACK[1], K. PAWASKAR[1], C. WIN[1], A. M. LEE[2];
[1]Univ. of Minnesota- Twin Cities, Minneapolis, MN; [2]Pharmacol., Univ. of Minnesota, Minneapolis, MN

Abstract: Cholinergic signaling in the ventral tegmental area (VTA) is required for dopamine-mediated alcohol reward, and the only cholinergic input to the VTA is the cholinergic midbrain nuclei- The pedunculopontine tegmental nucleus (PPN) and laterodorsal tegmental nucleus (LDT). Pharmacological inactivation of the PPTg reduces alcohol self-administration and PPTg

lesions block alcohol conditioned place preference, suggesting that the PPN regulates alcohol behaviors. However, *the cell-types underlying these behaviors are still unknown, and the contribution of cholinergic PPN & LDT neurons in alcohol-related behaviors have not been directly investigated*. We hypothesize that the cholinergic neurons of the PPN & LDT will be activated by alcohol administration, increase in activation across chronic alcohol exposure, and that inhibition of PPN cholinergic neurons will reduce alcohol consumption. To investigate the PPN & LDT cell-type activation by acute and chronic alcohol exposure, male and female C57BL/6J mice (n=3/group/sex) were injected i.p. daily with 2.0 g/kg, 4.0 g/kg alcohol, or saline for 1-15 days. Immunohistochemistry co-expression of c-fos activation and cholinergic markers was analyzed blinded using ImageJ. Activation of the non-cholinergic neurons across 15 days of i.p. alcohol or saline injections was identified using RNAscope to measure co-expression of c-fos with cholinergic, glutamatergic, and GABAergic mRNA transcripts and analyzed using cell-profiler software. Our results show that both cholinergic and non-cholinergic neurons of the PPN & LDT are activated by alcohol compared to saline controls, and that the proportion of activated cholinergic and non-cholinergic neurons changes between 1, 5, and 15 days of exposure. To determine the contribution of PPN & LDT cholinergic projections to the VTA on alcohol consumption, we used a viral targeting strategy to exclusively express an inhibitory DREADD on the cholinergic neurons of the PPN & LDT that project to the VTA in female C57BL/6J mice (n=12/group/sex). Chemogenetic inhibition of these cholinergic projections on day 5 and 15 of a 15-day 15% ethanol limited access model reduces alcohol consumption in females, which persists for 2 days after inhibition. Together, these data suggest that PPN & LDT cholinergic neurons regulate alcohol consumption, and is a promising avenue for future alcohol research.

Disclosures: S. Mulloy: None. **E.M. Aback:** None. **K. Pawaskar:** None. **C. Win:** None. **A.M. Lee:** None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 231.03

Topic: G.09. Drugs of Abuse and Addiction

Support: R37 AA015614
P50 AA022534

Title: Effect of Binge Ethanol Exposure at Postnatal Day 7 on Neuronal Numbers in the Anterior and Reticular Thalamic Nuclei of Adult Mice.

Authors: *S. MAYFIELD, G. CHAVEZ, C. BIRD, C. VALENZUELA;
Univ. of New Mexico, Univ. of New Mexico Dept. of Neurosciences, Albuquerque, NM

Abstract: Fetal alcohol exposure is a pervasive cause of intellectual disability. The range of effects caused by prenatal ethanol exposure are grouped under the umbrella term, Fetal Alcohol

Spectrum Disorders (FASDs). Exposure to alcohol during any period of pregnancy can damage the fetal brain, including the last trimester of pregnancy, where the brain growth spurt takes place. Ethanol exposure during late pregnancy produces learning and memory deficits that can last into adulthood. Studies suggest that these deficits may be a consequence of damage to the posterior limbic memory system. This system includes specialized neuronal populations in several connected regions: the subiculum, retrosplenial cortex, mammillary bodies and anterior thalamic nuclei. The excitability of anterior thalamic nuclei neurons is regulated by inhibitory neurons located in the thalamic reticular nucleus. Research from multiple laboratories has demonstrated that binge-like ethanol exposure during the third trimester equivalent of human gestation activates apoptotic pathways in the thalamus of rodents. In this study, we tested the hypothesis that third trimester-equivalent ethanol exposure would result in a long-lasting reduction of neuronal numbers in the anterior thalamic nuclei and/or thalamic reticular nucleus. To test this hypothesis, we exposed VGAT-Venus mice (expressing fluorescently tagged GABAergic interneurons) at postnatal day 7 (P7) to heavy, binge-like ethanol in vapor chambers (blood ethanol level ~0.4 g/dl; ~90 mM). Pups were left undisturbed until adolescence (~P60-70) when their brains were processed for immunohistochemistry with an antibody against the neuron-specific antigen, NeuN. Neuronal numbers were quantified exhaustively in a blind fashion, in images obtained with a Nuance spectral imaging system, using the point selection tool in Fiji (NIH Image J software). We found that ethanol exposure did not have a statistically significant effect on neuronal numbers in the anterodorsal, anteroventral, or reticular thalamic nuclei of either male or female mice group. Ongoing studies are investigating whether neurons in these thalamic nuclei display functional alterations that may contribute to the persistent learning and memory deficits caused by binge-like ethanol exposure during the mouse equivalent to the last trimester of human pregnancy.

Disclosures: **S. Mayfield:** None. **G. Chavez:** None. **C. Bird:** None. **C. Valenzuela:** None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

Location: SDCC Halls B-H

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Program #/Poster #: 231.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA, R01-AA026844
NIAAA, R01-AA013892

Title: Neural correlates of alcohol use disorder and treatment-related recovery

Authors: *J. S. MARTINS[1], N. ERINGROS[2], R. SINHA[3], D. SEO[4];
[1]Yale Univ., Yale Univ., New Haven, CT; [2]Yale Med. Sch., Yale Med. Sch., New Haven, CT; [3]Yale Univ. Sch. Med., Yale Univ. Sch. Med., New Haven, CT; [4]Yale Sch. of Med., Yale Sch. of Med., New Haven, CT

Abstract: Background: Chronic stress precipitates the development of alcohol use disorder (AUD), which is associated with altered neural responses to stress and alcohol cues. Yet, the extent to which these alterations are restored with treatment and whether stress intervention helps recover these functions remain unclear. Using functional magnetic resonance imaging (fMRI), the current study investigated altered neural patterns and correlates of recovery using standard cognitive-behavioral treatment combined with breathing-based stress reduction. Methods: Thirty demographically and clinically matched AUD patients (AUD) and 55 moderate drinkers (MD) (Mage=32.3, 43 females). Participants completed an fMRI task in which stress, alcohol, and neutral-relaxing cues were presented in a randomized block design, and craving and stress ratings were collected. After the scan, AUD patients underwent an 8-week outpatient treatment and were daily assessed for alcohol intake, craving, stress, and stress management using a smartphone app. A subset of AUD patients (n=17) also completed a second fMRI after treatment with the same task using a different set of pictures with emotional intensity commensurable to the first scan. Results: All fMRI results were whole-brain FWE corrected at $p<.001$ and cluster-corrected at $p<.05$. In the initial fMRI, AUD vs. MD showed greater alcohol craving during alcohol ($p<.001$) and stress ($p=.006$) and hypoactive ventromedial prefrontal cortex (VmPFC) but hyperactive limbic responses (amygdala, hippocampus, and thalamus) to stress and alcohol cues. When comparing initial vs. post-treatment fMRIs among AUD, craving ratings were reduced during stress ($p=.002$) and alcohol cues ($p<.001$), along with reduced stress ratings during stress ($p=.002$) after treatment. Additionally, amygdala and insula responses to stress and alcohol cues were reduced but VmPFC and subgenual anterior cingulate cortex (sgACC) responses were increased during stress and alcohol cues, respectively. VmPFC recovery during stress was associated with greater improvements in stress management ability during treatment ($r=.71$, $p<.001$). Conclusions: AUD patients have altered neural circuits of stress and emotion regulation, marked by decreased VmPFC, and sgACC but increased limbic responses, a neural pattern that appears to improve after treatment. Especially, the recovered VmPFC responses were associated with greater improvements in stress regulation ability during treatment. Our findings suggest that targeting stress dysfunction with behavioral treatment may help restore altered neural brain functions in AUD patients and promote better treatment outcomes.

Disclosures: **J.S. Martins:** None. **N. Eringros:** None. **R. Sinha:** None. **D. Seo:** None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA034696
NIH Grant R01 AA027544
NIH P30 DA048742-01A1

Title: Plasticity of GABAergic signaling in VTA GABA neurons following chronic intermittent ethanol exposure

Authors: *E. H. MITTEN;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Chronic ethanol exposure produces a variety of molecular adaptations within the ventral tegmental area (VTA), with investigations largely focusing on VTA dopamine (DA) neurons. VTA gamma-aminobutyric acid (GABA) neurons are the second largest neuron population of the VTA. VTA GABA neurons regulate the excitability of VTA DA neurons as well as other neuronal populations through local and long range projections. An accumulation of evidence suggests that VTA GABA neurons exhibit increased activity following chronic ethanol exposure. While increased glutamate neurotransmission has been shown to contribute to this phenotype, adaptations to inhibitory signaling have yet to be fully elucidated. To investigate plasticity of GABAergic signaling in VTA GABA neurons, male and female GAD67-eGFP mice (C57BL/6) were subjected to 3-4 weeks of chronic intermittent ethanol vapor exposure (CIE). Subsequently, slice electrophysiological experiments targeting VTA GABA (GFP-positive) neurons were performed 1-7 days following the final day of exposure. Animals exposed to air were used as controls. All data were separated by sex and analyzed by two-way ANOVA to assess for sex differences. If no sex differences were detected, data was pooled and analyzed using a Kolmogorov-Smirnov test or Welch's t-test, as appropriate. We found that CIE suppressed the frequency ($p = < 0.0001$) and potentiated the amplitude ($p = 0.0375$) of spontaneous GABA(A) receptor-dependent inhibitory postsynaptic currents in VTA GABA neurons ($n = 13$-14). GABA(A)-evoked somatodendritic whole cell currents were not altered by CIE (3 uM muscimol, $p = 0.5134$, $n = 12$-14; 30 uM muscimol, $p = 0.7630$, $n = 12$-14), suggesting that postsynaptic GABA(A) channel density is unaffected. In contrast, GABA(B)-evoked somatodendritic whole cell currents were significantly reduced following CIE (200 uM baclofen $p = 0.0007$, $n = 14$-16). Work from our group and others has established GIRK channels as critical mediators of GABA(B) receptor dependent signaling. In addition, GIRK channels are thought to be direct targets of ethanol's action, with the GIRK3 subunit being involved in ethanol-induced plasticity in other neuron populations. Therefore, we assessed GABA(B)-evoked currents in GIRK3 KO mice following CIE. Preliminary results suggest that GIRK3 KO mice that underwent CIE do not exhibit a reduction in GABA(B)-evoked currents. Ongoing investigations are directed at identifying presynaptic contributions that would produce a reduction in sIPSC frequency, further elucidating the role of GIRK3 in the loss of GABA(B)-evoked currents, and the behavioral relevance of these forms of ethanol-induced neuroplasticity.

Disclosures: E.H. Mitten: None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 231.06

Topic: G.09. Drugs of Abuse and Addiction

Support: R01AA027213

Title: Recruitment of the central nucleus of the amygdala during an ethanol-rewarded operant task

Authors: *M. CASTRO[1], T. DONG[2], D. J. OTTENHEIMER[3], P. JANAK[2];
[1]Johns Hopkins Univ., [2]Psychological & Brain Sci., Johns Hopkins Univ., Baltimore, MD;
[3]Dept. of Biol. Structure, Univ. of Washington, Seattle, WA

Abstract: Alcohol (EtOH) use disorder (AUD) is a common issue in the United States, affecting at least 17 million people per year. The central nucleus of the amygdala (CeA) is proposed to contribute to dysregulated alcohol intake. While primarily implicated in promoting drinking in response to aversive properties of EtOH withdrawal, the CeA is also implicated in EtOH consumption initiated by positive reinforcement mechanisms. Previously, we found that a subpopulation of CeA neurons was modulated during EtOH consumption in a free-drinking task. Here we sought to determine if CeA neurons are also active during the appetitive phase of reward seeking. As such, we used a discrete trials task in which rats (n=6 4F/2M, experiments start at age>P60) had to make 3 lever presses within 60 sec of lever insertion (DT3) after which the lever was retracted and 0.2 ml 10% EtOH was delivered in the adjacent port (30 rewards or 50 trials per session; 30s ITI); trials when the rat did not complete the ratio in time were labeled omissions. We recorded spike activity of CeA single units, with special interest in activity during the cues of lever insertion and retraction, as well as the appetitive and consummatory phases of the DT3 task. Across 1247 CeA neurons collected over the course of 15 recording sessions, we found that many neurons had a significant change in firing rate in response to either one or more of these phases of the task, with the greatest modulations at lever retraction (21% units increase activity; 22% units decrease activity), and EtOH consumption (18% units increase activity; 36% units decrease activity). On trials when reward was delivered, the excitatory neural response to lever insertion was approximately 240% greater than on trials when subjects failed to earn a reward, while the inhibitory neural responses did not differ between these events. Our results support the idea that the CeA is engaged during an EtOH-rewarded task during both the appetitive and consummatory phases, in animals who are experienced with, but not dependent on, alcohol.

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Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

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Program #/Poster #: 231.07

Topic: G.09. Drugs of Abuse and Addiction

Support: F32AA029289
AA025582

Title: Interoceptive processing and alcohol relapse-like behavior: roles of the nucleus reuniens and nucleus of the solitary tract

Authors: *D. LOVELOCK[1], D. J. PATEL[1], M. WINDRAM[1], M. A. HERMAN[1], J. BESHEER[2];
[1]Bowles Ctr. for Alcohol Studies, [2]Psychiatry; Bowles Ctr. for Alcohol Studies, UNC Chapel Hill, Chapel Hill, NC

Abstract: A major cause of relapse and drug-seeking after abstinence is exposure to environmental contexts that have become powerfully associated with drug intake. However, the neurobiological relationship between contextual cues and the driving of drug seeking behavior are not well-understood. Drugs of abuse produce interoceptive/subjective effects that can serve as interoceptive cues that may be an important contributor in the initiation and maintenance of drug seeking and relapse. To this end, the studies in this project aim to fill this gap in knowledge by using a context reinstatement procedure and focusing on neural circuitry proposed to be involved in interoceptive processing, the nucleus reuniens (Re) to anterior insula projection (AI), and the nucleus of the solitary tract (NTS) to Re projection. In the first experiment Gi DREADDs were delivered to the nucleus reuniens via microinjection in male and female rats, and after recovery rats were trained on alcohol administration (15% EtOH, FR2). Subjects were then implanted with cannulae aimed at the AI and returned to self-administration. Subsequently, rats underwent extinction in an environmental context distinct from self-administration. Finally, rats were tested in the original context in a two-phase reinstatement test involving a 15 minute seeking phase where cues were presented but alcohol was not delivered, followed by a reinitiation of drinking phase where alcohol was delivered. On test days clozapine-N-oxide (CNO, 3 uM, 0.3ul) or vehicle was delivered to the anterior insula 5 minutes prior to the beginning of the test. In male rats CNO increased alcohol seeking in the first phase of the test while reducing alcohol consumption in the reinitiation phase, while no effects were seen in females. In the second experiment, the same design was repeated with the Gi DREADD microinjection targeting the NTS and CNO delivered to the Re via cannula. Inhibition of the NTS-->Re projection did not alter alcohol seeking or reinitiation of drinking. Taken together, these results suggest that while the Re may play a role in alcohol relapse via modulation of AI activity, it is not likely that bodily interoceptive information routed through the NTS is critical for said function of the Re. Overall these experiments build towards understanding how interoceptive information may be important to inform relapse and relapse-like drinking.

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Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

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Program #/Poster #: 231.08

Topic: G.09. Drugs of Abuse and Addiction

Support: AA024292-05

Title: Habenula cholinergic neurons, synaptic responses in theinterpeduncular nucleus,and alcohol reinforcement

Authors: *J. WANG[1], S. CALIGIURI[3], M. ISHIKAWA[2], P. J. KENNY[3];
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Abstract: Nicotine and alcohol enhance cholinergic transmission in the brain by direct and indirect mechanisms, respectively. There is high co-occurrence of alcohol and tobacco use disorders, suggesting that cholinergic transmission may be a shared neural substrate for abuse of both substances. Cholinergic neurons in the medial habenula (MHb) project to the interpeduncular nucleus (IPn), and the MHb-IPn circuit contains some of the highest densities of α5 subunit-containing nicotinic acetylcholinergic receptors (α5* nAChRs) in mammalian brain. Activation of α5* nAChRs in the MHb-IPn circuit mediates aversive behavioral responses to nicotine thought to constrain tobacco smoking. Little is known about the role of α5* nAChRs in the MHb-IPn circuit in regulating alcohol consumption. We found that alcohol increased the tonic firing rate of MHb cholinergic neurons in ex vivo slice preparations from male and female mice. Alcohol also increased nAChR signaling in the IPn of freely moving mice, as measured using in vivo fiber photometry and a genetically encoded fluorescent reporter of nAChR-mediated cholinergic transmission. Notably, alcohol had a greater stimulatory effect on nAChR signaling in the IPn of female than male mice. Within the IPn, alcohol enhanced GABAergic transmission through a mechanism involving α5* nAChR signaling in female but not male mice. Finally, we found female mice self-administered greater quantities of alcohol than males and that whole-body or IPn-specific disruption of α5* nAChR signaling abolished this sex-dependent difference in alcohol intake. These data demonstrate that ethanol enhances nAChR-mediated cholinergic transmission in the MHb-IPn circuit to a much greater degree in female than male mice and that this response plays a critical role in sex-dependent differences in alcohol consumption.

Disclosures: J. Wang: None. **S. Caligiuri:** None. **M. Ishikawa:** None. **P.J. Kenny:** None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 231.09

Topic: G.09. Drugs of Abuse and Addiction

Support: JSPS KAKENHI Grant 20K16661

Title: Is Non-Alcoholic Beer Useful for Alcoholics?: A study using functional brain imaging of patients with alcohol use disorder in Japan

Authors: *S. FUKUSHIMA[1,2,3], N. ORIBE[1,4], T. MUTOU[1], T. YUZURIHA[1], T. UENO[1,4]; [1]Natl. Hosp. Organization Hizen Psychiatric Med. Ctr., Kannzaki/ Saga, Japan; [2]Michinoo Hosp., Nagasaki, Japan; [3]Nagasaki Univ., Nagasaki, Japan; [4]Kyuusyuu Univ. Hosp., Fukuoka, Japan

Abstract: Introduction: Alcohol use disorder (AUD) is a pattern of alcohol use that involves problems controlling their drinking. Craving appears to play an important role in compulsive use across addictive substances, predicting negative outcomes such as increased use and earlier relapse in AUD. The purpose of this study was to elucidate how brain function abnormalities in patients with AUD in response to visual cue tasks, mainly in the neural circuits of the reward system in the brain, vary with severity. **Methods**: To date, 6 patients with severe AUD, 1 patient with moderate AUD and 6 healthy controls (HC) participated in this study. Blood Oxygen Level Dependent (BOLD) functional activation was measured with 1.5T magnetic resonance imaging (MRI). The block task was used as the stimulus, and the visual cue tasks consisted of three stimulus videos (videos of the participants drinking a non-alcoholic beverage, a pseudo-alcoholic beverage, and an alcoholic beverage, respectively). We used SPM12 software to analyze BOLD signals of fMRI data within each subject, as well as a group. Subject BOLD signals were compared between groups using SPSS.
Results: An analysis using the posterior cingulate gyrus as the region of interest revealed a significant difference in the response of patients with severe AUD to shouchu (traditional Japanese distilled spirit) videos compared to the HC ($p=0.006$). We are planning to increase the number of subjects and present the results on the day of the study.

Disclosures: S. Fukushima: None. **N. Oribe:** None. **T. Mutou:** None. **T. Yuzuriha:** None. **T. Ueno:** None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 231.10

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R00 AA025677
Whitehall Foundation Research Grant 2022-05-99

Title: Impacts of cell type-specific neuropeptide receptor signaling on sex differences in anxiety behavior and alcohol drinking

Authors: Y. MA, H. SARDAR, A. R. MORNINGSTAR, M. A. BELNAP, M. E. BENABOU, R. N. FAJARDO, I. F. KANDIL, A. C. Y. YU, H. WANG, N. Z. CHARFAUROS, P. A.

MUNOZ-RODRIGUEZ, M. Z. V. CORTEZ, J. A. KAUER, *W. J. GIARDINO;
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Abstract: Alcohol use disorder is characterized by episodes of excessive drinking separated by periods of withdrawal- a state of hyperarousal that includes propensity for insomnia, seizure susceptibility, and increased anxiety. The negative emotional features of hyperarousal are thought to perpetuate addiction via negative reinforcement (escalating alcohol intake to relieve symptoms of withdrawal). Neuropeptides are molecules with specialized roles in modulating arousal via actions on G-protein-coupled receptors, and neuropeptides like hypocretin/orexin (Hcrt), corticotropin-releasing factor (Crf), and cholecystokinin (Cck) are implicated in addiction. Based on the distinct forms of arousal promoted by Hcrt actions on discrete cell types, and via two unique receptor subtypes (HcrtR1, HcrtR2), we hypothesized that Hcrt signaling in neuropeptide-defined cell types differentially impacts baseline anxiety, long-term intermittent alcohol drinking, or withdrawal-heightened anxiety. To test these hypotheses, we crossed floxed HcrtR mice with Crf-Cre or Cck-Cre mice to delete HcrtR1 or HcrtR2 from Crf or Cck neurons. All mice were homozygous for the floxed HcrtR allele and either wild-type or heterozygous for Cre. Adult female and male mice were acclimated to single housing prior to one week of baseline data collection that included anxiety tests (elevated plus maze; EPM, open field test; OFT) as well as body weight and food/fluid consumption measurements. The next 8 weeks, mice underwent the two-bottle choice long-term intermittent alcohol drinking paradigm (three 24hr sessions/week, every-other-day). Daily recordings of alcohol, water, and food were used to calculate weekly averages for intake and preference variables. In week 8, mice were tested again for anxiety during acute withdrawal (4-8hrs after alcohol drinking). Following behavior, brains were processed for validation of Crf/Cck-specific deletion of HcrtR1 or HcrtR2, focusing on the extended amygdala (bed nucleus of stria terminalis; BNST). Preliminary analyses of alcohol intake and preference in Crf/HcrtR1 mice revealed significant effects of sex where female mice consumed more than males ($p < 0.0001$). Alcohol intake analysis also identified a trend toward a significant effect of genotype in which Crf-HcrtR1 deletion reduced alcohol drinking. Preliminary analyses of the EPM revealed a significant effect of withdrawal and a trend toward a significant effect of genotype (greater open arm time in mice with Crf deletion of HcrtR1). These data suggest that HcrtR1 signaling in Crf neurons may be required for excessive alcohol intake and anxiety-like behavior.

Disclosures: Y. Ma: None. **H. Sardar:** None. **A.R. Morningstar:** None. **M.A. Belnap:** None. **M.E. Benabou:** None. **R.N. Fajardo:** None. **I.F. Kandil:** None. **A.C.Y. Yu:** None. **H. Wang:** None. **N.Z. Charfauros:** None. **P.A. Munoz-Rodriguez:** None. **M.Z.V. Cortez:** None. **J.A. Kauer:** None. **W.J. Giardino:** None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

Location: SDCC Halls B-H

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Program #/Poster #: 231.11

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH-COBRE P20 GM103642; NSF 1736026
NIH-BP-ENDURE R25NS080687

Title: Neurophysiological correlates of alcohol response in the Mushroom Body of Drosophila melanogaster

Authors: *N. FUENZALIDA-URIBE[1], C. S. IRIZARRY-HERNANDEZ[2], I. DIAZ-NIEVES[3], A. GHEZZI[3];
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Abstract: In nature, the fruit fly, Drosophila melanogaster, feeds on decaying fruits containing varying concentrations of alcohol —a by-product of fermentation. Consumption of low concentrations of alcohol can result in increased fitness, whereas higher concentrations can cause significant developmental delays and cognitive dysfunction. Thus, it is not surprising that adult flies can adapt to the presence of alcohol in food and minimize some of its detrimental effects. However, the neural mechanisms by which these adaptations occur remain unresolved. Here, we use a Drosophila model to explore the neurophysiological correlates of alcohol tolerance in the Mushroom Body of the fly—a key neuropil in the brain that integrates sensory signals. Using a functional imaging approach, we studied the neurophysiology of the MB calyx in response to ethanol. Flies expressing the calcium sensor protein GCamp6s in MB neurons were treated with ethanol vapor (~95%) until anesthetized (~40 minutes). Twenty-four hours later, we recorded the intracellular calcium dynamics in the MB calyx. Adapted flies showed a decreased response to a short ethanol exposure (3 seconds) compared to control flies, suggesting that this brain area is involved in the neurophysiological processing of ethanol tolerance. The reduction in neural signaling correlates with the changes observed in Drosophila behaviors related to ethanol exposure, suggesting a possible neural substrate for the behavioral adaptation to alcohol during tolerance.

Neurophysiological correlates of alcohol tolerance in the Mushroom Body of *Drosophila* melanogaster

NICOLAS FUENZALIDA-URIBE, CLAUDIA IRIZARRY-HERNANDEZ, IAN DIAZ-NIEVES, ALFREDO GHEZZI

IN NATURE, THE FRUIT FLY, *DROSOPHILA MELANOGASTER*, FEEDS ON DECAYING FRUITS CONTAINING VARYING CONCENTRATIONS OF ALCOHOL —A BY-PRODUCT OF FERMENTATION. CONSUMPTION OF LOW CONCENTRATIONS OF ALCOHOL CAN RESULT IN INCREASED FITNESS, WHEREAS HIGHER CONCENTRATIONS CAN CAUSE SIGNIFICANT DEVELOPMENTAL DELAYS AND COGNITIVE DYSFUNCTION. THUS, IT IS NOT SURPRISING THAT ADULT FLIES CAN ADAPT TO THE PRESENCE OF ALCOHOL IN FOOD AND MINIMIZE SOME OF ITS DETRIMENTAL EFFECTS. HOWEVER, THE NEURAL MECHANISMS BY WHICH THESE ADAPTATIONS OCCUR REMAIN UNRESOLVED. HERE, WE USE A DROSOPHILA MODEL TO EXPLORE THE NEUROPHYSIOLOGICAL CORRELATES OF ALCOHOL TOLERANCE IN THE MUSHROOM BODY OF THE FLY—A KEY NEUROPIL IN THE BRAIN THAT INTEGRATES SENSORY SIGNALS. USING A FUNCTIONAL IMAGING APPROACH, WE STUDIED THE NEUROPHYSIOLOGY OF THE MB CALYX IN RESPONSE TO ETHANOL. FLIES EXPRESSING THE CALCIUM SENSOR PROTEIN GCAMP6S IN MB NEURONS WERE TREATED WITH ETHANOL VAPOR (~95%) UNTIL ANESTHETIZED (~40 MINUTES). TWENTY-FOUR HOURS LATER, WE RECORDED THE INTRACELLULAR CALCIUM DYNAMICS IN THE MB CALYX. ADAPTED FLIES SHOWED A DECREASED RESPONSE TO A SHORT ETHANOL EXPOSURE (3 SECONDS) COMPARED TO CONTROL FLIES, SUGGESTING THAT THIS BRAIN AREA IS INVOLVED IN THE NEUROPHYSIOLOGICAL PROCESSING OF ETHANOL TOLERANCE. THE REDUCTION IN NEURAL SIGNALING CORRELATES WITH THE CHANGES OBSERVED IN *DROSOPHILA* BEHAVIORS RELATED TO ETHANOL EXPOSURE, SUGGESTING A POSSIBLE NEURAL SUBSTRATE FOR THE BEHAVIORAL ADAPTATION TO ALCOHOL DURING TOLERANCE.

Disclosures: **N. Fuenzalida-Uribe:** None. **C.S. Irizarry-Hernandez:** None. **I. Diaz-Nieves:** None. **A. Ghezzi:** None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R21AA028189
NIH Grant K01AA28059

Title: Chemogenetic Perturbation of the Posterior Cerebellum Reduces Voluntary Ethanol Consumption

Authors: P. A. ZAMUDIO, *J. J. WOODWARD;
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Abstract: Although historically neglected in the addiction field, recent studies implicate the cerebellum as a key brain region involved in the development and maintenance of addiction. The cerebellum communicates with the prefrontal and orbitofrontal cortex and with midbrain and basal ganglia structures such as the ventral tegmental area and dorsal striatum that are critically involved in control of goal-directed behaviors. Importantly, the posterior cerebellum is important for cognitive flexibility and has recently been implicated in drug-related memory. Thus, we hypothesized that the cerebellum, through its multiple connections to brain circuitry that underlie reward, is important in regulating alcohol consumption. To test this, we expressed inhibitory DREADDs (AAV8/hM4D-mCherry) or a control (AAV8-mCherry) in either anterior (IV-V) or posterior (VI-VIII) cerebellar cortex lobules of mice and measured consumption during alcohol drinking sessions. In a home-cage 24 hour-intermittent consumption paradigm, DREADD activation in posterior lobules in male mice, but not in females, significantly decreased alcohol consumption (hM4Di:62 ± 3.6 % of baseline, n=38 $p<0.0001$; AAV8-mCherry: 99.6 ± 5.4 % of baseline, n=8 $p=0.93$) and preference (hM4Di:77.8 ± 4.1 % of baseline $p<0.0001$; AAV8-mCherry: 107.6 ± 5.8 % of baseline, $p=0.23$). In contrast, activation of inhibitory DREADDs did not affect sucrose or quinine consumption in neither anterior nor posterior lobules. Similarly, in an operant paradigm, where mice were trained on a fixed ratio one schedule of reinforcement, the weekly average number of licks per session was significantly decreased upon DREADD activation in posterior lobules (hM4Di:73.7 ± 3.7 % of baseline, n=7, $p=0.0004$; AAV8-mCherry: 90.87 ± 5.1 % of baseline,n=7, $p=0.12$). Importantly, motor coordination, as measured by performance on an accelerated rotarod, was unaffected by chemogenetic manipulations. However, distance travelled in locomotor boxes was significantly decreased after chemogenetic perturbation of both anterior and posterior lobules. These results indicate that neural computation within the posterior cerebellar cortex plays an important role in the motivation for alcohol consumption in male C57BL/6J mice. Future studies will focus on defining specific downstream neuronal circuits underlying the effect.

Disclosures: P.A. Zamudio: None. **J.J. Woodward:** None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

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Title: Neural correlates of the addictions neuroclinical assessment (ANA) incentive salience factor in alcohol use disorder

Authors: E. M. BURNETTE, S. J. NIETO, E. N. GRODIN, L. A. RAY;
Dept. of Psychology, UCLA, Los Angeles, CA

Abstract: Alcohol use disorder (AUD) is a highly heterogeneous disorder, resulting in a number of sub-phenotypes with its own unique profile of drinking pattern and motivation for drinking. The Addictions Neuroclinical Assessment (ANA) is a recently-developed clinical framework offering a more holistic understanding of three neurofunctional and behavioral domains - specifically, incentive salience, negative emotionality, and executive function - that reflect the neurobiological dysfunction seen in addiction disorders such as AUD. The current study involves secondary data analysis of a two-week experimental medication trial of the neuroimmune modulator ibudilast for AUD. Forty-five non-treatment-seeking participants with AUD (17 F / 28 M) completed a battery of validated behavioral assessments forming the basis of their incentive salience score, computed via factor analysis, as well as a functional neuroimaging (fMRI) task assessing their neural reactivity to visual alcohol cues. Whole-brain generalized linear model analyses were conducted to examine associations between neural alcohol cue-reactivity and incentive salience. Age, sex, medication (ibudilast vs. placebo), and smoking status (smoker vs non-smoker) were included as covariates.Incentive salience was significantly positively correlated ($p < 0.05$) with the contrast in neural activation in response to images of alcoholic beverages over neutral beverages in reward-learning and affective regions including the right anterior and posterior cingulate cortices, bilateral precuneus, and bilateral precentral gyri. Results indicate that the ANA incentive salience factor is reflected in brain circuitry important for reward learning and emotion processing. Identifying a sub-phenotype of AUD characterized by increased incentive salience to alcohol cues allows for precision medicine approaches, i.e. treatments specifically targeting craving and reward for alcohol. Importantly, this study serves as a preliminary bio-behavioral validation for the ANA framework. Further studies validating the neural correlates of other ANA factors, as well as replication in larger samples, appear warranted.

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Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

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NIGMS P20GM103645 (Kaun)

Title: Distinct dopamine microcircuits underlying alcohol-induced locomotor activity and alcohol-associated memories in Drosophila melanogaster

Authors: *K. D. CALDARONE[1,2], S. L. SONG[3], N. T. SAVORY[3], T. M. A. NGUYEN[1], S. POLLACK[1], K. R. KAUN[3], K. M. SCAPLEN[1,2,3];
[1]Dept. of Psychology, [2]Ctr. for Hlth. and Behavioral Sci., Bryant Univ., Smithfield, RI; [3]Dept. of Neurosci., Brown Univ., Providence, RI

Abstract: Dopamine has been implicated in highly conserved mechanisms underlying reward and punishment-based learning, salience, motivation, and locomotion. Alcohol and drugs of abuse disrupt dopaminergic activity and remold pathways leading to enduring memories and maladaptive goal-directed behaviors. Previous work in *Drosophila* identified discrete dopamine neural circuits required for the acquisition and expression of alcohol-associated memories (Scaplen et al 2020). More recent evidence suggests that subsets of dopamine neurons also mediate alcohol-induced locomotor activity. It is unclear, however, whether dopamine neurons identified as important for alcohol reward are also important for the locomotor response to alcohol. Using an automated group activity monitor called flyGrAM, we sought to investigate the role of dopamine neurons in modulating alcohol-induced activity and the potential overlap in neural circuits for memory and locomotion. We show that both the PAM and PPL1 subsets of dopamine neurons innervating the Mushroom Body, a memory encoding central brain structure, play dynamic roles in modulating alcohol-induced locomotor activity. Inactivation of dopamine neurons had the most profound effect during the later stages of alcohol intoxication and at higher doses, suggesting that more dopamine neurons are recruited at higher doses to counter the sedating effects of alcohol. High-content behavior analysis using FlyTracker, a post-processing computer vision software, suggests that distinct subsets of dopamine neurons modulate activity metrics such as velocity, angular velocity, and distance to the walls of the arena. Interestingly, the discrete PAM and PPL1 subsets that are required for the expression of alcohol-associated memories are not required for alcohol-induced locomotor activity. Future work will use post-processing computer vision software such as Ctrax and machine learning-based systems such as JAABA to analyze more refined social and behavioral features to better characterize the precise modulatory role of dopamine neurons on alcohol-induced activity.

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Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

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Program #/Poster #: 231.15

Topic: G.09. Drugs of Abuse and Addiction

Support: McDonnel Fellowship, Washington University In St Louis
Rita Allen Foundation

Title: Ventral pallido-subthalamic adaptations underlying compulsivity in alcohol use disorders

Authors: *Y. M. VACHEZ[1], L. Z. FANG[2], M. C. CREED[3];
[1]Washington Univ. Sch. of medicine in St. Louis, Saint Louis, MO; [2]Div. of Biomed. Sci., Mem. Univ., St John's, NL, Canada; [3]Dept. of Anesthesiology, Neurosciences and Psychiatry, Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Alcohol use disorder is a chronic relapsing disorder characterized by impulsive and compulsive decision-making processes driven by a disinhibition of behavior control.The subthalamic nucleus (STN) is of particular importance for appropriate decision making, especially its ventromedial portion (vmSTN), a limbic territory involved in mood and motivated behavior. Behavioral inhibition requires burst firing of the vmSTN while impulsivity correlates with decreased activity. The ventral Pallidum (VP), a limbic structure mainly inhibitory, projects to the STN and is theoretically the main inhibitory input of the vmSTN. VP activity reflecst perceived reward, is affected by ethanol consumption and is increased in impulsive patients. We hypothesized that VP GABAergic projections to the vmSTN become persistently strengthened following alcohol intake, promoting impaired decision making and compulsivity. In a mouse model of ethanol drinking, we combined behavioral assays to highlight maladaptive decision making, *ex vivo* electrophysiology patch clamp coupled with pharmacology and optogenetic modulation to elucidate how ethanol drinking alters the VP to vmSTN synapse strength, and *in vivo* optogenetic experiment to modulate the VP to vmSTN activity during behavioral assays. Together, these results will elucidate neural substrates of inhibitory controls and how they are altered in alcohol use disorders.

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Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant U01AA028710

Title: Sex dependent effects of voluntary wheel running exercise on acetylcholine efflux in the mPFC and behaviors following adolescent intermittent ethanol exposure

Authors: *M. J. FECIK, P. T. NUNES, L. M. SAVAGE;
Psychology-Behavioral Neurosci., Binghamton Univ., Binghamton, NY

Abstract: Heavy usage of alcohol during adolescence can induce changes to both the brain and behavior that persist into adulthood. Voluntary exercise (VEx) has been used to rescue some ethanol-induced deficits in rats. The current project investigated whether adolescent intermittent ethanol exposure (AIE) induced differential cortical-dependent behavioral deficits, as well as decreased acetylcholine (ACh) efflux in the medial prefrontal cortex (mPFC) and the effect of VEx on these measures. Long Evans rats, male and female, were given an intragastric gavage of 20% ethanol between postnatal days (PD) 25 to 55 to induce binge levels of intoxication, or water in the case of controls, followed by an mPFC cannulation. Following surgery, some rats had running wheels attached to their cages from PD 75-105, while the other rats were housed in standard cages. Rats were subsequently assessed for spatial working memory during spontaneous alternation with in-vivo microdialysis for ACh efflux in the mPFC and later an operant attention set-shifting task to assess cognitive and behavioral flexibility. Running wheel activity was subject to dramatic sex differences, with female rats running about twice as much as male rats. Exclusively in female rats, AIE resulted in a blunting of ACh efflux and a trend toward a rescue of these deficits with following exercise. In addition, in female rats, exercise was shown to improve reversal learning in rats not exposed to ethanol in adolescence. We did not detect AIE deficits in behaviorally evoked ACh efflux into the mPFC in male rats, contrasting previous data, and there were no AIE impairments on spontaneous alternation performance nor did exercise improve it. Furthermore, there were no AIE impairments on cognitive flexibility or behavioral flexibility. These data suggest that AIE deficits to both frontocortical neurochemistry and its dependent behaviors are variable. Thus, a cohort of separate rats will be tested on an operant sustained attention task, which is sensitive to phasic cholinergic activity, which we hypothesize will be more sensitive to AIE-induced neuropathology.

Disclosures: M.J. Fecik: None. **P.T. Nunes:** None. **L.M. Savage:** None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

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Program #/Poster #: 231.17

Topic: G.09. Drugs of Abuse and Addiction

Support: T32 NS007491
T32 MH065215
R37 AA019455 (DGW)
F32 AA029592 (MAD)

Title: Aversion-resistant ethanol intake after limited access operant self-administration: Potential roles of activity in the bed nucleus of the stria terminalis

Authors: *M. A. DOYLE[1], A. S. PARK[1], M. E. TROUTMAN[2], M. E. ALTEMUS[1], D. N. ADANK[1], L. LANTIER[2], C. A. SICILIANO[1], D. G. WINDER[1];
[1]Vanderbilt Ctr. for Addiction Res., [2]Mouse Metabolic Phenotyping Ctr., Vanderbilt Univ., Nashville, TN

Abstract: Alcohol use disorder (AUD) is a chronic, relapsing disease that is highly comorbid with anxiety and depression. These states of negative affect experienced during withdrawal are hypothesized to drive alcohol seeking and relapse behavior. Here, we used an operant conditioning task known as Structured Tracking of Alcohol Reinforcement (STAR) to assess changes in ethanol intake and aversion resistance following a forced abstinence period. Male and female C57BL/6J mice were trained to self-administer a 20% ethanol solution via a retractable sipper. Following a training period, mice self-administered ethanol in daily 1-hour sessions for 14 days before starting a forced abstinence period (28 days). Mice then underwent the STAR procedure. Here, mice were returned to ethanol self-administration for 3 days before increasing concentrations of quinine (0.25, 0.5, 0.75, and 1.0 mM) were added to the ethanol in order to assess aversion-resistant intake behaviors. In the initial self-administration phase, no statistical differences in acquisition or intake were observed between sexes, highlighting a significant advantage for this approach over traditional two-bottle choice assays, where females are known to have higher basal levels of ethanol intake compared to males. Following the forced abstinence period, quinine sensitivity was assessed. Male, but not female mice exhibited a significant shift towards quinine resistance after abstinence, suggesting a potential sex-dependent effect of abstinence on compulsive-like alcohol intake behaviors. Because the bed nucleus of the stria terminalis (BNST) is known to undergo plastic changes during forced abstinence following exposure to alcohol or drugs of abuse, we used fiber photometry to record BNST GCaMP transients as a method of assessing changes in BNST activity across initial ethanol intake sessions and during forced abstinence (days 1, 7, and 28). These recordings may yield insights into sex differences in BNST adaptations. Ultimately, it is the goal of this work to identify the mechanisms through which an aversion resistant phenotype may develop and assess potential interventions to prevent this outcome.

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Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

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Program #/Poster #: 231.18

Topic: G.09. Drugs of Abuse and Addiction

Title: Motor cortical innervation of interneuron populations in the mouse insula

Authors: *D. N. ADANK, A. TAYLOR, J. R. LUCHSINGER, D. G. WINDER;
Vanderbilt Univ., Nashville, TN

Abstract: Motor cortical innervation of interneuron populations in the mouse insula
Danielle N. Adank, Joseph R. Luchsinger, Danny G. Winder Vanderbilt University, Center for Research Addiction, Nashville, TN, 37232, United States Vanderbilt University, Vanderbilt Brain Institute, Nashville, TN, 37232, United States
Alcohol use disorder is characterized as a chronically relapsing disorder. Relief of negative affective states is thought to be a major driver of relapse. Several brain regions including extended amygdalar structures have been proposed as regulators of abstinence and relapse. The insular cortex (IC) has emerged as an important component in this network because of its involvement in negative affect and interoception and its rich interconnections with the extended amygdala. We have previously demonstrated the IC as a critical player in negative affective behaviors that emerge during alcohol-forced abstinence in mice and in active coping bouts during restraint stress. Indeed, our previous mapping studies have demonstrated somatomotorcortical areas as a major source of afferents to IC neurons that project to the bed nucleus of thestria terminalis (BNST), a component of the extended amygdala. Active coping events during stress are associated with the activation of insular projection neurons. These bouts were also associated with transient reductions in extracellular GABA levels. IC GABA is a regulator of chronic stress adaptation, suggesting the possibility that, in addition to motor projections to output neurons, motor sources may impinge on interneuron populations in IC. To test this hypothesis, we have taken converging anterograde and retrograde viral tracing strategies. First, we utilized an anterograde tracing approach to analyze primary motor cortex (MOp) projection targets in the IC. Our data suggest that MOp projecting neurons synapse onto GABAergic cells in the IC, with specificity to somatostatin interneurons(SST-INs) relative to parvalbumin and VIP expressing classes. Next, we utilized a convergent retrograde strategy with rabies viral tracing to explore specific input onto IC SST interneurons. These data indicate that the MOp provides efferents to both intrinsic SST and projection neuronal populations. The MOp is essential for planning movement execution in mice, and in future studies, we aim to see how exercise manipulates negative affect and compulsivity during alcohol abstinence via MOp-IC microcircuitry.

Disclosures: D.N. Adank: None. **J.R. Luchsinger:** None. **D.G. Winder:** None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

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Program #/Poster #: 231.19

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA Grant R37AA019455

Title: Insula to BNST circuit adaptations following restraint stress and alcohol withdrawal associated negative affect

Authors: *A. TAYLOR[1], D. N. ADANK[1], P. A. YOUNG[1], J. A. BROWN[1], Y. QUAN[1], D. G. WINDER[2];
[2]Vanderbilt Univ., [1]Vanderbilt Univ., Nashville, TN

Abstract: Mental illnesses such as anxiety and depression impact almost 50 million adults in the United States (US). An estimated 95 million adults in the US meet the criteria for Alcohol use disorder (AUD), which is highly comorbid with anxiety and depression. Stress and negative affective states are both thought to contribute to AUD. Research to elucidate the precise neural changes that occur during negative affective states, and their regulation by alcohol is greatly needed. Our previous work in mouse models has implicated a projection from the insular cortex to the bed nucleus of the stria terminalis (BNST) in active coping during restraint stress, and in the emergence of negative affective behaviors during alcohol abstinence. Here, we assess the impact of restraint stress, as well as chronic drinking forced abstinence (CDFA) on synaptic and excitable properties of insular neurons that control the BNST using C57BL6/J mice. Using whole cell patch clamp electrophysiology, we find a sex-specific increase in inhibitory drive onto mid- and BNST-projecting, but not anterior insula cells in male mice following acute restraint stress. These data suggest that interneurons within the insular cortex may be more active during acute restraint stress. Using the calcium indicator GCaMP virally expressed in either parvalbumin (PV), somatostatin (SST) or vasoactive intestinal peptide (VIP) expressing interneurons and fiber photometry, preliminary data suggests that the activity of PV and SST but not VIP insular interneuron activity is correlated with active struggling bouts during restraint stress. We next investigated Insula to BNST circuitry 24hrs or 2 weeks following CDFA in female mice. We find that BNST-projecting insula cells are transiently more excitable following acute ethanol withdrawal. In future experiments we will be assessing the mechanisms underlying the observed hyperexcitability of this neuronal population. Furthering knowledge on how acute and chronic stress reorganizes neural circuitry will provide insight into how negative affective states may lead to relapse.

Disclosures: A. Taylor: None. **D.N. Adank:** None. **P.A. Young:** None. **J.A. Brown:** None. **Y. Quan:** None. **D.G. Winder:** None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 231.20

Topic: G.09. Drugs of Abuse and Addiction

Support: R21AA028602

Title: Chemogenetic inhibition of mouse amygdala intercalated cells during binge-like alcohol drinking

Authors: *N. SHAND[1], T. PERRY[2], H. CARVOUR[3], S. MONROE[3], A. K. RADKE[2];
[1]Miami Univ., [2]Psychology, [3]Miami Univ., Oxford, OH

Abstract: Chemogenetic inhibition of mouse amygdala intercalated cells during binge-like alcoholdrinking Natalie Shand, Thomas Perry, Harrison Carvour, Sean Monroe, Anna K. RadkeDepartment of Psychology and Center for Neuroscience & Behavior, Miami University, Oxford, Ohio.Post-traumatic stress disorder (PTSD) and alcohol use disorder (AUD) have a high rate ofcomorbidity. While this comorbidity is well documented, its neural correlates are still poorlyunderstood. Recent studies have shown that anxiety disorders and AUD are similarlycharacterized by altered amygdalar structure and activity but less is known about the specificcircuits within the amygdala that promote drinking. The intercalated cells of the amygdala (ITC)are GABAergic interneurons situated around the basolateral amygdala (BLA). ITCs are knownto regulate fear behaviors, such as extinction, that are relevant to PTSD, however, their role inalcohol use has not been assessed. In the current study, we investigated the effect of inhibitingITCs on ethanol (EtOH) consumption. To this end, we used a two-bottle choice, limited accessdrinking in the dark paradigm to assess binge-like EtOH consumption and preference.Expression of the viral vector AAV8- hSyn-DIO-hM4D(Gi)-mCherry (Addgene) was targeted toITC cells via injection into male and female FoxP2-Cre mice. Mice expressing hM4Di or eGFPdrank 15% EtOH vs. water for 2 h over 10 sessions. The DREADD receptor ligand clozapine-n-oxide (CNO, 1 mg/kg i.p.) or vehicle was injected 15 min before the commencement of drinking(CNO on session 3, 5, 7, and 9; VEH on sessions 4, 6, 8, and 10). Our results demonstrate thatinhibition of ITC neurons within the amygdala had no impact on alcohol consumption orpreference for both sexes. Ongoing experiments are examining the role of ITC mu-opioidreceptors in alcohol drinking behaviors. Future studies will also explore whether early life stressexposure alters the relationship between ITC cell activity and alcohol drinking.Keywords: alcohol, sex differences, amygdala, intercalated cells, drinking in the dark

Disclosures: N. Shand: None. **T. Perry:** None. **H. Carvour:** None. **S. Monroe:** None. **A.K. Radke:** None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 231.21

Topic: G.09. Drugs of Abuse and Addiction

Support: NWO-Vidi 2017/2018

Title: Pay attention to this change: The role of Lateral Hypothalamus in alcohol memories

Authors: *I. ALONSO-LOZARES[1], P. WILBERS[1], S. TEIJSSE[1], L. ASPERL[1], D. SCHETTERS[1], P. JEAN-RICHARD-DIT-BRESSEL[2], A. J. MCDONALD[1], H. D. MANSVELDER[3], T. J. DE VRIES[1], N. J. MARCHANT[1];

[1]Amsterdam UMC, Amsterdam, Netherlands; [2]UNSW, Kensington, Australia; [3]VU Univ. Amsterdam, Netherlands, Amsterdam, Netherlands

Abstract: In alcohol use disorder (AUD) the positive value of a drug memory persists during abstinence, leading to relapse when re exposed to cues that trigger such memories. As such, addiction can be seen as a disorder of motivation and associative learning. Lateral hypothalamus (LH) is a brain region critical for memory and motivation. Recent studies suggest the GABAergic subpopulation of neurons in LH are key players in memory processes, although the specific mechanisms through which LH-GABA encode and express memories are still understudied. In this study, we aim to describe how alcohol reward memories are encoded and expressed in LH GABAergic neurons. In experiment 1, we used fiber-photometry to monitor LH-GABA calcium transients during acquisition and expression a cue-alcohol associations (n = 6). In experiment 2 (n = 9 per group), we inhibited these neurons during acquisition of the cue-alcohol association with optogenetics, while in experiment 3, we inhibited these neurons during the expression of the cue-alcohol association (n = 9 experimental group, n = 8 control group). Finally, we unilaterally inhibited ventral tegmental area or nucleus accumbens shell inputs to LH with chemogenetics, while recording from LH-GABA using fibre photometry (n = 18). To target LH-GABA neurons, we used a dual virus approach, combining one AAV encoding GAD-cre and another encoding cre-dependent gCaMP7f (photometry experiments) or GtACR2 or mCherry (optogenetics experiments) into the LH of Long-Evans rats. To unilaterally inhibit inputs to LH, we injected retro-flp in LH and flp-dependent hm4di or mCherry in VTA or NAcS. We first trained the rats on an alcohol conditioning task in which they learned to associate one conditioned stimulus (CS+) with alcohol availability in a magazine (20% EtOH in water), while a different conditioned stimulus (CS-) was presented without consequence. We then tested expression of the memory by presenting the CS+ and CS- without consequence, in extinction. Our results show LH-GABA initially respond to all incoming stimuli regardless of their valence, but this increased activity is maintained to reward-predictive stimuli (CS+), while activity is decreased to the behaviourally irrelevant stimuli (CS-). Moreover, in extinction this decrease is no longer present, but activity remains above baseline, potentially indicating that these neurons might be signalling reward prediction error in extinction. Finally, we also show that LH-GABA are functionally involved in the acquisition of alcohol memories. Disentangling how encoding and expression of alcohol memories happens in the brain could open the door to new therapeutic targets for individuals with AUDs.

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Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 231.22

Topic: G.09. Drugs of Abuse and Addiction

Support: Ronald E. McNair Postbaccalaureate Achievement Program

Title: Effect of ethanol oral administration on relative use of multiple memory systems in rats

Authors: *D. GONZALEZ, E. LORENZ, D. PATEL, K.-C. LEONG;
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Abstract: The 2020 National Survey on Drug Use and Health estimated that 10.2% of Americans suffer from alcohol use disorder. Alcohol consumption can have numerous effects on memory. The mammalian brain consists of two often competing major memory systems: the hippocampus-dependent cognitive memory system and dorsal striatum-dependent habit memory system. The formation and retrieval of cognitive and habitual memories can be acquired using a place or response strategy, respectively. The purpose of the present study was to investigate the effects of ethanol on the relative use of the cognitive and habitual memory systems. Furthermore, it is unclear whether ethanol biases relative use of these memory systems during the consolidation or retrieval phase. The dual-solution water plus-maze task offers a behavioral paradigm to determine the relative preference of memory systems in rats. Adult male Sprague-Dawley rats were trained over two days on the dual-solution water maze task and were tested on the third day of the paradigm. Training sessions consisted of six trials per day in which the rodent swam from the same start arm (South) towards a hidden escape platform located in the same goal arm (East). On test day, the relative use of place or response learning was assessed on a probe trial in which rats were started from the opposite start arm (North). To determine ethanol's effects of retrieval (Experiment 1), animals were orally administered 20% ethanol (2 g/kg) 30 minutes before the test trial. To determine ethanol's effects on consolidation (Experiment 2), animals were orally administered 20% ethanol (2 g/kg) immediately after each training session. Results from Experiment 1 showed that ethanol exposure prior to probe test biases animals towards preferential use of the response strategy to complete the task. In Experiment 2, post-training ethanol exposure did not bias animals towards the use of place or response strategy on test day. The present study indicates that ethanol biases the relative use of multiple memory systems towards the use of the habit memory system through interactions with the retrieval process but not the consolidation process. The ability of alcohol to produce strong preferential use of the habit memory system over the cognitive memory system has important implications for the understanding of alcohol use disorder, general addiction, and memory function.

Disclosures: D. Gonzalez: None. **E. Lorenz:** None. **D. Patel:** None. **K. Leong:** None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

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Program #/Poster #: 231.23

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R00AA025384
NIH Grant R01AA028770

Title: Impact of alcohol vapor exposure on neural responses to alcohol cues

Authors: A. JOSEPH, F. HANAK, A. SELIMOVIC, A. SOOD, J. M. RICHARD;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Environmental cues play an important role in relapse and addiction-related behaviors. Chronic intermittent ethanol (CIE) vapor exposure is a commonly used model of alcohol dependence, but the impact of CIE vapor inhalation on neural and behavioral responses to cues is poorly understood. Previously, we examined the effects of CIE vapor inhalation on behavioral responses to cues paired with alcohol availability (Carpio et al., 2021). Male and female Long Evans rats were trained in a discriminative stimulus task with 15% ethanol reward prior to exposure to CIE vapor inhalation or control conditions. When rats were subsequently tested in the presence of cues, we found that CIE increased the likelihood of cue responses, but only in rats who were allowed to consume alcohol during acute withdrawal during the CIE exposure phase. This increase in cue reactivity occurred under extinction conditions, and when alcohol reward was present. Here, our goal was to assess potential neural correlates of this increase in behavioral cue reactivity using c-Fos immunofluorescence. Rats underwent a final cue test under extinction conditions and were perfused and their brains fixed either immediately or 60-90 minutes after this test. Our initial investigation focused on c-Fos in the medial prefrontal cortex (mPFC). Overall, we observed similar levels of cFos expression in mPFC in CIE and control rats that were perfused immediately after the cue test, preventing the induction of c-Fos expression following cue related neural activity. In contrast, we observed an increase in mPFC c-Fos in CIE rats that were perfused after a delay compared to controls. This pattern was highly variable, and subsequent studies will determine whether this is due to potential sex differences in the impact of CIE. Overall, these results suggest the mPFC as a promising candidate region for mediating the impact of CIE on behavioral cue responses. Future work will examine other brain regions implicated in the effects of CIE, alcohol cues, and their interactions.

Disclosures: A. Joseph: None. **F. Hanak:** None. **A. Selimovic:** None. **A. Sood:** None. **J.M. Richard:** None.

Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

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Program #/Poster #: 231.24

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH grant R00 DA04510
NIH U01 AA029971
Alkermes Pathway Research Award

Brain Research Foundation
Whitehall Foundation
Stanley Cohen Innovation Fund

Title: Chemesthetic perception gates orosensory acceptance of alcohol

Authors: S. MUKERJEE, D. B. COHEN, V. R. MAHAJAN, A. R. BROWN, C. A. SICILIANO;
Pharmacol., Vanderbilt Univ., Nashville, TN

Abstract: Ethanol is highly unusual among acutely toxic chemicals in that it will be repeatedly consumed by essentially all animal species when given access. The central pharmacological effect of ethanol mediates its powerful reinforcing properties but the neural mechanisms that control palatability of ethanol and explain individual differences in innate preferences prior to intoxication are unknown. Further, when ethanol is not available heavy drinkers often consume poisonous ethanol-containing solutions, such as gasoline and mouthwash, suggesting that chronic ethanol use disrupts innate chemical defense systems responsible for oral rejection of toxins. Ethanol has a complex flavor profile, evoking a range of gustatory sensations in addition to a burning-tingling sensation which becomes dominant at high concentrations. This sensation, termed oral chemesthesis, is distinct from traditional taste qualities and is detected by the trigeminal innervation. Here we reveal the circuits that sense concentrations of chemical irritants in the oral cavity and directly report to the brainstem to determine whether ethanol is consumed or rejected. Neural coding of oral chemesthesis is under-studied, we first identified the structural and functional brain regions that respond to 50% ethanol, a relatively high concentration known to elicit strong chemesthesis. These results revealed that the trigeminal ganglion is centrally positioned for relaying chemical information from the mouth to the brain. Further, using pharmacological and chemogenetic interventions combined with *in vivo* imaging we identified peripheral to central circuits and cellular targets that influences palatability of ethanol thereby altering motivation and consumption. Altogether, the present findings elucidate how ethanol bypasses the gating mechanism in the mouth to allow ingestion of a substance with a dominant burning-tingling irritant like flavor. Further, the cellular targets uncovered in these studies illuminate novel drivers of substance use behaviors and may provide a mechanism for hypofunction of this system within chronic ethanol users.

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Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

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Alkermes Pathways Research Award
Brain Research Foundation
Whitehall Foundation
Stanley Cohen Innovation Fund

Title: Structured tracking of alcohol reinforcement (STAR) reveals distinct cortical and brainstem biomarkers of compulsion

Authors: *A. BROWN[1], H. BRANTHWAITE[1], Z. FARAHBAKHSH[1], S. MUKERJEE[1], P. MELUGIN[1], H. NOAMANY[2], C. SICILIANO[1];
[1]Pharmacol., Vanderbilt Univ., Nashville, TN; [2]Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Ethanol functions as a powerful reinforcer through central pharmacological actions, and the motivational processes engaged during ethanol seeking are highly conserved across species. While many studies focus on the pathophysiology of alcohol use disorder, the need for models that are valid at the basic and translational level has become increasingly apparent. Here, we develop a novel modular behavioral paradigm: Structured Tracking of Alcohol Reinforcement (STAR). STAR provides a robust framework for quantitative assessment of alcohol use disorder-related behaviors while still allowing flexibility in overall experimental design required for investigation of the neural mechanisms underlying reinforcement learning and aberrant motivational processes. We use the STAR framework to define longitudinal phenotype dynamics in alcohol use disorder-relevant behaviors over the course of repeated alcohol use and binge drinking. The adaptable design allows for any number of other methodologies, investigative or intervening, to be easily integrated to tailor for the specific question being asked. Further, using STAR in combination with tissue analytics, we reveal putative neuro-biomarkers of heightened alcohol abuse. Tissue from medial prefrontal cortex (mPFC) and dorsal periaqueductal gray area (dPAG) was neurochemically screened through LC-MS to investigate biomarkers across the drinking phenotypes that were implicated in alcohol use disorder-related behaviors. These results revealed multiple analytes in both regions that differed significantly across phenotypes. For example, dopamine- and 5-HT-related analytes in both the mPFC and dPAG were positively correlated with ethanol intake, but not during punished sessions, whereas excitatory and inhibitory transmitters in the dPAG were positivity correlated with punishment resistance. Overall, our results provide a novel and robust platform for studying the development of drinking behavior in mice that is both flexible and easily integrated with existing technologies, making it a strong model for studying the development of increased alcohol use and compulsion from a behavioral, circuit, and population perspective. Lastly, our data points to several neural analytes in cortical and brainstem regions that imply the possibility of underlying neuro-biomarkers for vulnerability to compulsive drinking behaviors.

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Poster

231. Neural Circuit Mechanisms Underlying Alcohol Use

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Program #/Poster #: 231.26

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA020919
NIH Grant DA035958

Title: Neuroimmune-mediated alteration of typical alcohol response in mesolimbic dopamine system

Authors: *C. A. SMALL[1], P. E. WILLIAMS[1], J. D. OBRAY[2], J. T. YORGASON[1], S. C. STEFFENSEN[1];
[1]Brigham Young Univ., Provo, UT; [2]Med. Univ. of South Carolina, Charleston, SC

Abstract: The effects of ethanol (EtOH) on the brain are closely linked to neuroimmune responses mediated by microglia and the possible infiltration of the blood-brain barrier (BBB) by peripheral macrophages, which has been reported in studies with high doses of EtOH or with chronic EtOH exposure. We have shown that dopamine (DA) transmission is increased by acute EtOH via the inhibition of ventral tegmental area (VTA) GABA neurons and inhibited during withdrawal to chronic EtOH due to hyperexcitation of VTA GABA neurons. This study aimed to determine the effect of acute EtOH on mesolimbic microglia activation and the effects of macrophage depletion on the typical physiological response to EtOH. We hypothesized that acute EtOH would lead to an increase in neuroinflammation by activating resident microglia to an inflammatory polarization (M1) and that peripheral macrophage depletion would result in atypical DA transmission in mesolimbic DA system. By measuring volume/surface area of microglia in the ventral tegmental area (VTA) and nucleus accumbens (NAc) following EtOH, we found that EtOH increased microglia activation in the VTA and NAc, shifting microglia toward an M1 polarization, consistent with our hypothesis. We also found that depleting macrophages in the blood approximately 50% with liposomal clodronate reduced EtOH inhibition of VTA GABA neuron firing rate and reduced subsequent DA release in the NAc. We also found similar inhibition of typical EtOH effects on locomotor activity compared to clodronate control liposomes. Overall, these findings suggest that the typical physiological response to ETOH in the mesolimbic DA system is mediated, at least in part, through neuroimmune processes. This challenges the dogma that acute EtOH has exclusively central effects on DA neuronal activity and release.

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Poster

232. Nicotine: Pharmacology and Circuits Effects

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Program #/Poster #: 232.01

Topic: G.09. Drugs of Abuse and Addiction

Support: TRDRP T30IP0931
TRDRP T32DT5202

Title: Lynx2 Proteins Mediate Nicotine Relapse-Related Behaviors

Authors: *M. BAUTISTA, J. P. FOWLER, C. D. FOWLER;
Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Tobacco use results in several adverse health consequences and remains a leading cause of preventable death and disease in the United States. Of those who attempt to quit smoking, very few are successful each year, thereby illustrating the moderate efficacy of currently available nicotine cessation aids. Interestingly, endogenous allosteric modulators have emerged as a novel target of interest for drug development. Lynx2 proteins are a negative allosteric modulator of the nicotinic acetylcholine receptor (nAChR) and have been shown to affect nAChR activity in the presence of an agonist. Given that the nAChRs underlie nicotine-associated behaviors, lynx2 may play a role in modulating drug seeking. In this study, we used *Lynx2* knockout mice and their wildtype littermates to explore the role of lynx2 proteins in nicotine relapse-related behaviors. Lynx2 knockout mice and their wildtype littermates were examined for differences in intravenous nicotine self-administration and incubation of craving, a measure of relapse. Our data indicate that lynx2 knockout mice have altered nicotine relapse-related behaviors in comparison to their wildtype littermates. Together, these findings demonstrate that lynx2 proteins play a role in nicotine seeking, thereby further validating this target for drug development efforts. This work was supported by funding from the Tobacco-Related Disease Research Program (TRDRP T30IP0931 to CDF and TRDRP T32DT5202 to MB).

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Poster

232. Nicotine: Pharmacology and Circuits Effects

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Program #/Poster #: 232.02

Topic: G.09. Drugs of Abuse and Addiction

Support: Tobacco and Related Disease Research Program (TRDRP) 22RT-0103, T31P1427
UCI School of Medicine Startup Funds (TR001414)

Title: Nicotine plus cue-primed reinstatement is enhanced in adolescent Sprague-Dawley rats containing the human CHRNA6 3'UTR polymorphism (rs2304297) in the alpha(α)6 nicotinic acetylcholine receptor subunit

Authors: *D. CARRENO[1], S. LOTFIPOUR[2];
[1]Univ. of California, Irvine, [2]Dept. of Emergency Med., Univ. of California, Irvine, Irvine, CA

Abstract: Background: Nearly 40 million adults in the United States are current smokers, the majority of whom began smoking during adolescence. Large-scale human candidate gene studies have indicated a genetic variant in the alpha(α)6 nicotinic acetylcholine receptor subunit (nAChR), encoded by CHRNA6, may play a key role in adolescent smoking. We hypothesize the CHRNA6 3'UTRC123G single nucleotide polymorphism (SNP), rs2304297, selectively enhances nicotine + cue-primed reinstatement, but not nicotine- or cue-only reinstatement in GG (risk) versus CC (non-risk) allele carriers. Methods: Using CRISPR-Cas9 genomic engineering, we developed a humanized rat line with the exact human gene variant of the CHRNA6 3'UTRC123G locus in Sprague Dawley rats. Genetically modified adolescent male and female rats were food trained under a fixed-ratio one (FR1) schedule of reinforcement and progressively increased to FR5TO20. Animals were implanted with catheters and began intravenous nicotine self-administration (15 μg/kg/infusion) at FR5. Upon reaching stable responding, progressive ratio was tested, followed by extinction of reinforcement behavior by removal of drug and cues. After reaching extinction criteria, reinstatement testing began for cue only, nicotine only, and nicotine + cue in a Latin square design. Animals were returned to extinction conditions 2 days minimum between testing. Results: No genotype effects were observed for food reinforcement, during acquisition of intravenous nicotine self-administration at FR5 or progressive ratio schedule of reinforcement. All animals showed a preference for reinforced versus non-reinforced responding. Male and female CC and GG-allele carriers exhibited equivalent nicotine reinforcement and extinction. Male GG rats exhibited enhanced nicotine + cue-primed reinstatement as compared with male CC rats, which was not observed in females. Conclusions: Our findings indicate the male GG-carriers as compared with CC-carriers exhibit enhanced nicotine + cue-primed reinstatement without altering natural food reward, nicotine reinforcement, cue- or nicotine-only reinstatement. The findings support the in vivo functionality of a non-coding CHRNA6 3'UTRC123G SNP in nicotine seeking behavior, which may help with future drug cessation strategies.

Disclosures: D. Carreno: None. **S. lotfipour:** None.

Poster

232. Nicotine: Pharmacology and Circuits Effects

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Topic: G.09. Drugs of Abuse and Addiction

Support: TRDRP/UCOP Grant 22RT-0103
T31IP1427

Title: Nicotine-induced dopamine release in adolescent humanized CHRNA6 3'UTR^{C123G}mutant rats

Authors: S. LOTFIPOUR, ***A. FACUNDO**, D. CARRENO;
Univ. of California, Irvine, Irvine, CA

Abstract: Introduction: Adolescent nicotine consumption via electronic cigarettes has become increasingly popular, contributing to polysubstance abuse amongst adolescents. The rewarding aspects of smoking are attributed to the neuronal dopaminergic system in the limbic circuitry. Nicotine modulates neuronal nicotinic acetylcholine receptor (nAChR) systems which are crucial for reward processing and drug reinforcement. Previous preclinical studies using genetic animal models found alpha(α)6-containing nAChRs in the ventral tegmental area (VTA) to be necessary for nicotine-induced locomotion, dopamine release, and nicotine self-administration. A single nucleotide polymorphism (SNP) in the 3'-untranslated region (UTR) of the α6 nicotinic receptor subunit gene *CHRNA*6, rs2304297, was associated with nicotine/tobacco and general substance use during adolescence. To understand the mechanistic role of the *CHRNA*6 3'UTR^{C123G} SNP in adolescent nicotine use, our lab used CRISPR-Cas9 genomic engineering to develop a humanized rodent line including either the G*G* risk allele or CC non-risk allele of the human α6 3'UTR^{C123G} SNP α6^{GG} and α6^{CC}, respectively). We hypothesized the *CHRNA*6 3'UTR^{C123G} SNP would sex- and genotype-dependently enhance nicotine-induced dopamine release in the brains of adolescent male and female α6^{GG} and α6^{CC} carriers. **Methods:** In this study, adolescent male and female α6^{CC} and α6^{GG} *CHRNA6* 3'UTR^{C123G} Sprague-Dawley rats underwent a 4-day sub-chronic, low-dose (0.03 mg/kg/0.1 mL) nicotine pretreatment paradigm to assess nicotine-induced dopamine release in the nucleus accumbens shell (NAcS) using *in vivo* microdialysis coupled with high-performance liquid chromatography-electrochemical detection (HPLC-ECD). **Results:** Our preliminary data suggest nicotine-induced dopamine release in α6^{CC} and α6^{GG} groups, with a blunted potentiation in α6^{GG} groups. **Conclusion:** Future efforts will be made to increase our group size. This will help elucidate how the *CHRNA*6 3'UTR^{C123G} SNP influences the observed nicotine-seeking phenotypes in adolescents and the postulated gateway effect of nicotine towards abuse of other illicit substances.

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Poster

232. Nicotine: Pharmacology and Circuits Effects

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Topic: G.09. Drugs of Abuse and Addiction

Support: ACADIA Pharmaceuticals grant
U. Houston-Clear Lake Biobehavioral Research Fund

Title: A 5-HT_{2A} inverse agonist reverses nicotine withdrawal effects on sleep stages.

Authors: *J. M. JAMES[1], J. C. SHAHIN[1], H. L. CHAPMAN[1], D. H. MALIN[1], P. TSAI[1], A. SUZAKI[1], E. S. BURSTEIN[2], C. P. WARD[1];
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Abstract: Sleep disturbances are common in nicotine withdrawal, increasing risk for relapse to smoking. This study determined if the selective 5-HT2A serotonin receptor inverse agonist MDL 100907 (volinanserin) can increase time spent in restorative NREM sleep and reduce sleep fragmentation. All surgery was conducted under isoflurane anesthesia. Male Sprague-Dawley rats (N = 33) were implanted with EEG and EMG electrodes and with osmotic minipumps continuously infusing either 9 mg/kg/day s.c. nicotine bitartrate in saline or saline alone. After 7 days, pumps were removed to induce spontaneous nicotine withdrawal syndrome. Rats were injected i.p. with 1 mg/kg MDL 100907 (volinanserin) in a vehicle of saline/DMSO/Tween80 or with a vehicle alone, 17 hours post-pump removal. The three treatment groups were: No Nicotine/Vehicle (n = 11), Withdrawal/Vehicle (n = 13) and Withdrawal/MDL 100907 (n = 9). Rats were monitored during peak withdrawal (18 to 22 hours post-pump removal) within the sleep-intensive, lights-on cycle. The EEG and EMG waves were scored by SleepSign software for the time spent in and number of separate bouts of Wake, NREM and REM. For accuracy, the resulting tracings were also scored manually under blind conditions. For % of total time in each stage (M ± SEM), all p values are based on Fisher's LSD post-hoc test following one-way ANOVA, * p = .022, †.05 < p < 1.0 vs. Withdrawal/Vehicle. Time % in NREM was 71.16 ± 2.45† in the No Nicotine/Vehicle group, 63.18 ± 2.45 in the Withdrawal/Vehicle group and 75.89 ± 2.78* in the Withdrawal/MDL 100907 group. Time % in REM was 12.60 ± 5.96 in the No Nicotine/Vehicle group, 12.02 ± 2.31 in the Withdrawal/Vehicle group and 8.08 ± 2.78† in the Withdrawal/MDL 100907 group. Time % in Wake was 16.24 ± 2.24 in the No Nicotine/Vehicle group, 24.79 ± 5.80 in the Withdrawal/Vehicle group and 16.03 ± 3.36 in the Withdrawal/MDL 100907 group. The effect of nicotine withdrawal in the absence of MDL 100907 is indicated by the difference between Withdrawal/Vehicle and No Nicotine/Vehicle. Reversal of the withdrawal effect is indicated by comparison with the difference between Withdrawal/Vehicle and Withdrawal/MDL 100907. The nicotine withdrawal effect on % time in Wake was reversed by 102.5%; the effect on % time in NREM was reversed by 159.7%. Compared with nicotine dependent rats injected with vehicle, dependent rats injected with MDL 100907 had 35.6% fewer separate Wake bouts, p = .063; 59.5% fewer REM bouts, p = .004 and 48.0% fewer NREM bouts, p < .001. Inactivating 5-HT2A receptors may favor NREM sleep and reduce sleep fragmentation, reversing the disruptive effects of nicotine withdrawal.

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Poster

232. Nicotine: Pharmacology and Circuits Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 232.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH DA039658

Title: Gpr3 as a novel therapeutic target for nicotine addiction

Authors: *S. N. CHEEKS[1], M. R. BAUTISTA[1], B. BLOUGH[2], E. A. GAY[2], C. D. FOWLER[1]; [1]Neurobio. and Behavior, UC, Irvine, Irvine, CA; [2]Ctr. for Drug Discovery, RTI Intl., Research Triangle Park, NC

Abstract: GPR3 as a Novel Therapeutic Target for Nicotine Addiction
Samantha Cheeks[1], Malia Bautista[1], Bruce Blough[2], Elaine Gay[2] and Christie Fowler[1]
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Although many individuals express a desire to quit smoking tobacco, currently available therapeutics have proven to be only moderately efficacious, where only one in ten individuals are able to quit long term. With the recent emergence of e-cigarettes, a new generation of individuals are developing symptoms of addiction. Thus, a pressing need exists for target identification and validation to derive novel therapeutics. We have previously shown that the projection from the medial habenula to the interpeduncular nucleus attenuates nicotine reinforcement, and thus, selective modulation of this brain pathway may provide an innovative therapeutic approach. Here we seek to validate whether the orphan G_s-protein coupled receptor GPR3, which is expressed selectively in the medial habenula, can alter nicotine intake in a mouse model. Adult, male and female C57BL6/J mice were assessed in the intraveneous nicotine self-administration protocol. After establishing consistent responding for nicotine, mice were administered the GPR3 agonist, EGBB-158, in a Latin-square design across sessions. EGBB-158 was also examined for its effects on operant responding for food reward. We found that administration of the GPR3 agonist, EGBB-158, attenuated nicotine intake in the intravenous nicotine self-administration procedure. Interestingly, a higher dose of EGBB-158 also decreased food self-admiministration, suggesting that EGBB-158 may additionally serve as a potential therapeutic to decrease food consumption. Administration of EGBB-158 did not alter locomotion, providing evidence that the effects were not due to generalized behavioral inhibition. Taken together, these studies establish GPR3 as a novel, promising target for nicotine dependence.
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Disclosures: S.N. Cheeks: None. **M.R. Bautista:** None. **B. Blough:** None. **E.A. Gay:** None. **C.D. Fowler:** None.

Poster

232. Nicotine: Pharmacology and Circuits Effects

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 232.06

Topic: G.09. Drugs of Abuse and Addiction

Support: T31KT1859 UC

Title: Cannabidiol prevents withdrawal symptoms and relapse to nicotine vapor in rats

Authors: C. CROOK, S. DIRIK, A. R. MARTINEZ, T. E. HUGHES, M. R. IYER, C. E. BLUCHER, P. SCHWEITZER, G. DE GUGLIELMO, ***M. KALLUPI**;
UCSD Depart. Psychiatry, San Diego, CA

Abstract: Preclinical models of voluntary nicotine electronic cigarette have shown that 3 weeks of daily (1 h) nicotine vapor self-administration produce addiction-like behaviors, including somatic signs of withdrawal, anxiety-like behavior, and relapse-like behavior during abstinence. Cannabidiol (CBD) is known to modulate nicotinic receptor function. However, preclinical evaluation of its efficacy on nicotine vapor dependence is still lacking. The goal of this study was to test the preclinical efficacy of chronic CBD treatment by preventing nicotine vapor dependence, using measures of withdrawal, anxiety, and relapse. Rats self-administered daily 0.5mg/ml of nicotine vapor in 1 h sessions via operant vapor chambers over 3 weeks. One group of rats received CBD injections (30 mg/kg/day) for 2 weeks, or its vehicle, starting after stable nicotine intake was achieved. The control group included rats that self-administered air vapor and received CBD or vehicle injections of sesame oil. Throughout the experiment, withdrawal, anxiety, and relapse induced by stress and cues associated to nicotine vapor were performed. CBD (30 mg/kg/day) prevented rats from exhibiting somatic signs of withdrawal and anxiety like behaviors during acute abstinence. In rats with a history of nicotine vapor self-administration, presentation of stress and stimuli predictive of drug availability reinstates drug seeking, triggering relapse. The animals that self-administered nicotine vapor and chronic treatment of CBD did not reinstate their behavior.This preclinical study suggests that CBD treatment may be beneficial as a strategy to alleviate the withdrawal symptoms and relapse in animal models of nicotine vapor self-administration.

Disclosures: C. Crook: None. **S. Dirik:** None. **A.R. Martinez:** None. **T.E. Hughes:** None. **M.R. Iyer:** None. **C.E. Blucher:** None. **P. Schweitzer:** None. **G. de Guglielmo:** None. **M. Kallupi:** None.

Poster

232. Nicotine: Pharmacology and Circuits Effects

Location: SDCC Halls B-H

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Program #/Poster #: 232.07

Topic: G.09. Drugs of Abuse and Addiction

Support: Collaborative Study on the Genetics of Alcoholism COGA U10 AA008401
Multi 'Omics Integration and Neurobiological Signatures of Alcohol Use Disorder (AUD) R01 AA027049
Integrating Epigenomics in Human Brain and Genomics of Nicotine Dependence R01 DA042090
UK Biobank Resource under Application Number 48123

Title: Integrating Brain Imaging Phenotypes, Genomics, and Substance Use

Authors: *Y. CHANG[1], V. E. THORNTON[2], A. CHALOEMTOEM[2], J. BIJSTERBOSCH[1], A. ANOKHIN[2], L. FOX[1], L. J. BIERUT[2];
[2]Psychiatry, [1]Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Cigarette smoking is associated with persistent neurochemical and pathological changes in brain. This study uses UK Biobank data to examine smoking behaviors, brain measures, and genetics to understand whether these associations represent predisposing features or consequences of smoking behaviors.
Using the currently available subset of UK Biobank data of 40,000 individuals, we studied the association between cigarette smoking and brain imaging-derived phenotypes (IDPs). We examined 4 brain measure IDPs and 930 regional IDPs (474 structural and 450 diffusion MRI-derived phenotypes and 6 resting-state IDP groups and 4 total brain measure IDPs. Individuals were divided into those with a history of daily smoking and those who never smoked more than 100 cigarettes in their lifetime. We performed regression analyses to measure the association between 936 IDPs and a history of daily smoking versus never smoking. In those regions associated with a lifetime history of smoking, we then examined pack years of smoking in people with a history of smoking to determine if there was a dose response effect. Then we created smoking-related polygenic risk scores (PRSs) of ever smoking using summary statistics from GWAS & Sequencing Consortium of Alcohol and Nicotine use to determine is this score was associated with smoking behaviors and IDPs.
A lifetime history of daily smoking had a strong association with total brain volume, grey and white matter volume, and ventricular CSF. A total of 119 of 474 structural IDPs and 137 of 450 diffusion IDPs were associated (after total brain correction, 28 and 65 remain, respectively). The brain regions most significantly associated were putamen, caudate, pallidum for both hemispheres of the brain. For diffusion tracts, fornix, corona radiata, and longitudinal fasciculus were most significantly associated with the adverse direction of effect (decreased white matter integrity). The dose-effect of smoking (pack-years) showed that a higher dose of cigarettes was associated with decreased brain volume and white matter integrity. PRS of ever smoking showed a strong association with a lifetime history of daily smoking, but no significant association with brain IDPs.
In summary, this evidence supports a causative effect of daily smoking resulting in widespread pathologic changes in the brain, and these changes do not represent genetic predisposing factors to smoking behaviors.

Disclosures: **Y. Chang:** None. **V.E. Thornton:** None. **A. Chaloemtoem:** None. **J. Bijsterbosch:** None. **A. Anokhin:** None. **L. Fox:** None. **L.J. Bierut:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Patent 8,080,371,"Markers for Addiction".

Poster

232. Nicotine: Pharmacology and Circuits Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 232.08

Topic: G.09. Drugs of Abuse and Addiction

Support: SSA/TRDRP 27IR-0047A

Title: Nicotine-dependent rats exhibit acute increased food intake that is mediated by neural and metabolic changes

Authors: *K. SHANKAR[1,2], S. BONNET-ZAHEDI[2], O. GEORGE[2];
[1]The Scripps Res. Inst., La Jolla, CA; [2]Psychiatry, UC San Diego, La Jolla, CA

Abstract: The current dogma within the field of nicotine addiction is that nicotine is an anorectic agent that decreases food intake and increases energy metabolism, leading to decreased body weight gain. However, we recently showed that acute intake of nicotine produces a short-term (15 minute) increase in feeding behavior in nicotine-dependent rats via intravenous self-administration, while still displaying long-term (days) anorexigenic effects. We used a two-pronged approach to identify central and peripheral signaling changes that may be contributing to this behavioral phenomenon. As our primary behavioral results show differences between non-dependent and dependent animals, our studies focused on comparisons of acute nicotine intake in dependent *vs.* non-dependent rats. Analysis of regional cFos signaling changes revealed increases in hyperphagia-inducing regions including the lateral hypothalamus, arcuate nucleus, and solitary tract nucleus, as well as decreases in hypophagia-inducing regions including the ventromedial hypothalamus and lateral parabrachial nucleus. Because some of the brain regions we analyzed integrate signals from peripheral hormones and peptides, we studied changes in peripheral metabolic signaling using a model of passive nicotine administration. In looking at changes in feeding-related hormones, we found that chronic nicotine exposure produced tolerance to insulin, and acute injection of nicotine in dependent animals decreased signaling of GLP-1, an anorectic hormone. Our data shows that acute nicotine intake in nicotine-dependent rats is associated with activation of hunger-related brain regions and dysregulation of satiety-regulating hormones.

Disclosures: **K. Shankar:** None. **S. Bonnet-Zahedi:** None. **O. George:** None.

Poster

232. Nicotine: Pharmacology and Circuits Effects

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 232.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NRF-2021R1A2C2008083

Title: Nicotine Rather Than Non-Nicotine Substances in 3R4F WCSC Increases Behavioral Sensitization and Drug-Taking Behavior in Rats

Authors: *S. KIM, S. SOHN, E. S. CHOE;
Dept. of Biol. Sci., Pusan Natl. Univ., Busan, Korea, Republic of

Abstract: Nicotine increases reinforcing effects of cigarette smoking by upregulating glutamate and dopamine releases via stimulation of nicotinic acetylcholine receptors (nAChRs) in the dorsal striatum (CPu). The present study was conducted to evaluate whether non-nicotine substances in cigarette smoke potentiate nicotine-induced behaviors by increasing glutamate and dopamine concentrations in the CPu. Changes in the levels of glutamate and dopamine in the CPu were analyzed using a glutamate colorimetric assay and dopamine enzyme-linked immunosorbent assay, respectively, after repeated administration of nicotine or whole cigarette smoke condensate (WCSC) in male Sprague-Dawley rats. Changes in locomotion and drug-taking behavior were analyzed using the measurements of locomotor activity and self-administration under a fixed ratio 1 schedule in response to repeated administration of nicotine or WCSC. Repeated subcutaneous (s.c.) injections of nicotine (0.25 mg/kg/day) for seven consecutive days significantly increased the levels of glutamate and dopamine in the CPu. Similar results were obtained from repeated injections of WCSC (0.25 mg/kg nicotine/day, s.c.) extracted from 3R4F Kentucky reference cigarettes. Parallel with the increases in the neurotransmitter levels in the CPu, both nicotine and WCSC increased locomotor activity and self-administration (0.03 mg/kg nicotine/infusion). However, repeated injections of WCSC did not change the nicotine-induced increases in neurotransmitter levels, locomotor activity, and self-administration. Nicotine rather than non-nicotine substances in WCSC play a major role in potentiating behavioral sensitization and drug-taking behavior via elevation of glutamate and dopamine concentrations in the CPu of rats.

Disclosures: S. Kim: None. **S. Sohn:** None. **E.S. Choe:** None.

Poster

232. Nicotine: Pharmacology and Circuits Effects

Location: SDCC Halls B-H

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Program #/Poster #: 232.10

Topic: G.09. Drugs of Abuse and Addiction

Support: RO1DA047785
T32DA043469

Title: Glp-1 epidermal stem cell-based gene therapy attenuates nicotine reward

Authors: *L. RIEDY[1], Q. KONG[2], J. LEE[3], X. WU[3], M. XU[2,1];
[1]Committee on Neurobio., [2]Dept. of Anesthesia and Critical Care, [3]Ben May Dept. for Cancer Res., Univ. of Chicago, Chicago, IL

Abstract: Smoking and the use of other tobacco products remains the leading cause of preventable death worldwide. Nicotine is the main reinforcing component of tobacco that drives the use of cigarette and other tobacco products. Glucagon-like-peptide-1 (GLP-1) is a peptide produced both peripherally in the intestinal tract and centrally in the hindbrain and serves to regulate blood glucose homeostasis and feeding behavior. GLP-1 receptor activation has also been shown to attenuate a variety of drug mediated behaviors including the acquisition and reinstatement of nicotine conditioned place preference (CPP) and nicotine intravenous self-administration. GLP-1 receptor agonists are degraded rapidly with short circulating half-lives, and therefore, require repeated and costly dosing. We have developed a skin cell-based gene delivery platform to genetically alter mouse epidermal stem cells such that they promote the long-term, continuous production and reversible release of GLP-1. We have previously shown that this skin cell-based gene therapy can successfully reverse diet-induced weight gain and insulin resistance in a type 2 diabetes mouse model as well as attenuate acquisition and reinstatement of alcohol abuse. In the present study, we performed nicotine CPP using 0.4 mg/kg nicotine in GLP-1 skin grafted (n=7) and non-grafted nicotine control (n=7). GLP-1 skin grafted mice do not acquire nicotine CPP as compared to non-grafted nicotine controls ($p < 0.05$). We then performed drug induced reinstatement of nicotine CPP using an acute priming dose of 0.25 mg/kg nicotine in additional non-grafted nicotine (n=8) and saline (n=4) control male mice. Non-grafted nicotine controls showed robust acquisition and reinstatement of nicotine CPP whereas non-grafted saline controls did not. We hypothesize that GLP-1 skin grafted mice will be protected from reinstatement of nicotine CPP. Finally, to assess nicotine oral self-administration, we performed a nicotine 2 bottle choice (2BC) in non-grafted controls (n=16). We determined an ascending dose response curve at 0, 10, 25, 50, and 100 ug/mL nicotine showing increased nicotine consumption at each ascending dose. We hypothesize that GLP-1 skin grafted mice will show attenuated self-administration of nicotine water across all doses.

Disclosures: L. Riedy: None. **Q. Kong:** None. **J. Lee:** None. **X. Wu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder. **M. Xu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder.

Poster

232. Nicotine: Pharmacology and Circuits Effects

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 232.11

Topic: G.09. Drugs of Abuse and Addiction

Title: Examination of pharmacotherapies for nicotine use in a rodent model of diabetes.

Authors: *S. ORTEGON[1], P. GINER[1], L. M. CARCOBA[2], L. E. O'DELL[2];
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Abstract: There is a lack of information on the effectiveness of pharmacotherapies used to treat diabetes on nicotine use. As a first step in addressing this issue, we examined the effects of insulin and bromocriptine (Cycloset) on nicotine intravenous self-administration (IVSA) and glucose levels in a rodent model of diabetes. Insulin is a commonly prescribed hormone treatment that normalizes glucose levels in patients with diabetes. Bromocriptine is a dopamine agonist that that normalizes post-meal increases in blood glucose levels. Briefly, female and male rats received a chronic high-fat diet (HFD) plus a low dose of streptozotocin (STZ; 25 mg/kg) that induces insulin resistance. Control rats received a regular diet (RD) plus a low dose of STZ. STZ is toxic to insulin-producing beta cells and produces an increase in glucose levels. The rats were then given extended access to IVSA of nicotine (0.03 mg/kg for 6 weeks) in an operant box where they continued to consume their respective diet and performed nose poke responses for water deliveries. Each nicotine dose was delivered for 4 days with 3 intermittent days of abstinence in their home cage. Plasma glucose levels were assessed every day of the IVSA regimen. The rats received systemic administration of bromocriptine (3.0, 3.0, 10 mg/kg) and then insulin (0.75 U/kg) each day for one week at 6 pm at the onset of their night cycle. The results revealed that bromocriptine dose-dependently increased nicotine intake and peripheral glucose levels, and these effects were greater in HFD+STZ rats relative to RD+STZ controls. In contrast, insulin administration reduced nicotine IVSA and glucose levels in HFD+STZ rats versus controls. Our findings suggest that pharmacotherapies that reduce glucose levels may be more effective at reducing nicotine use in patients with diabetes.

Disclosures: S. Ortegon: None. **P. Giner:** None. **L.M. Carcoba:** None. **L.E. O'Dell:** None.

Poster

232. Nicotine: Pharmacology and Circuits Effects

Location: SDCC Halls B-H

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Program #/Poster #: 232.12

Topic: G.09. Drugs of Abuse and Addiction

Support: MOE T2EP30220-0020
Yale-NUS College IG19-BG106
Yale-NUS College IG18-BG101
Yale-NUS College SUG

Title: Alpha-3 nicotinic acetylcholine receptors regulate acute tolerance to nicotine and impact behaviors associated with nicotine addiction and comorbid disorders.

Authors: *A. S. MATHURU[1,2,3], C. KIBAT[1], T. GOEL[2];
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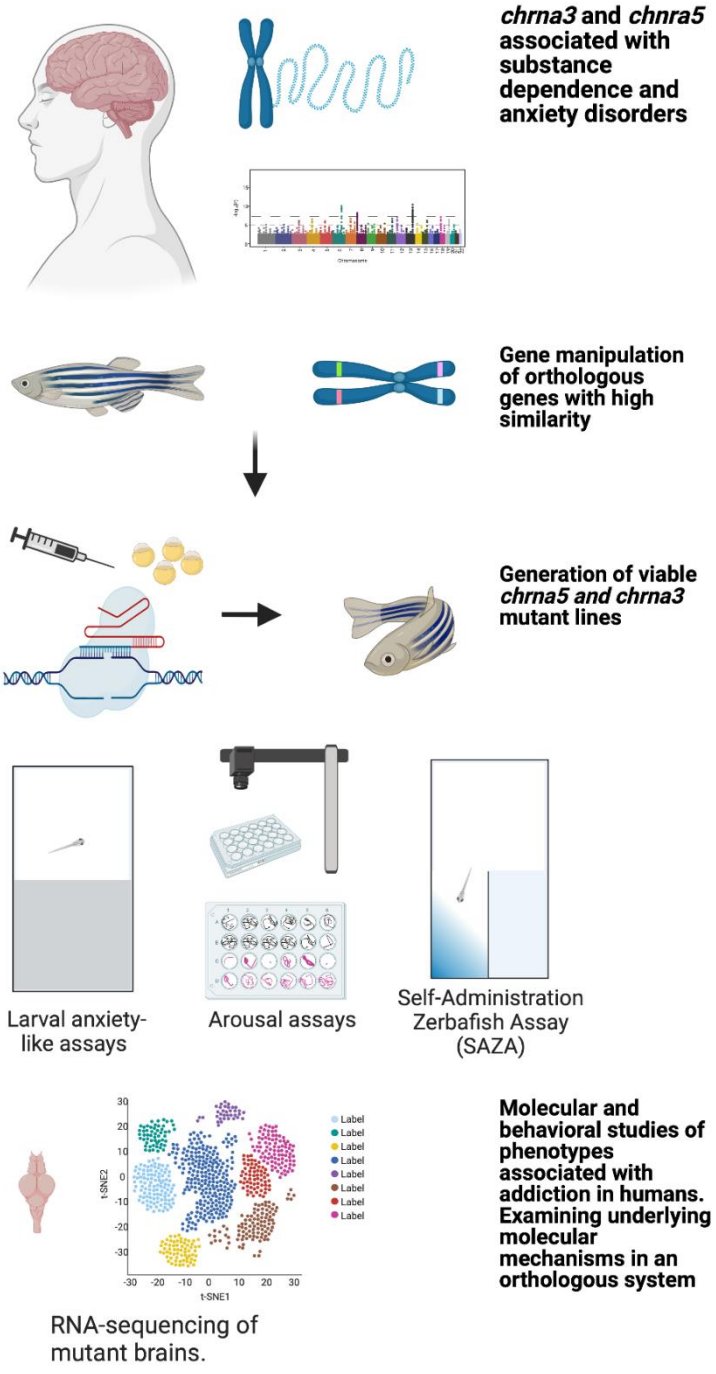
Abstract: Nicotine dependence is often comorbid with susceptibility to multi-substance addiction and psychiatric ailments such as anxiety disorders and clinical depression. This points towards potential common genetic players and neural circuits. Here, we show that zebrafish are powerful systems to uncover the underlying neurobiology. Human genetic studies have associated *chrna3* and *chrna5* genes that code for the α3 and α5 nicotinic acetylcholine receptor (nAChR) subunits with nicotine dependence, alcohol dependence, anxiety disorders, and neuroticism. We generated zebrafish homozygous mutant lines in both these genes using the CRISPR/Cas-9 technique. We developed a two-choice Self-Administration Zebrafish Assay (SAZA) to quantify voluntary preference for nicotine and alcohol. In the first series of experiments, we found that wild-type zebrafish, like alcohol self-administering mammals, displayed hormesis or an inverted U-shaped preference for increasing concentrations of alcohol. This finding validated a key aspect of the vertebrate response to alcohol in zebrafish. Next, we found that *chrna5* mutant fish phenocopied rodents mutant for *Chrna5* and were tolerant to high concentrations of nicotine and alcohol. Finally, we examined the behaviors of *chrna3* mutants. The two *chrna3* mutants showed overlapping, but slightly different behaviors. One mutant missing transmembrane and intracellular domains showed no avoidance of nicotine at high concentrations, displayed increased anxiety-like behaviors, had changes in appetitive behavior, and showed disrupted circadian behavior. The second mutant, with a premature stop codon resulting in a loss of ligand binding domain, showed an increased preference for nicotine at low concentrations, and a mild effect on anxiety-like behaviors, appetite, and circadian behavior. RNA-sequencing of brains suggested large transcriptional program change in the mutants that may explain these broad impacts. Together, our results provide evidence for the use of zebrafish in neurogenetic studies to model human addiction and comorbid disorders.



Disclosures: **A.S. Mathuru:** None. **C. Kibat:** None. **T. Goel:** None.

Poster

233. Mechanisms of Attention: Rodent Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 233.01

Topic: H.01. Attention

Support: PHS Grant RO1DA045063

Title: Vulnerable for addiction-like behavior: Disrupted cholinergic signaling and exaggerated (neuro)immune response in sign-tracking rats

Authors: *H. CARMON[1], V. V. PARIKH[2], E. HALEY[3], N. C. TRONSON[1], M. F. SARTER[1]; [1]Univ. of Michigan, ANN ARBOR, MI; [2]Psychology & Neurosci., Temple Univ. Grad. Neurosci. Program, Philadelphia, PA; [3]Temple Univ., Philadelphia, PA

Abstract: Some rats (sign-trackers; STs) are prone to attribute incentive salience to reward cues, which can manifest as a propensity to approach and contact Pavlovian cues, and for addiction-like behavior. STs also exhibit poor attentional performance, relative to their counterparts, the goal-trackers (GTs), mediated by attenuated cholinergic activity. Poor cholinergic-attentional control contributes to the propensity of STs to approach and utilize Pavlovian drug cues. In STs, increases in neuronal activity fail to translocate choline transporters (CHTs) into synaptosomal plasma membrane, thereby limiting the capacity of cholinergic terminals to sustain cholinergic activity. Here we investigated post-translational modifications responsible for disrupted CHT trafficking in STs, and the hypothesis that attenuated cholinergic activity in STs causes exaggerated (neuro)immune responses. We determined levels of ubiquitinylated CHTs because ubiquitin-mediated processes can account for attenuated externalization of intracellular CHTs, ranging from proteolysis to enhanced internalization or alternative compartmentalization. The proportion of ubiquitinylated CHTs, extracted from cortex and striatum, was robustly higher in STs than in GTs, with no overlap in the data from the two phenotypes. Moreover, modified CHTs located in intracellular domains, but not in synaptosomal plasma membrane, completely accounted for the phenotype-specific ubiquitylation levels. To explore the hypothesis that elevated levels of brain CHT ubiquitinylation are associated with, and perhaps secondary to, an elevated neuroinflammatory state in STs, we challenged STs and GTs with the endotoxin lipopolysaccharide (LPS). Following LPS challenge, ubiquitinylation levels of CHTs in cortex and striatum were drastically increased in GTs, but not STs, suggesting that in unchallenged STs, ubiquitinylated CHTs already are at maximum levels and unresponsive to an additional immune challenge. The effect of LPS in GTs, however, support a relationship between neuroinflammation and CHT ubiquitinylation. Furthermore, elevated levels of CHT ubiquitinylation in STs were associated with elevated levels of cytokines in the brain, but not spleen. Together, these findings suggest potentially escalating, bidirectional interactions between disrupted cholinergic signaling and elevated (neuro)immune responses as an integral component of the cognitive-motivational trait indexed by sign-tracking.

Disclosures: H. Carmon: None. **V.V. Parikh:** None. **E. Haley:** None. **N.C. Tronson:** None. **M.F. Sarter:** None.

Poster

233. Mechanisms of Attention: Rodent Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 233.02

Topic: H.01. Attention

Support: NIH grant P50NS091856

Title: Cortico-striatal transfer of movement commandsin rats with opposed attentional biases.

Authors: *C. AVILA[1], M. SARTER[2];
[1]Univ. of Michigan, Ann Arbor, MI; [2]Psychology, Univ. of Michigan Dept. of Psychology, Ann Arbor, MI

Abstract: Moving across dynamic surfaces and obstacles requires integrating cues that guide movements, such as turning commands. Failures to detect and integrate such cues (i.e., misses) can lead to life-threatening falls and hospitalization. Cholinergic modulation of fronto-cortical inter-and output neurons was previously demonstrated to mediate the detection of attention-demanding cues. We hypothesize that such cues are transferred into the striatum via cortico-striatal glutamatergic (GLU) projections and integrate with striatal movement sequencing. We investigated this hypothesis by recording GLU signaling in the striatal projection field of frontal cortical efferents, the dorsomedial striatum, in rats performing a task involving turn and stop-and-go signals. Moreover, we observed real-time GLU signaling in rats classified as sign-trackers (STs) and goal-trackers (GTs). Since STs were previously shown to exhibit relatively poor cortico-cholinergic attentional control, we predicted that turn cues are missed more frequently than in GTs and, if utilized, evoke truncated GLU activity. Amperometric recordings of GLU were time-locked to task events. STs did not miss more turn cues than GTs. In GTs, detected turn cues were associated with sharp increases in GLU activity, reaching 10-17 µM increases over baseline and peaking within 250-350 ms from cue onset. Missed cues did not evoke such transients in GTs. In STs, both detected and missed turn cues evoked comparable GLU transients. Using a dual-vector approach to silence cortico- striatal projections, we verified the cortical origin of the GLU activity recorded in these experiments. Moreover, silencing this pathway increased the number of misses in GTs, not STs. This evidence supports the hypothesis that cortico-striatal transfer of movement cues is essential for cue-guided movement. However, in rats exhibiting poor attentional control as a trait (STs), such transfer may be functionally replaced by bottom-up modulation of GLU release. Such “replacement” may be adequate for performing well-practiced, cued movements but is predicted to disrupt performance when in unfamiliar and dynamic environments. These findings suggest that selective signaling in GTs may account for superior attentional regulation due to elevated non-relevant noise filtering. Upon corticostriatal silencing, peak GLU amplitudes and cue-triggered turning were attenuated in GTs but not STs, further supporting that STs do not engage this top-down circuitry. Together, these results highlight phenotypic differences in cue-directed behavior and may elucidate the neural correlates of attentional control in complex movement.

Disclosures: C. Avila: None. **M. Sarter:** None.

Poster

233. Mechanisms of Attention: Rodent Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 233.03

Topic: H.01. Attention

Title: Cholinergic regulation of attentional effort

Authors: J. W. DE GEE[1,2], ***Z. H. MRIDHA**[1,2], M. THOMPSON[1,2], K. JASPE[1,2], M. J. MCGINLEY[1,2,3];
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Abstract: Attention is limited in capacity and costly to utilize. Therefore, organisms are driven by ongoing behavioral incentives to choose which stimuli to attend to (selective attention), and how much to attend to them (attentional effort). We previously showed that pupil-linked arousal helps regulating attentional effort to exploit increases in task utility (de Gee et al., 2022). Fluctuations in pupil size at constant luminance track the brain's global arousal state, and the activity of major neuromodulatory systems, including noradrenaline, acetylcholine, and perhaps also serotonin and dopamine. It is currently unknown which of these neuromodulatory systems are responsible for regulating attentional effort.
In the attentional effort task, mice learn to lick for sugar-water reward to report detection of the unpredictable emergence of temporal coherence in an ongoing tone-cloud. To detect all acoustic signals, mice would need to sustain an infeasibly high level of attentional effort across the 75-minute sessions. To probe adaptive effort allocation, the sugar-water reward size switched between high and low values in blocks of 60 trials. Thus, mice should increase attentional effort during blocks high reward blocks, to exploit the high utility, and reduce effort in low reward blocks, to conserve energy. We simultaneously recorded pupil size, walking speed, and acetylcholine concentration in auditory cortex via two-photon imaging of GRABACh (GPCR-based sensor).
We report behavioral and physiological signatures of adaptive shifts in behavior in 4 mice (24 experimental sessions). Mice better detected the weak sensory signal in the high vs low reward context. We focused on two modes of cholinergic activity: the tonic mode, as captured by the ACh concentration before trial onset, and the phasic mode, as captured by the magnitude of evoked ACh responses around the time of choice (lick). Irrespective of block type, evoked ACh responses were bigger for hits versus false alarms (28.1% increase, $p = 0.008$). This indicates that phasic cholinergic signaling helps optimize task performance. Importantly, irrespective of outcome, evoked ACh responses were bigger in the high versus low reward blocks (23.8% increase, $p = 0.001$). These results were not due to differences between conditions with respect to walking speed or lick rate.
In sum, we find that cholinergic signaling regulates attentional effort in mice. In ongoing work, we are determining the roles other neuromodulatory systems in mediating these adaptive shifts in cognitive behavior.

References:
de Gee et al. 2022. Mice regulate their attentional intensity and arousal to exploit increases in task utility. bioRxiv.

Disclosures: **J.W. De Gee:** None. **Z.H. Mridha:** None. **M. Thompson:** None. **K. Jaspe:** None. **M.J. McGinley:** None.

Poster

233. Mechanisms of Attention: Rodent Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 233.04

Topic: H.01. Attention

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Title: Disrupted choline clearance and sustained acetylcholine release in vivo by a common choline transporter coding variant associated with poor attentional control in humans

Authors: *S. KLAUSNER[1], E. DONOVAN[1], C. AVILA[1], V. PARIKH[2], C. FENOLLAR-FERRER[3], R. D. BLAKELY[4], M. SARTER[1];
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Abstract: The high-affinity choline transporter (CHT; SLC5A7) is essential for the synthesis and release of acetylcholine (ACh), a neurotransmitter that plays a vital role in attentional mechanisms, and inhibition of CHT can cause attentional impairments. A common CHT coding variant (rs1013940; Ile89Val) has been previously associated with reduced attentional control and attenuated frontal cortex activation in humans, as well as ADHD. Using a CRISPR/ Cas9 approach, we generated mice that express the naturally occurring CHT coding variant I89V to explore, in vivo, the effects on CHT-mediated choline clearance and ACh release, as well as behavior. CHT-mediated clearance of choline in male and female mice expressing one or two Val89 alleles was reduced by over 80% in cortex and over 50% in striatum in comparison to WT mice. In CHT Val89 mice, choline clearance was further reduced by neuronal inactivation. Five and 10 minutes after repeated depolarization at a low, behaviorally relevant frequency, deficits in ACh release indicated an attenuated presynaptic reloading capacity of cholinergic neurons in mutant mice. The Val89 variant did not impact the density of CHT protein in either total synaptosomal lysates or plasma-membrane-enriched fractions, suggesting a selective impact on CHT function. In order to determine potential effects of the minor allele on attention in mice, animals were trained to perform a sustained attention task and presented with a visual disruptor

to reveal potential differences in attentional control. Wild type mice responded to the distractor by adopting a more conservative response bias, that is, they exhibited a greater propensity for reporting the absence of a signal. In contrast, Val89 mice did not change their response bias, indicative of relatively diminished top-down attentional control, and perhaps equivalent to the relatively greater distractor vulnerability previously demonstrated in Val89 humans. Using a structural model based on a high-resolution structure of a CHT homolog (V. parahaemolyticus sodium/galactose symporter, vSGLT), we hypothesize that the loss of function of CVT 189V derives from an impact of the variant on conformational changes needed following choline binding to achieve normal transport rates. Our findings support the idea that the perturbed attentional performance in individuals expressing CHT Val89 is due to a diminished, sustained cholinergic signaling capacity. Moreover, we provide evidence that the CHT Val89 mutant mouse serves as a valuable model to further explore heritable risk for cognitive disorders arising from cholinergic dysfunction.

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Poster

233. Mechanisms of Attention: Rodent Models

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Topic: H.01. Attention

Support: NIH Grant F31NS122428

Title: Using fluorescent neuromodulator reporters to predict cortical acetylcholine and norepinephrine levels from arousal state-related behavioral variables

Authors: *E. NEYHART[1], N. ZHOU[2], C. SMITH[3], M. A. GALDAMEZ[2], Z. H. MRIDHA[4], J. DE GEE[4], M. J. MCGINLEY[2], J. REIMER[2];
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Abstract: Spontaneous transitions between high and low arousal, indexed by behavioral variables like pupil size and locomotion, have been indirectly linked to cortical levels of acetylcholine (ACh) and norepinephrine (NE). However, we don't yet know how long ACh & NE are available to act in the cortex following their release and whether this availability is spatially homogeneous during spontaneous state transitions. The current study used in vivo 2-photon imaging to record ACh and NE activity in various cortical areas of mice using the fluorescent neuromodulator reporters GACh3.0 and GrabNE2h. We first performed controls to validate the sensors in vivo and found that GACh responds faithfully to changes in ACh levels and displays high signal to noise ratio (SNR). We then determined that ACh levels track locomotion and pupil size very closely, and this relationship remains similar across cortical areas. Although GrabNE responds faithfully to NE in vivo, its SNR is lower than that of GACh.

We developed a method to reduce noise in the GrabNE signal and determined that cortical NE levels are closely correlated with pupil size, and that locomotion-related NE levels remain elevated for longer than ACh levels. Finally, we examined the relationship of cortical ACh and NE to activity of cholinergic basal forebrain (BF) and adrenergic locus coeruleus (LC). Our results indicate that BF and LC both show increased activity directly before locomotion- and dilation-induced peaks in ACh and NE (respectively). From this data we aim to develop a model whereby cortical neuromodulator levels can be predicted from arousal state-related behavior variables.

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Poster

233. Mechanisms of Attention: Rodent Models

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Topic: H.01. Attention

Support: Lundbeck Foundation Grant R276-2018-792

Title: Determining the role of norepinephrine in attention by chemogenetic manipulation and fiber photometry measurement of transmitter release dynamics

Authors: *L. P. POSSELT, S. HEIDE JØRGENSEN, K. KLIEM, A. TOFT SØRENSEN, U. GETHER;
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Abstract: Norepinephrine (NE) signaling in the frontal cortex is highly involved in attentive, adaptive and goal-directed behavior. Dysfunctional NE signaling is associated with a large range of neurological and psychiatric diseases including attention deficiency hyperactivity disorder (ADHD). While previous studies have consistently demonstrated that NE deficiency severely impairs attention, it remains unknown how excessive NE levels affect attentive behavior. Understanding the neuronal mechanism of NE-signaling in attention could lead to improved diagnosis and treatment of attentional deficits.
Here, we take advantage of an excitatory Designer Receptor Exclusively Activated by Designer Drugs (DREADD), representing a chemogenetic tool allowing selective and temporal activation of neurons *in vivo*. By expressing DREADD in the Locus Coeruleus, we explore how attention is affected by selective activation of NE neurons. Additionally, through expression of the recently developed noradrenergic fluorescent biosensor GRABNE1m in the medial prefrontal cortex, we are able to interrogate NE release patterns in the medial prefrontal cortex in real-time and directly synchronize these patterns with behavioral outcomes measured during an attentive task.We confirm that activation of the DREADD expressing neurons leads to a robust release of NE, which produces a strong increase in basal GRABNE1m fluorescence in the medial

prefrontal cortex. Interestingly, this increase in fluorescence signal is found to correlate strongly with a severely impaired attentional performance. The behavioral outcome as well as the increase in fluorescence are found to be dose-dependent without affecting any gross motor functions. We conclude that excessive NE levels in the frontal cortex impair attentional performance. Current studies allow further analysis and the possibility to directly link NE dynamics on a smaller timescale of milliseconds to minutes to more discrete behavioral outputs. Future studies will aim to clarify the functional role of NE dynamics in other brain regions involved in attentive behavior.

Disclosures: L.P. Posselt: None. **S. Heide Jørgensen:** None. **K. Kliem:** None. **A. Toft Sørensen:** None. **U. Gether:** None.

Poster

233. Mechanisms of Attention: Rodent Models

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Topic: H.01. Attention

Support: NIH Grant R01MH105592
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Title: Neural Activity of the Locus Coeruleus-Medial Prefrontal Cortex Circuit During Sustained Attention

Authors: *S. S. ADIRAJU[1], J. MIRANDA-BARRIENTOS[1], H. L. HALLOCK[2], S. OH[1], J. VALERINO[3], A. DUBROSSE[1], G. V. CARR[4], K. MARTINOWICH[1];
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Abstract: Sustained attention, the ability to focus on a stimulus for long periods, is a critical component of cognition, and is impaired across several neuropsychiatric disorders, including attention-deficit/hyperactivity disorder (ADHD) and schizophrenia. Individuals with these disorders exhibit aberrant neuronal activity in the medial prefrontal cortex (mPFC) during sustained attention tasks, suggesting a critical role of mPFC activity in sustained attention. The locus coeruleus (LC), the primary source of norepinephrine (NE) synthesis in the brain, is implicated in sustained attention, and Atomoxetine, a selective NE reuptake inhibitor is used as a treatment for ADHD. Importantly, neuronal activity in mPFC is impacted by LC-NE regulation. Specifically, LC stimulation modulates excitability of mPFC neurons and increases power of oscillations recorded in mPFC. However, the cellular and molecular mechanisms by which neural activity in the LC-mPFC circuit controls sustained attention have yet to be fully elucidated. Here, we characterized neural activity in the LC-mPFC circuit in the rodent Continuous Performance Task (rCPT) in which mice are trained in a touch-screen operant

chamber to discriminate between a rewarded stimulus (S+) and a non-rewarded stimulus (S-). The rCPT consists of three stages where attention demands increase as mice advance through the stages. Performance is assessed using d', a ratio between correct and incorrect responses (screen touches) across trials where higher d' reflects higher sustained attention. To characterize neural activity in the LC-mPFC circuit at different spatial and temporal resolution, we used *in vivo* electrophysiology and endoscopic imaging across stages of the rCPT. First, we simultaneously recorded local-field potentials (LFPs) in LC and mPFC to acquire population-level activity with high temporal resolution. We found increased power of low frequency oscillations and higher theta-gamma coupling as mice advanced through rCPT stages, demonstrating synchronous activity related to attention. Ongoing experiments are imaging calcium activity at cellular resolution from virally-expressed GCaMP6 in mPFC neurons to identify groups of neurons where neural activity correlates with rCPT performance. Finally, we are imaging NE release dynamics from viral expression of the biosensor GRAB-NE in mPFC neurons. Here, we expect to correlate NE release dynamics with attentional performance in the rCPT. Together, these findings will identify functional connectivity and modulatory interactions of the LC-mPFC circuit during sustained attention.

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Poster

233. Mechanisms of Attention: Rodent Models

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JHU Catalyst Award (SPM)

Title: Ancient PV+ midbrain neurons control selective spatial attention

Authors: *N. B. KOTHARI, W.-K. YOU, A. BANERJEE, Q. ZHANG, S. P. MYSORE; Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: The neural circuit mechanisms underlying the control for selective visuospatial attention remain largelyunknown. Here, we probe in mice, the function of an evolutionarily conserved inhibitory midbrain nucleus calledthe pLTN (periparabigeminal lateral tegmental nucleus). We first show that pLTN consists of parvalbuminexpressing (PV+) GABAergic neurons and is connected bidirectionally with the midbrain sensorimotor hub, thesuperior colliculus, establishing parallels with pLTN's extensively studied avian counterpart. Next, in head-fixed,passive mice, we show that bilateral silencing of pLTN neurons using cell-type

specific chemogenetics severelydisrupts electrophysiological signatures of stimulus competition in the SC, establishing a potential role for pLTNin spatial decision-making. Finally, in freely behaving mice performing a human-inspired flanker task of spatialattention, we demonstrate that bilateral silencing of pLTN neurons using cell-type specific chemogeneticsseverely disrupts attentional target selection and causes hyper-distractibility. These deficits in attentionalselection occur without deficits in motor plan selection, single target perception, or motor execution. Together,our results establish the midbrain pLTN as a critical mechanistic seat for the control of selective spatial attention.

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Poster

233. Mechanisms of Attention: Rodent Models

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Program #/Poster #: 233.09

Topic: H.01. Attention

Support: CIHR Grant
Ontario Graduate Scholarship

Title: Prefrontal parvalbumin neurons modulate target discrimination during focused visual attention

Authors: *T. DEXTER, D. PALMER, A. HASHAD, B. ALLMAN, W. INOUE, L. SAKSIDA, T. BUSSEY;
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Abstract: Parvalbumin expressing neurons (PVIs) are a subclass of inhibitory cells that contribute to balanced cortical excitation. PVIs modulate the spike timing of local excitatory neurons and are necessary for the generation of synchronous high frequency neural oscillations. Specifically, the role of PVIs in facilitating oscillations in the gamma frequency (30 - 60hz) has implicated this population in modulating cognition. In the prefrontal cortex, disrupting the activity of PVIs in rodents has been shown to impair processes such as cue detection, working memory and cognitive flexibility. By combining *in vivo* optical techniques with touchscreen cognitive testing, the objective of this study was to identify the role of prefrontal PVIs during focused visual attention in mice. To assess focused attention, we used the touchscreen rodent continuous performance task (rCPT). This task allows us to study various aspects of attention, including sustained attention, response inhibition, and visual discrimination. We used fiber photometry to measure the calcium dynamics of prefrontal PVIs in male and female mice during rCPT acquisition and cognitive probes. Further, we used optogenetics to either inactivate or stimulate prefrontal PVIs in male and female mice during the rCPT. *In vivo* calcium imaging revealed that prefrontal PVI activity increases in response to the target stimulus, and significantly

decreases following a correct response. Further, the intensity of PVI activity increases under distracting conditions that include the presentation of more target stimuli, indicating that prefrontal PVI activity conveys information specific to rewarded stimuli. *In vivo* optogenetics was used to inactivate prefrontal PVIs or stimulate these neurons at a high (30hz) or low (5hz) frequency during the response phase of the task. Both inactivation and low frequency stimulation (5hz) of PVIs significantly reduced animals' ability to accurately discriminate the target stimulus. Alternatively, stimulating PVIs at a high (30hz) frequency significantly improved animal's ability to successfully discriminate target stimuli from non-targets. We also observe that an animal's baseline performance (high or low) is predictive of whether optogenetic manipulation can impair or enhance task performance, respectively. This implies that the effectiveness of optogenetic stimulation on altering attention may depend on the baseline organization of prefrontal cortex activity, and that animals with unoptimized prefrontal cortex function can be improved by high frequency stimulation of PVIs.

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Poster

233. Mechanisms of Attention: Rodent Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 233.10

Topic: H.01. Attention

Title: Claustral projections to anterior cingulate cortex modulate responsiveness to sensory stimuli.

Authors: *I. MARSH YVGI[1,2], A. GAL[2], N. PEREZ RIVLIN[1], H. TURM[1], M. GOLDMAN[1], A. CITRI[1];
[1]Hebrew Univ. of Jerusalem, Jerusalem, Israel; [2]Physiol., UCSF school of medicine, San Fransisco, CA

Abstract: We are constantly exposed to extensive sensory input but respond behaviorally only to a subset of relevant information, enabling us to pursue our everyday tasks. Task engagement and selective attention are features modulating this cognitive capacity, although the neural correlates of which are not yet fully understood. We have recently found that a population of neurons in the claustrum projecting to the anterior cingulate cortex (ACC) strongly controls the responsiveness of the animal to task stimuli. We found that the population activity of the claustral ACC projecting neurons (ACCp), recorded via fiber photometry from 20 wild type male mice, is inversely correlated with the responsiveness of the animal to external cues. No correlation has been found in an additional population that we have tested. We have shown this in a task that requires a tradeoff between response inhibition and sensory sensitivity, finding that low ACCp activity correlates with low response inhibition and high rate of impulsive errors, while high

ACCp activity correlates with low task engagement and low responsiveness to sensory stimuli. Using chemogenetics , in five mice, we show that this relationship is causal, such that elevating ACCp activity decreases engagement in the task. Mice performed significantly lower rates of impulsive errors compared to saline control (ANOVA effect of treatment, $p = 0.0052$). We further find that mice exhibit different strategies of coping with the task, which vary in their dependence on ACCp activity. Here we present our work, including future directions, aiming to extend our knowledge of the circuit to a detailed mechanism by which this circuit is regulated, as well as the capacity to apply the mechanistic insight obtained from this study for clinical utility in treatment of impulsivity or apathy in a variety of neuropsychiatric disorders.

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Poster

233. Mechanisms of Attention: Rodent Models

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Program #/Poster #: 233.11

Topic: H.01. Attention

Support: NIH Grant 5U19NS107616-04

Title: Non-social cognitive profile of an oxytocin receptor knockout mouse model

Authors: *P. LEONE, B. GAMALLO-LANA, A. MAR;
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Abstract: Considerable research highlights a critical role of the neuropeptide oxytocin in the regulation of diverse mammalian social behaviors. Recent work has challenged the specificity of oxytocin to social function and hypothesized more domain-general roles of oxytocin in cognition and behavior. However, few studies have investigated the role of oxytocin receptor signaling on core cognitive phenotypes. This study examines the impact of constitutive loss of oxytocin receptor function in mice performing a battery of tests of visual discrimination, attention and behavioral flexibility.Adult, male and female oxytocin receptor knockout (OXTR-KO) mice were food-restricted and assessed on touchscreen-based paradigms. Tests included two-alternative visual discrimination learning for either orientation bar or compound second-order stimuli, two-choice reversal learning, as well as a rodent analogue of the continuous performance test (rCPT). The rCPT involves brief presentation of individual visual stimuli on the screen over a sequential series of trials. In all these paradigms mice learn to discriminate and respond to select target stimuli that earn a sweetened milk reward, while inhibiting responses to unrewarded non-target stimuli.We found that visual discrimination learning and reversal learning were unaffected in OXTR-KO relative to their littermate wild-type control mice. However, OXTR-KO mice showed slower acquisition and a reduced discriminability index (d') and target response ratio (hit rate) in the rCPT. These deficits did not vary across rCPT session time, but were sex-

dependent. Discriminability index reductions were primarily observed in male OXTR-KO mice whereas lower hit rates were seen mainly in female OXTR-KO mice. To identify behaviors associated to these alterations, mice were continuously recorded in their home cages. These results suggest that oxytocin receptor signaling contributes to non-social aspects of focused attention to rapidly presented stimuli associated with a food reward. This study adds to the growing evidence for a more domain-general role of oxytocin in cognitive processes.

Disclosures: P. Leone: None. **B. Gamallo-Lana:** None. **A. Mar:** None.

Poster

233. Mechanisms of Attention: Rodent Models

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Program #/Poster #: 233.12

Topic: H.01. Attention

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I01 BX004500
IK6 BX005714
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Title: The effects of filorexant, a dual orexin receptor antagonist, on evoked prefrontal cortical and hippocampal gamma oscillations in a model of NMDA receptor hypofunction

Authors: *E. B.-L. MANESS[1], D. D. AGUILAR[1], J. A. BURK[2], J. M. MCNALLY[1], R. E. STRECKER[1];
[1]Psychiatry, Harvard Med. School/VA Boston Healthcare Syst., West Roxbury, MA;
[2]Psychological Sci., William & Mary, Williamsburg, VA

Abstract: Gamma frequency oscillations (30 to 100 Hz, centered around 40 Hz) are associated with enhanced wakefulness, sensory processing, and cognition. Evoked oscillatory gamma activity is impaired in individuals with schizophrenia, highlighting dysfunctional gamma-generating cells in cortical circuitry that are thought to underlie not only the psychosis associated with schizophrenia, but the cognitive deficits as well. Our recent findings (Maness doctoral thesis, 2022) have shown that sub-sedative doses of the dual orexin receptor antagonist filorexant are sufficient to improve attentional impairments produced by the psychomimetic NMDA receptor antagonist MK-801. The aim of the present experiment was to determine if these attention enhancing qualities of filorexant could stem from the rescue of abnormalities in evoked gamma oscillatory activity in the prefrontal cortex and hippocampus. To test this, mice (N = 5) were surgically implanted with local field potential electrodes targeting the medial prefrontal cortex and dorsal hippocampus, administered filorexant (0, 10, 30, or 60 mg/kg, ip) and/or MK-801 (0, 0.3, 0.5, or 1 mg/kg, ip). The mice were then exposed to repeated 40 Hz trains of auditory stimuli. MK-801 alone suppressed both phase synchrony and narrowband gamma power (35 to

45 Hz) during 40 Hz auditory steady state stimulation in both the prefrontal cortex and hippocampus. By itself, filorexant also reduced hippocampal, but not prefrontal cortical, gamma power during the 40 Hz auditory stimulus. When co-administered with 0.5 mg/kg of MK-801, none of the concentrations of filorexant were able to normalize deficient gamma entrainment. In conclusion, dual orexin receptor antagonism may improve attentional behavioral outcomes in an NMDA receptor hypofunction model of schizophrenia through mechanisms not relevant to the restoration of evoked gamma activity in these attention-associated brain areas.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 234.01

Topic: H.03. Decision Making

Title: Deciphering learning rules underlying choice behavior in D. melanogaster using a model-driven approach

Authors: *R. MOHANTA[1,2], G. C. TURNER[2];
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Abstract: Navigating the world requires an animal to choose between different available actions or stimuli. Dynamic environments and reward uncertainty further complicate these choices. Therefore, animals typically adapt their behavior by accumulating information from past experiences, but the exact nature of the computations performed and their neural implementations are currently unclear. Extensive genetic tools and well-characterized neuronal connectivity in fruit flies (*D.melanogaster*) provide a unique opportunity to explore the circuit basis of cognitive factors underlying decision-making. Recent work from the lab (Rajagopalan et al., 2022) suggests that flies in a Y-maze can use reward expectation to produce operant matching behavior in a probabilistic two-alternative forced-choice (2AFC) task. However, to expand our understanding of the cognitive computations performed by fruit flies, we require a large number of choice trajectories from single flies. We develop a high-throughput Y-maze assay that allows us to track the choice behavior of 16 flies simultaneously. To understand the choice behavior, we then take two complementary approaches to explore various learning rules - a model-fitting approach and a novel de-novo learning rule synthesis approach. In the first approach, we test increasingly complex Q-learning rules that include consideration of future time horizons, forgetting of learned odor-value over time, aversion/perseverance on reward-omission, and independent response extinction. We also compare these models to habit-value arbiter frameworks that combine perseverance and value-driven behavior. We find that models that incorporate or approximate perseverance in choice behavior better explain and predict choice

outcomes for individual flies. In our second approach, we develop a more flexible framework that does not assume a specific value learning rule but instead uses neural networks to infer the learning rule from behavior and use it to predict choice trajectories. We find that even small recurrent neural networks with less than < 5 neurons trained to estimate action-values based on past choice experience can accurately predict decisions across flies. We then analyze the behavior of these networks as a first-order discrete dynamical system to reveal underlying attractor dynamics and derive interpretable learning rules using sparse identification of non-linear dynamics. This approach uncovers the same perseverance behavior observed in our behavioral analysis using structured Q-learning rules. Our results suggest that flies' choices may be influenced by habit-forming tendencies beyond naive reward seeking.

Disclosures: R. Mohanta: None. **G.C. Turner:** None.

Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 234.02

Topic: H.03. Decision Making

Title: Learning rules underlying dynamic foraging in Drosophila melanogaster

Authors: *A. RAJAGOPALAN ECHAMBADI[1,2], R. DARSHAN[1], J. E. FITZGERALD[1], G. C. TURNER[1];
[1]Janelia Res. Campus, ASHBURN, VA; [2]Dept. of Neurosci., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Foraging animals must use decision-making strategies that dynamically account for environmental uncertainty. To cope with this uncertainty, species across the animal kingdom have developed strikingly convergent strategies that use information about multiple past choices and reward to learn representations of the current state of the world. However, the underlying learning rules that update these representations have remained unclear. Here, working in the relatively simple nervous system of the fruit fly, Drosophila melanogaster, we combine behavioral measurements, mathematical modeling, and neural circuit perturbations to show that dynamic foraging depends on a learning rule that incorporates reward expectation. Using a novel olfactory dynamic foraging task, we characterize the behavioral strategies used by individual flies when faced with unpredictable rewards. We show, for the first time, that they perform operant matching. Animals that follow operant matching divide their choices in proportion to the number of rewards they receive from each option. We build on past theoretical work and demonstrate that this strategy requires the existence of a covariance-based learning rule in the mushroom body - a hub for learning in the fly. Such covariance-based rules are defined by their incorporation of stimulus expectation, reward expectation, or both. These three learning rule variants explain the fly's matching behavior similarly well, but they are distinguishable by optogenetic experiments that manipulate how reward is delivered. In particular, the behavioral

consequences of an optogenetic perturbation experiment that equated dopamine neuron activity with reward suggest that this learning rule exclusively incorporates reward expectation in this circuit. Our results identify a key element of the algorithm underlying dynamic foraging in flies and suggest a comprehensive mechanism that could be fundamental to these behaviors across species.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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Program #/Poster #: 234.03

Topic: H.03. Decision Making

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Title: Neural circuit models for accumulating evidence through sequences in a navigation-based, decision-making task

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Abstract: Accumulating evidence to make a decision is a fundamental cognitive process. Traditional models hypothesize that evidence is accumulated along a low-dimensional attractor, resulting in persistent, ramping activity of individual neurons. Yet, an increasing number of studies have found that neurons fire transiently and sequentially, motivating new models of evidence accumulation that result in choice-selective sequences of neural firing. Such sequences have been observed across cortical and subcortical regions in a navigation-based, accumulation of evidence task, where mice should turn to the side with more visual cues. Inspired by this task, we develop two new classes of models that accumulate evidence through sequences and that make predictions to differentiate the underlying computations in each region. The first is a position-gated bump attractor, where the set of active neurons jointly encodes position and evidence non-monotonically. The bump is maintained by local excitation and long range inhibition, and its location is shifted by the inputs and feedforward excitation between positions. The second is two competing chains, where position is encoded along the chain and evidence is encoded monotonically; inputs to one side increase the firing amplitude in the corresponding chain while inputs to the other side decrease this amplitude. This is achieved by excitation from

external inputs to one side, feedforward excitation within a chain, and inhibition from the other chain or the inputs to the other side. Model simulations make three distinguishing predictions that can be tested in existing recordings and new optogenetic experiments. 1) For a single neuron, the bump model predicts non-monotonic tuning to evidence as in the hippocampus, while the chains model predicts monotonic tuning to evidence as in the anterior cingulate cortex. 2) In the population, the bump model predicts different neurons will fire depending on the trajectory through position and evidence space, while the chains model predicts the same neurons will fire but with different amplitude depending on the evidence trajectory. 3) For optogenetic stimulation of a single cell, the bump model predicts excitation of cells with tuning to nearby evidence levels and inhibition of cells tuned to distant evidence levels. The chains model predicts this excitation will increase firing in subsequent neurons in the same chain, and if the chains are mutually inhibitory, decrease firing in subsequent neurons in the opposing chain. Overall, this work presents two novel and experimentally distinguishable model classes for how sequences of neural activity may accumulate evidence for decision making.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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Title: Modeling communication and switching nonlinear dynamics in multi-region neural activity during decision making

Authors: *O. KARNIOL-TAMBOUR[1], D. M. ZOLTOWSKI[2], E. M. DIAMANTI[1], L. PINTO[3], D. W. TANK[2], C. D. BRODY[4], J. W. PILLOW[1];
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Abstract: Understanding how multiple brain regions interact to produce behavior is a major challenge facing systems neuroscience, with many regions causally implicated in critical tasks such as sensory processing and decision making. A precise description of interactions between regions during decision making remains an open problem. Moreover, neural dynamics are nonlinear, non-stationary, and can vary dramatically across sessions, days, and animals. Here we propose multi-region, switching dynamical systems (MR-SDS), a probabilistic model of multiple

latent interacting systems that evolve with switching nonlinear dynamics and communication. MR-SDS includes directed interactions between brain regions, allowing for estimation of state-dependent communication signals, and accounts for sensory inputs effects, history effects, and heterogeneity across days and animals. We validate our model using two multiregion simulations, including a simulation of multiregion evidence accumulation, and then apply it to two multi-region imaging datasets involving mouse decision making. The first is a single-cell-resolution recording of hundreds of simultaneously recorded neurons across 3 distant cortical regions, and the second is a mesoscale widefield dataset of 8 adjacent cortical regions imaged across both hemispheres. On these multi-region datasets, our model outperforms existing piece-wise linear multi-region models and reveals multiple distinct dynamical states and a rich set of cross-region communication profiles.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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DOD VBFF
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Title: Encoding models for quantifying multi-area interactions on single trials during flexible categorical decisions

Authors: *K. W. LATIMER[1], O. ZHU[1], V. SHIRHATTI[1], S. DAVID[1], D. J. FREEDMAN[2]; [1]Neurobio., [2]Neurobio. and Computat. Neurosci., Univ. of Chicago, Chicago, IL

Abstract: We propose a framework for quantifying multi-area shared variability during behavioral tasks with statistical encoding models. Specifically, we apply tensor-regression to extend generalized linear mixed models for spike trains to estimate task-driven population activity and covariability between regions, including the time window of covariation. Our model quantifies trial-to-trial variability in population recordings using a combination of latent factors and fixed coupling terms describing how spikes from one brain area predict future spiking in another. We apply the model to simultaneous population recordings (approximately 50 units from each area) from the lateral intraparietal area (LIP), frontal eye fields (FEF), and superior colliculus (SC) from macaque monkeys performing a rapid categorization task. On each trial, the monkey views a random dot motion stimulus and they must quickly saccade to a color target

associated with a learned motion direction category. Population responses in each area reliably encode task information such as the category identity, target configuration, and saccade direction. The model reveals low-dimensional interactions between areas (i.e., communication subspaces) spanning multiple timescales. For example, we find stronger coupling with faster timescales from SC to LIP (approximately 20-50 ms window of spike integration) than from FEF to LIP (100-200 ms). We apply the model fit to visualize the low-dimensional interactions on single trials to occur within the LIP-FEF-SC network that support formation of a decision in the categorization task. We compare interpretations of inter-area communication using common pairwise measures with our model fitting all three areas in the dataset at once. We also study the impact of including latent factors on coupling estimation: latent factors may capture features of shared input from non-recorded regions that could otherwise bias estimates of inter-area effects. Together, these results show that tensor regression provides a powerful toolset for analyzing communication subspaces in large-scale neural recordings during flexible tasks.

Disclosures: K.W. Latimer: None. **O. Zhu:** None. **V. Shirhatti:** None. **S. David:** None. **D.J. Freedman:** None.

Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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Topic: H.03. Decision Making

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Title: Early life adversity alters sensitivity to motivational value of reward in an instrumental learning paradigm

Authors: *S. KHOJA, G. D. CARVALHO, L. Y. CHEN;
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Abstract: Adversarial experiences during childhood represent a major burden on public health. Early life adversity (ELA) can disrupt brain development and neural circuit formation such that it has been established as a contributing factor to various psychiatric disorders. However, circuit mechanisms by which ELA can contribute to behavioral dysregulation reminiscent of psychiatric disorders are unclear. Recent studies have shown that ELA delayed the maturation of dendritic spines on neurons in the dorsolateral striatum (DLS) but not in the dorsomedial striatum (DMS). Multiple studies have shown that the DMS and DLS, part of two distinct corticostriatal circuitries, are responsible for regulating goal-directed and habitual behavior respectively. The regulation between goal-directed behavior that is based on action-outcome (A-O) associations and habitual behavior that is based on stimulus-response (S-R) associations determines our flexibility over action control in a dynamic environment. Based on these findings, we hypothesize that ELA affects behavioral flexibility by altering the balance between goal-directed

and habitual behavior. To this end, we employed the limited bedding and nesting (LBN) paradigm in mice at postnatal day 2-9 (P2-P9) that mimics characteristics of ELA in humans. This was followed by a dual-contextual instrumental lever-press task with reinforcements scheduled at a random ratio (RR) for goal-directed behavior and random interval (RI) for habitual behavior, followed by two days of outcome devaluation. Additionally, post-LBN, we also undertook whole-cell patch-clamp electrophysiology to investigate the effects of ELA on neurotransmitter release probability and synaptic transmission in the DMS and DLS. We calculated the devaluation index in both male and female ELA mice in the RI and RR contexts and found that they exhibited differential sensitivity to outcome devaluation in a context-dependent manner. At the synaptic level, there were alterations in neurotransmitter release probability and postsynaptic transmission in ELA mice. The differences between male and female ELA mice suggest sex-dependent effects of ELA on behavioral flexibility, for which underlying mechanisms need to be explored. By establishing a link between ELA and circuit mechanisms underlying behavioral flexibility, our findings will begin to identify novel molecular mechanisms that can represent strategies for treating behavioral inflexibility in individuals who experienced early life traumatic incidents.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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Topic: H.03. Decision Making

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Title: Mapping whole brain neural dynamics subserving association learning in awake behaving mice using functional magnetic resonance imaging

Authors: *A. LAWEN, D. LICHTMAN, I. KAHN;
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Abstract: Behavior broadly, and learning particularly, requires the engagement of diverse brain systems. Recording activity from multiple regions is therefore crucial for characterizing the neural mechanisms that underlie learning. Human task-based functional magnetic resonance imaging (fMRI) is a well-established method. However, compared to other imaging techniques, task-based fMRI has not been used as widely in mice. We developed an fMRI experimental setup that allows for longitudinal whole-brain mapping and high-resolution behavioral monitoring of mice from the naïve state to task proficiency (n=8 males, 16-18 sessions per mouse, 1 session per day). Mice performed an association learning task with rewards varying in size: 43% of trials consisted of a conditional stimulus (CS; light cue) followed by unconditional stimulus (US; water reward), henceforth CS-US trials; 43% of trials were comprised of a

surprising reward without a predictive cue, henceforth NoCS-US trials; finally, 14% of trials consisted of a cue that predicted reward absence. Reward volumes were varied pseudo-randomly (low, 1 μl; medium, 3 μl; or high, 6 μl), aiming not only to establish associative learning but also to assess reward prediction error as a driver of learning. Lick behavior was tracked using MR-compatible videography. Lick frequencies in CS-US intervals were higher in comparison to NoCS-US intervals in proficient mice, indicating the establishment of a CS-US association that was not noticeable in the naïve state ($n=8$; $p<0.001$ Wilcoxon signed-rank test). We found that the blood oxygenation level dependent (BOLD) signal in both the Ventral Tegmental Area (VTA) and the Nucleus Accumbens (NAc) was correlated with anticipatory licking buildup over sessions. Most notably, VTA and NAc responses were modulated by reward volume and reward anticipation status ($p<0.05$, family wise error correction for both modulations), suggesting the formation of stimulus driven reward prediction. Other brain regions responded differentially between anticipated and surprising rewards, such as the anterior cingulate area and retrosplenial cortex, which is consistent with their previously defined roles in attention shifting and memory retrieval, respectively. Our setup and imaging approach produced behavioral and neural results that were consistent at the individual level. Collectively, these results provide evidence in mice that can be more directly compared to humans considering other imaging modalities. Finally, additional analyses targeting brain-wide responses will characterize the contributions of additional brain regions to learning and performance in this task.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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Title: Neural correlates of reinforcement learning across the brain

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Abstract: Learning from rewards is a key component of cognitive flexibility in a changing world. Reinforcement learning is often studied in humans and animals using the “dynamic two-armed bandit” task. In each trial of this task the subject selects one of two possible actions, each of which is associated with a different time-varying probability of reward. To maximize reward, subjects must learn which action currently has the higher reward probability, and bias their

choices towards that action. This task helps to study value-guided decisions allowing to isolate brain activity that is specifically related to the underlying cognitive process, unlike tasks relying on sensory stimuli that guide decisions. Although many brain regions might be involved in this task, studies to date have typically focused on a relatively small number of brain regions. This makes comparison between regions tricky as the details of tasks in different studies may differ. Here, we developed a version of the two-armed bandit task for head-fixed mice. We fitted and compared ten learning models of mouse behaviour in this task, and found that the differential forgetting Q-learning (DFQ) model best matched mouse behavior. We used high-density silicon probes to make acute extracellular recordings from ~17000 neurons across 8 brain regions. We found correlates of DFQ model internal states in the secondary motor cortex, as well as in the prelimbic cortex, throughout the trial. We then applied optogenetics to inactivate the secondary motor cortex and the prelimbic cortex, and observed that MOs is causal for the execution of the choice, while the prelimbic cortex plays a role in learning. Our work provides a large-scale survey of multiple brain regions, both cortical and subcortical, in the value-guided decision-making process, and highlights a special role of the secondary motor cortex and the prelimbic cortex among them.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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Topic: H.03. Decision Making

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Title: Cortical contributions to value-based decisions in the two-armed bandit task

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Abstract: The formation and updating of decision-making strategies during dynamic foraging is an essential skill for survival in mice. However, it is unclear which brain regions are involved in this process. To begin assessing this, we trained water-restricted Ai32;PV-Cre mice on a two-armed bandit task, where they lick right or left to seek water rewards which are delivered on an alternating 70%:10% and 10%:70% probabilistic reward schedule. Once trained, each of 40 evenly spaced locations in the dorsal cortex is inhibited on a trial-by-trial basis to elucidate its role in the task. This approach allows us to determine systematically and causally the function of

different cortical locations for value-based decisions. Previous studies have shown that silencing the anterior lateral motor cortex (ALM) inhibits contralateral licking. Consistent with these earlier works, we found that inactivation of ALM decreases contralateral licking during the two-armed bandit task. The effectiveness of the ALM inactivation depended on which side has higher reward probability, suggesting conflicting drives between the mouse's decision-making strategies and the effect of neural activity perturbation. Perturbations of other brain regions led to milder behavioral consequences or, in some cases, no effect.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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Title: WITHDRAWN

Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

Location: SDCC Halls B-H

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Title: Activity- and plasticity-based meta-reinforcement learning via orbitofrontal cortex

Authors: *R. HATTORI[1], N. G. HEDRICK[1], A. JAIN[2], S. CHEN[1], H. YOU[1], M. HATTORI[1], J.-H. CHOI[3], B. K. LIM[3], R. YASUDA[2], T. KOMIYAMA[1];
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Abstract: The meta-reinforcement learning (meta-RL) framework, which uses RL over fast and slow timescales, has been successful in training deep RL networks that generalize to new environments. This framework uses slow plasticity-based RL to build a recurrent network that performs fast trial-by-trial RL using recurrent activity dynamics. Here we show that the orbitofrontal cortex (OFC) of the prefrontal area is critically involved in both fast and slow aspects of meta-RL in mice. We trained mice and artificial meta-RL networks on a probabilistic reversal learning task across sessions during which they showed meta-learning and improved their trial-by-trial RL policy. We showed that synaptic plasticity in OFC was necessary for this across-session meta-learning of RL but not for the within-session trial-by-trial RL in experts. After the meta-learning, OFC population activity robustly encoded history-based value signals, and transient OFC inactivation impaired the ability of mice to exploit the value on each trial. Longitudinal tracking of OFC neural activity during training by in vivo 2-photon calcium imaging revealed that the across-session meta-learning slowly stabilizes population value coding in accordance with the ongoing behavioral policy. All these experimental results from OFC were consistent with simulations from artificial meta-RL networks. Our results indicate that two distinct RL algorithms with distinct neural mechanisms and timescales coexist in OFC to support adaptive decision making.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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Topic: H.03. Decision Making

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Title: Medial prefrontal cortex neuron activity in relation to diminishing reward-based action selection in rats

Authors: *S. NONOMURA, T. TAKAYASU, T. KANEKO, H. AMITA, K. INOUE, M. TAKADA;
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Abstract: Action-outcome contingency, in an unstable and dynamic environment, is gradually changed by repeating an action which makes the outcome value diminish. We often face the situation in which there is a dilemma whether to keep or switch an action that may cause no reward definitively. It is well known that a circuitry related to the medial prefrontal cortex (mPFC) plays a pivotal role in action selection based on the outcome value. However, it remains

unknown how neurons giving rise to the mPFC circuitry process the information about the action selection with a reward progressively diminishing. Here, we focused on the pathway from mPFC to the rostromedial striatum (rmStr), a major component of the mPFC circuitry and investigated its functional role in diminishing reward-based action selection. To this end, we developed a novel behavioral task in which rats under a head-fixed condition had to choose a right or left pedal with the corresponding forelimb to acquire a better reward when the reward value was changed. Reward diminishing occurred when rats chose the same action associated with a certain reward repeatedly for 5-15 successive trials. The reward-diminishing rate followed a hyperbolic discounting rule. First, we confirmed that the rats which were trained with this task successfully switched their actions (i.e., the right to left or left to right pedal) when the reward diminished by repeating the same action. To examine whether mPFC neurons contribute to action selection based on reward diminishing, we next made ibotenic acid (IBO) lesions in the bilateral mPFC of these rats. Before IBO lesions, the probability of switching actions within three trials after the onset of diminishing rewards was 18.1%. After the lesions, the probability was increased to 31.3% ($p < 0.001$). This indicates that mPFC neurons play a role in negatively controlling action switching to minimize reward loss. To define an involvement of the mPFC-rmStr pathway in diminishing reward-based action selection, we finally analyzed alterations in animal behavior and mPFC neuron activity by optogenetically manipulating mPFC-rmStr neuron activity. We injected a viral vector expressing channelrhodopsin-2 (ChR2) into mPFC and performed optical stimulation in rmStr while identifying the mPFC neurons antidromically. A set of data on behavioral and electrophysiological changes are currently being obtained to elucidate how signals representing the diminishing outcome value are processed in the mPFC-rmStr pathway for action switching.

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Poster

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Title: From Retrospective to Prospective: Integrated Value Representation in Frontal Cortex for Predictive Choice Behavior

Authors: *K. HAMAGUCHI[1], H. TAKAHASHI-AOKI[1,2], D. WATANABE[1];
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Abstract: To make a deliberate action in a volatile environment, the brain must frequently reassess the value of each action (action-value). Choice can be initially made from the experience of trial-and-errors, but once the dynamics of the environment is learned, the choice can be made from the knowledge of the environment. The action-values constructed from the experience (retrospective value) and the ones from the knowledge (prospective value) were identified in various regions of the brain. However, how and which neural circuit integrates these values and executes the final action remains unknown.
A subregion of the mouse frontal cortex, anterior lateral motor (ALM) cortex, is involved in the planning and control of orofacial movements (*1-4*). Neurons in ALM showed persistent activity that predicts the future lick direction, often referred to as preparatory activity. The preparatory activity in ALM is maintained by the thalamocortical loop (*5*) which is under the influence of the basal-ganglia pathway (*6*). Therefore, the anatomical connection of ALM is suitable for integrating reward-expectation signals through its cortico-basal ganglia-thalamic loop and bias the future lick direction. However, it is not known whether ALM encodes either retrospective or prospective values or integrates both to make a choice.
To investigate the value representation of ALM in the animals that learned the structural knowledge about the task, we employed a task in which the change of reward condition (state) is highly predictable; The rewarding water port is alternated in every 10^{th} reward delivery (state transition). The overtrained mice spontaneously changed their action near the state transition, suggesting that the mice predicted the approach of state transition. The reinforcement learning model predicted two distinct forms of value dynamics depending on the training stages. In naïve mice, the expectation about reward will monotonically increase after each rewarded trial. In contrast, the expectation would be downregulated in the overtrained mice so to trigger the spontaneous choice alternation. The ALM preparatory activity showed a clear concordance with this prediction throughout learning. Photoinhibition of ALM in experts delayed reversal and suppressed the spontaneous choice alternation toward the state transition, returning the experts into a naïve state. Our results demonstrated the importance of ALM in the deliberative action selection that incorporates the knowledge about the task.
1. Komiyama et al., Nature 2010; 2. Guo et al., Neuron 2014; 3. Li et al., Nature 2015; 4. Bollu, et al., Nature 2021; 5. Guo et al., Nature 2017; 6. Wang et al., Neuron 2021.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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Title: Dorsal raphe neurons signal expected reward amount and reward delay during multi-attribute decision-making

Authors: *Y.-Y. FENG[1], E. S. BROMBERG-MARTIN[2], I. E. MONOSOV[3];
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Abstract: Serotonin (5-HT) is thought to be involved in emotion, motivation, and cognition and its dysfunction is strongly implicated in maladaptive risk-seeking and impulsivity often observed in many psychiatric disorders. However, to date, whether and how single neurons within 5-HT nuclei signal information to guide adaptive value-based decision making is unclear. To address this gap, we recorded 134 single neurons in the dorsal raphe nucleus (DRN), the primary source of forebrain 5-HT, as two monkeys performed a multi-attribute decision-making task in which they chose between offers whose expected reward amount, reward delay, and reward uncertainty varied independently. Recently, using this task paradigm, we found that single neurons in the lateral habenula (LHb), a key target and modulator of DRN, signal subjective value reflecting total preferences for multi-attribute offers (Bromberg-Martin, Feng, et al. 2022, Submitted). Given the strong bidirectional connections between DRN and LHb, we asked whether DRN neurons also signal subjective value, or alternatively, they preferentially signal particular decision attributes, such as those thought to mediate risk-seeking or impulsivity. We found that unlike LHb, DRN neurons did not signal the subjective value of offers. Instead, they preferentially signaled only particular decision attributes: reward amount and reward delay, but not reward uncertainty. To further probe whether DRN neurons integrate decision attributes into subjective value, on some trials, offers provided advance information about the reward outcome. Previous work has shown that monkeys prefer such information and that its value can depend on reward attributes, particularly reward uncertainty. Again, consistent with a lack of subjective value coding, we did not observe a significant number of DRN neurons that signal the value of information. Finally, while DRN neurons did not signal reward uncertainty during choice, previous work showed they can signal tonic levels of uncertainty while waiting for uncertain rewards (Grossman et al. 2022). In line with these results, after the choice, DRN neurons exhibited strong tonic and ramping uncertainty-related signals, suggesting that while they did not signal uncertainty to guide choice, they could provide uncertainty-related information to guide other behaviors, such as foraging or learning. Our results indicate that the DRN contributes to decision-making by signaling information about reward amount and reward delay during ongoing choices, placing important constraints on theories of 5-HT and its dysfunction in psychiatric disease which involve the idea that 5-HT signals subjective value.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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Title: Serotonin input to medial prefrontal cortex modulates dynamic decision making

Authors: C. D. GROSSMAN, ***J. Y. COHEN**;
Johns Hopkins Univ., Baltimore, MD

Abstract: The brain learns from rewards to behave flexibly in a dynamic world. This task is difficult, especially when the relationships between choices and rewards are noisy and subject to change. Normative models propose that behavior can be optimized by modulating learning rate as a function of uncertainty ("meta-learning"). One meta-learning model hypothesizes two forms of uncertainty that drive learning rate. In these models, "expected uncertainty" tracks the variability of action-outcome relationships and slows learning so that behavior is not driven suboptimally by noisy outcomes. "Unexpected uncertainty," on the other hand, detects violations in this variability and speeds to respond appropriately to a perceived change in action-outcome contingency. We recently found that the activity of serotonin neurons in the mouse dorsal raphe tracks both forms of uncertainty to modulate how quickly the brain learns from the consequences of its actions (Grossman et al., Curr Biol, 32, 586, 2022). How is this uncertainty signal used by downstream areas for learning and decision making? Neurons in the medial prefrontal cortex (mPFC) encode decision variables that mice used in a dynamic foraging task (Bari et al., Neuron, 103, 922, 2019), and may thus be a substrate for uncertainty-driven learning. Here, we asked how serotonin inputs modulate mPFC neurons during dynamic decision making. Using a similar lick-based foraging task in head-restrained mice, we manipulated dorsal raphe serotonin inputs to mPFC to test the hypothesis that this pathway modulates how value representations are updated during learning. We developed a novel modeling approach that parameterizes the effect of serotonin axon stimulation on uncertainty. Following artificial excitation of serotonin axons in mPFC, we observed changes in learning consistent with a decrease in expected uncertainty and subsequent increase in unexpected uncertainty that was captured by our model. Recordings of action potentials from mPFC neurons confirmed the presence of value signals related to available actions. Manipulation of surrounding serotonin neuron axons caused changes in the dynamics of these value-related firing rates at the single neuron and population level. These changes were consistent with the changes in uncertainty and learning captured by the meta-learning model. Thus, we provide a quantitative link between serotonin activity and cortical dynamics used for flexible decision making.

Disclosures: C.D. Grossman: None. **J.Y. Cohen:** None.

Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 234.16

Topic: H.03. Decision Making

Support: R01NS104834

Title: Locus coeruleus norepinephrine neurons modulate reinforcement learning

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Abstract: To make decisions in a dynamic and stochastic world, animals adjust their behavior in response to environmental feedback. To understand how this flexibility is achieved, it is essential to know how the nervous system learns from choice outcomes. Theories proposed that norepinephrine (NE) neurons in the locus coeruleus (LC) modulate learning from reinforcement. We tested this hypothesis by measuring activity from identified mouse LC-NE neurons during a behavioral task requiring ongoing learning from reward prediction errors (RPE). Thirsty, head-restrained mice made choices to lick leftward or rightward for reward with changing probabilities. We made electrophysiological recordings from identified LC-NE neurons by "tagging" channelrhodopsin-2-expressing LC-NE neurons in *Dbh*-Cre mice. We found two types of LC-NE neurons: type I neurons were excited by lack of reward and predicted choice switching after no reward. Type II neurons were excited by positive RPE. The two groups of neurons also showed distinct distributions in the dorsal-ventral axis, had different spike waveforms, excitability and correlation of activity among simultaneously-recorded neurons. To test the hypothesis that LC-NE neurons modulate learning from decision outcomes, we silenced their activity using the inhibitory opsin GtACR2. These inactivations caused increased probability of switching choices on future trials following no reward. Our data indicate biophysically and anatomically distinct modules in LC, and reveal a function for LC-NE neurons in modulating learning from experience.

Disclosures: Z. Su: None. **J.Y. Cohen:** None.

Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

Location: SDCC Halls B-H

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Topic: H.03. Decision Making

Support: NWO Grant VI.C.181.032

Title: Noradrenergic modulation of learning noise in value-based decision making

Authors: *F. WURM[1,2], M. ALEKSIC[1], M. JEPMA[3], V. WYART[4], S. NIEUWENHUIS[1,2];
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Abstract: Over the past few decades, reinforcement learning has proven to be an invaluable tool for understanding decision making and learning in humans. However, the behavioral phenomenon of choice variability defies standard reinforcement learning models and still lacks a mechanistic explanation. In a recent study, Findling et al. (2018) show that choice variability in humans is best accounted for by a novel reinforcement learning model which incorporates learning noise as an additional source of choice variability. Learning noise refers to the idea, that the updating of expectations is only precise to a certain degree. Moreover, this research suggests, that the modulation of learning noise (or precision) is governed by the level of noradrenergic (NE) activity in the brain. In this study, we investigate this proposal by combining a pharmacological manipulation of NE (atomoxetine) with electrophysiological recordings in an established variant of the two-armed bandit task. We hypothesize that increased NE levels are associated with increased computational noise and that this relation is reflected on the behavioral and neural level. We replicate previous findings and establish connections between model variables and EEG. However, contrary to our expectations, we do no find evidence for a modulation of these effects by noradrenaline.

Disclosures: F. Wurm: None. **M. Aleksic:** None. **M. Jepma:** None. **V. Wyart:** None. **S. Nieuwenhuis:** None.

Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 234.18

Topic: H.03. Decision Making

Support: R00 DA047419

Title: Endocannabinoid signaling influences the dopaminergic substrates of cognitive flexibility

Authors: *B. L. OLIVER[1], A. VILLA[1], S. LEE[2], K. DUONG[1], N. E. ZLEBNIK[1];
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Abstract: Dopamine (DA) in the mesolimbic system is crucial for reward-based learning. A reward prediction error is adapted and encoded via mesolimbic DA neurons to associate a cue with the value of a reward. During early reversal learning when stimulus-reward associations are altered, accumbal phasic DA release is essential for flexibly updating these associations and improving choice performance. The endocannabinoid system has emerged as a critical modulator of the mesolimbic DA system. Endogenous cannabinoids can influence cue-motivated behavior via midbrain dopaminergic projections to the NAc. Further, extracellular DA levels are shown to be reduced in the NAc following CB1 antagonism and increased following direct and indirect CB1 agonism. Therefore, endocannabinoid signaling may play a role in reward processing and reversal learning performance on a reward-motivated task via regulation of mesolimbic dopamine function. The current experiment uses fiber photometry to measure transient DA

signals in the NAc at different stages of learning during an operant 80:20 probabilistic reversal learning (PRL) task while receiving chronic treatments of the monoacylglycerol lipase (MAGL) inhibitor JZL-184 and/or the CB1 receptor antagonist AM251. Male and female C57BL6/J mice (N = 30) underwent surgery to unilaterally implant an optical fiber and express the genetically-encoded dopamine sensor, GRABDA, into the NAc. Mice were first trained to discriminate between two levers for a sucrose pellet reward. Mice then completed an acquisition phase followed by a reversal phase in which the correct and incorrect levers were inversed. DA was recorded during early and late acquisition, and early, mid, and late reversal sessions. Performance on the PRL task was analyzed to assess mean differences in the number of sessions required to reach the learning criterion for acquisition and reversal. Analysis of error probabilities (win-stay and lose-shift) were also conducted. Finally, DA release was compared between and within subjects across sessions to determine differences in DA transients following correct-rewarded trials. Results demonstrate that systemic manipulations of endocannabinoid signaling impair reversal learning performance and dysregulate associated NAc DA release. These findings give critical insight into the role of the endocannabinoid system in flexible reward-based learning and may have significant implications for the use of cannabinoids for recreational or therapeutic purposes.

Disclosures: B.L. Oliver: None. **A. Villa:** None. **K. Duong:** None. **N.E. Zlebnik:** None.

Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 234.19

Topic: H.03. Decision Making

Support: Human Frontier Science Program Organization (Grant CDA00009/2018)

Title: Tyrosine hydroxylase-positive neurons in the nucleus accumbens influence decision making in a mouse delay discounting T-maze task

Authors: J. J. BOTTERILL[1], A. KANANI[1], J. STEININGER[1], M. ZHAO[2], S. RIAZ[1], R. ITO[1], M. ARRUDA-CARVALHO[1];
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Abstract: Delay discounting (DD) is a phenomenon where individuals devalue a reward associated with a temporal delay. DD has historically been investigated in rats and we sought to develop a mouse DD task to study circuits involved in DD decision making using modern genetic tools. We used tyrosine hydroxylase transgenic mice (TH-Cre) to target TH+ neurons in the nucleus accumbens (NAc). Adult male and female TH-Cre mice underwent stereotaxic surgery and received Cre-dependent excitatory or inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) or a control fluorophore (mCherry). After a post-

surgical recovery period of 1 week, mice were habituated to a T-maze used for DD. Mice received 5 days of maze habituation with their cage mate and then underwent an individual training session where they received 5 large rewards (6 sucrose pellets) and 5 small rewards (1 sucrose pellet) located at the opposite arms of the T-maze. Mice then underwent 10 free-choice trials/day until reaching >80% preference for the large reward on two consecutive days. Once training criteria were met, mice underwent 6 days of DD where the large reward choice was associated with a 10s delay. The DREADD agonist C21 (2mg/kg) was injected 1 hr prior to behavioral testing on the final two days of DD (days 5 & 6). Inhibition of TH+ neurons in the NAc decreased preference for the small but immediate reward during DD sessions. Excitatory DREADDs had no effect on reward decisions, but significantly increased the latency to pick a reward. Mice also underwent the novelty suppressed feeding test and we found that excitatory DREADDs increased the latency to eat the sugar reward in the center of the arena. Taken together, these results suggest that NAc activity influences decision making in reward-based tasks.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 234.20

Topic: H.03. Decision Making

Support: NIH MH122838-01
BWF Career Award for Medical Scientists

Title: Are reduction in reward impulsivity due to methylphenidate administration dependent on nucleus accumbnes?

Authors: J. L. CRAMER[1], A. SENGUPTA[2], A. TERRY[2], N. LEE[3], M. J. FRANCOEUR[5], ***D. RAMANATHAN**[6,4];
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Abstract: Impulsivity, acting prematurely without considering future consequences, is associated with a variety of psychiatric disorders and is apparent after brain injury. Behavioral pharmacology studies suggest that both serotonergic and dopaminergic manipulations alter reward impulsivity, but results are conflicting based on task requirements, drug dose, route of delivery, and target receptors. Moreover, little is known about which reward-related brain regions are necessary to facilitate the neurochemical effects on impulsive behavior. We used a temporal discounting paradigm to evaluate the effects of methylphenidate

(dopamine/norepinephrine reuptake inhibitor) and citalopram (selective serotonin reuptake inhibitor) on reward impulsivity. Next, in the same group of animals we lesioned the nucleus accumbens to determine if its function is necessary for serotonergic or dopaminergic modulation of impulsive behavior. A cohort of 12 male Long-Evans rats were trained to perform a temporal discounting task in which rats choose to wait for a high value (3x water) reward or to receive a low value (1x water) reward immediately (500ms). In the first block of trials (≤ 65 trials) the high value reward is delayed 2s, and in the second block (>65 trials) is delayed 10s. We used an intraperitoneal injection of a saline control or drug (methylphenidate/ citalopram) delivered at 5 different doses (0.5, 1.0, 2.0, 5.0, 10.0 mg/kg) on alternating days (saline, drug, saline, drug). We administered the doses in a pseudo-Latin squares design (each dose repeated twice). There was a significant interaction between dose and block following methylphenidate injections ($F_{(5,55)}$=5.23, *p<0.001*) driven by an increase of high value choices on block 2 (10s delay) at the highest dose of methylphenidate (10mg/kg). Citalopram injections did not significantly alter behavior (main effect of block $F_{(1,11)}$=174.42, *p<0.001*). The same animals next received bilateral NMDA excitotoxic lesions centered on nucleus accumbens and repeated a modified (saline, 1.0 and 10.0 mg/kg) drug injection schedule of methylphenidate and citalopram. Rats were still sensitive to the temporal delay following nucleus accumbens lesions. Results will be reported on whether lesions affect temporal discounting related to methylphenidate and/or citalopram. We predict neurochemical modulation through methylphenidate at high doses is mediated through the nucleus accumbens. Large-scale neurophysiology studies paired with neurochemical manipulations may reveal more information about how methylphenidate reduces impulsive choice.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

Location: SDCC Halls B-H

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Topic: H.03. Decision Making

Support: Simons Foundation
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Alfred P. Sloan Research Fellowship

Title: Reduced Dopamine Reuptake Leads to Enhanced Reward Prediction Error During Ovulation

Authors: *D. K. GREWAL[1], C. E. M. GOLDEN[2], C. J. AOKI[2], C. M. CONSTANTINOPLE[2]; [2]Ctr. for Neural Sci., [1]New York Univ., New York, NY

Abstract: Gonadal hormones can activate the dopaminergic system, which is thought to instantiate reward prediction errors for reinforcement learning. However, the mechanisms by which endogenous hormones modulate dopamine signaling are unclear. We used 15 10-week-old female Long Evans rats (N=5/group) in various stages across the estrous cycle, the rat's reproductive cycle, to assess protein expression in the nucleus accumbens core (NAcc). The NAcc is a region in the striatum that receives dense dopaminergic input from the midbrain. Over 3,000 proteins were detected and over 400 were found to be differentially expressed in proestrus and estrus (ovulation stages) compared to diestrus (a period of low estrogenic hormone levels). Gene ontology analysis of the differentially expressed proteins identified a significant enrichment of proteins related to dopamine reuptake. Two of these proteins were downregulated in proestrus: the dopamine transporter (DAT) and serotonin transporter (SERT), which also reuptakes dopamine. Two were downregulated in estrus: SERT and sphingomyelin phosphodiesterase 3 (Smpd3). Smpd3 has been shown to translocate DAT to the plasma membrane, suggesting there would be less plasmalemmal DAT in rats in estrus compared to diestrus. To test this hypothesis, we performed immunogold labeling of DAT in 12 female rats in estrus and diestrus (N = 6/group) and used electron microscopy to evaluate their subcellular location. Staying blind to the estrous stage, we measured the distance of DAT (>50 putative DAT particles/rat) from the plasma membrane in over 600 micrographs in ImageJ. We controlled for the number and size of the silver-intensified immunogold particles to account for the variability in silver intensification, as well as the size of the visible axon. We found DAT to be more intracellular (i.e. further from the axonal plasma membrane) in estrus compared to diestrus ($p<0.05$). Fiber photometry of dopamine sensors in the NAcc (GRABDA) revealed larger magnitude transients during behavior in estrus and proestrus, compared to diestrus. Altogether, this indicates that gonadal hormones promote the downregulation of proteins that mediate dopamine reuptake in the NAcc in proestrus and estrus, potentially leading to greater dopamine availability in ovulation stages of the cycle. We speculate that enhanced dopamine signaling supports enhanced learning and behavioral flexibility during ovulation, when decisions are likely to be consequential for the animal.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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Program #/Poster #: 234.22

Topic: H.03. Decision Making

Support: NSF Grant 2137023
LSU CBS Briding Grant

Title: Ventral tegmental glutamate projections guide cortical encoding of reward-oriented tasks.

Authors: T. T. ADEYELU[1], T. VAUGHN[3], ***O. M. OGUNDELE**[2];
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Abstract: Reciprocal connections between the neocortex and ventral tegmental area (VTA) detect the valence of environmental stimuli and recall events to guide future decisions. Through this mechanism, the VTA-mPFC circuit executes measured behavioral responses to rewarding and (or) aversive events. VTA projections to the mPFC consist of dopamine (~35%), and anatomically dominant glutamate terminals (~65%). Because of the traditional focus on the dopamine pathways, the role of the glutamate neurons is less understood in the cortical encoding of adaptive behaviors. Double floxed AAV-DIO-eNpHR3.0-mCherry was injected by stereotaxic surgery into the VTA of male Vglut2Cre mice to express an inhibitory opsin (eNpHR3.0) in glutamate neurons. Silicone probes were also implanted in the mPFC to sample extracellular spikes in freely behaving mice. The behavioral task session has two trials, and mice fasted for ~4h. In an open field chamber, four reward cups were kept in fixed positions. In the first trial (5 min), a food reward was presented in a random location. After an inter-trial time in the home cage (1h), the reward was omitted in the second trial (5 min). The reward learning task was performed with and without selective VTA-glut inhibition (610nm, 10Hz pulses). After spike sorting, putative principal neurons were identified by waveform valley-to-peak duration (μs) and autocorrelogram. Most mPFC neurons exhibit dichotomous firing patterns in reward/aversion tasks. Infralimbic principal neurons encode reward learning (82.2% ↓FR) and context discrimination (90% ↓FR) by firing rate suppression and enhanced firing regularity. A robust increase in regularity distinguished the encoding reward learning from the modest regularity increase observed in context discrimination. Inhibition of VTA-glut neurons during reward acquisition caused rapid habituation to the reward. Likewise, mice did not show a preference for the target when the reward was omitted. Although VTA-glut inhibition did not alter the directionality of cortical ΔFR, errors in these tasks were linked to suppressed cortical inhibition (69.6% and 40.8% ↓FR). Thus, the fold decrease in FR during these tasks was significantly below baseline ΔFR scores. The VTA-glut-mPFC tract also guides the differential encoding of reward acquisition and reward-linked contexts. As a result of VTA-glut suppression, the fold change in cortical inhibition (ΔFR, $p=0.33$) and regularity ($p=0.04$) that distinguished these tasks were not observed. By modulating the timing and propensity of infralimbic cortical inhibition, we conclude that VTA glutamate inputs guide reward learning and reward-oriented context discrimination.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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JSPS Overseas research fellowship
AMED Grant JP20dm0207069

Title: Characterization of glutamate inputs to midbrain dopamine neurons

Authors: *R. AMO[1], K. F. TANAKA[2], N. UCHIDA[1], M. WATABE-UCHIDA[1];
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Abstract: Dopamine facilitates learning by broadcasting reward prediction error (RPE) as a teaching signal. Previous studies suggest that dopamine neurons encode a particular form of RPE, temporal difference [TD] error. Although there are alternative models for dopamine activity, we recently found that dopamine cue responses gradually move in time during learning, resembling TD errors (Amo *et al*., 2022). However, it is not understood how TD error is computed. Several studies suggested that specific information such as value expectation and reward is sent from a specific brain area to dopamine neurons. In contrast, a previous study found that neurons presynaptic to dopamine neurons represent mixed information of both expectation and reward (Tian *et al*., 2016). The study also found that a majority of presynaptic neurons are activated at rewarding cue, contradicting with the idea that only inhibitory inputs contribute computation of RPE. Although these observations suggest potential contribution of glutamate inputs to dopamine neurons for RPE coding, the actual computational mechanism remains to be elucidated. We address this problem by 1) identifying glutamate neurons presynaptic to dopamine neurons, and 2) characterizing activity of glutamate inputs. We focused on dopamine neurons projecting to the ventral striatum (VS), from which we reliably observed RPE coding. First, we developed the retrograde labeling system that can label glutamate input neurons to VS-projecting dopamine neurons using pseudotyped rabies virus together with AAV-mediated genetic manipulations and transgenic mice lines. With this system we mapped glutamate input neurons to VS-projecting dopamine neurons throughout the brain. We observed glutamate input neurons in various areas including the subthalamic nucleus, the lateral hypothalamus, the periaquedactal gray, and the cortex. Second, we extended this labeling system for recording of neural activity using an intersectional expression strategy. Our tracing and recording systems will be broadly applicable for studies of presynaptic neurons, and our study of glutamate inputs to dopamine neurons will provide new insights for the mechanism of dopamine RPE coding.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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Topic: H.03. Decision Making

Support: NIH Grant R01 NS116753
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Human Frontier Science Program LT000801

Title: Distributional value coding in the striatum

Authors: *A. LOWET[1], Q. ZHENG[2], M. MENG[1], S. MATIAS[1], J. DRUGOWITSCH[2], N. UCHIDA[1];
[1]Mol. and Cell. Biol., Harvard Univ., Cambridge, MA; [2]Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Machine learning research has achieved large performance gains on a wide range of reinforcement learning (RL) tasks by expanding the learning target from mean rewards to entire probability distributions of rewards — an approach known as distributional RL. We asked if the brain might use a similar distributional approach; while representations of mean reward abound across brain regions, little is known about whether, where, and how populations of neurons encode information about higher-order moments of reward distributions — much less the complete shapes of these distributions. To fill this gap, we used Neuropixels probes to acutely record striatal activity from well-trained, water-restricted mice (N=3 mice, n=5,978 neurons) performing a classical conditioning task in which six different odors were paired with three different reward distributions (amounts of water), with pairings randomized across animals. Critically, each reward distribution was associated with two unique odors, and two of the distributions had the same mean, allowing us to disentangle the effects of sensory stimulus, motor behavior, and expected value from higher-order moments of reward distributions. We identified the lateral nucleus accumbens (lNAc) as a hotspot for encoding distributional information. During the trace period, lNAc activity better supported discrimination between odors with different reward distributions than with the same reward distribution ($p < .001$, Wilcoxon rank-sum test), even when all odors had the same mean, consistent with the distributional RL hypothesis. To test whether neural activity truly factorized distributional information and odor-specific information, we trained a linear decoder on a subset of odors and tested it on a held-out subset. Classification performance in the lNAc generalized to the held-out conditions well above chance ($p < .001$, Wilcoxon rank-sum test), again even when all odors signified the same mean reward. Lastly, we built an artificial neural network (ANN) that took pseudo-trial spike counts as its input and predicted complete probability distributions as its output, and we trained it to minimize the Wasserstein distance between the decoded and ground-truth reward distributions. The ANN was able to decode the cued reward distributions with a high degree of accuracy — over and above what would be possible from odor identity or mean reward information alone — and generalized to odors not seen during training. Together, these results demonstrate that the lNAc represents learned reward distributions, suggesting a possible circuit implementation of distributional RL in the brain.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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Program #/Poster #: 234.25

Topic: H.03. Decision Making

Support: NIH Grant 5F32MH126505-02
NIH Grant 5R01NS108740-02

Title: Causal evidence for an actor-critic organization of dopamine-driven learning in the striatum

Authors: *M. G. CAMPBELL, S. XU, N. UCHIDA;
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Abstract: Dopamine neurons play a central role in the brain's reinforcement learning systems. An influential hypothesis is that dopamine neurons convey a reward prediction error (RPE) signal to the striatum which drives value learning. Value signals then in turn feedback to dopamine neurons, forming a closed loop RPE-value computation. However, there is growing appreciation that dopamine neurons comprise diverse sub-populations with properties that depend on their projection target and location within the midbrain. How these dopamine neuron sub-populations contribute to and interact with each other during value learning remains incompletely understood. To address this question, we combined multisite optogenetic stimulation with large-scale electrophysiology to simultaneously record from and manipulate populations of individual dopamine neurons in the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc) while tagging them by projection target. Using this technique, we found that optogenetic stimulation of dopamine axons in ventral (VS), but not dorsomedial (DMS) or dorsolateral (DLS) striatum drove increases in the phasic excitatory response to preceding odor cues (n = 8 mice, n = 109 dopamine neurons). This increase was observed in dopamine neurons regardless of projection target (n = 65 VS-projecting neurons: 0.75 +/- 0.14 spikes/sec increase above control odor during 0-300 ms after odor onset, $t(64) = 5.26$, $p = 1.8e\text{-}6$; n = 44 non-VS-projecting neurons: 0.50 +/- 0.12 spikes/sec, $t(43) = 4.16$, $p = 0.0002$). While only VS stimulation increased the cue response, stimulation of all three sites reinforced licking for water rewards (n = 4 mice, lick rate between odor onset and water delivery increased by dopamine axon stimulation in VS, DMS, and DLS for 3 out of 4 mice [$p < 0.05$, t-tests over sessions]). These results support an actor-critic architecture in which dopamine release in VS drives value learning which transfers to dopamine neurons projecting to other parts of the striatum. These widely broadcast signals then modify behavior through their action on downstream circuits without necessarily contributing to value updates.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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Topic: H.03. Decision Making

Support: NINDS 3 U19NS113201
NIMH 1 F32 MH126505

Title: Signatures of an evidence accumulation process in medical prefrontal cortex underlying patch foraging decisions

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Abstract: To ensure their survival, animals must decide how best to allocate their time while foraging for food and water. One of the most fundamental decisions during foraging is when to abandon a depleting 'patch' of resources. The Marginal Value Theorem (MVT) provides a normative solution to this problem — foragers should leave a given patch once the instantaneous in-patch reward rate drops below the average reward rate of the surrounding environment. However, this requires perfect knowledge of in-patch and environmental value, which animals do not have access to in the real world. Nevertheless, many species exhibit behavior that conforms to the MVT predictions. Here we examine how mice make such value-based decisions. We designed a virtual foraging task in which head-fixed mice traverse a 1-D track to locate water-rich patches. Within patches, rewards are delivered with varied, decaying probabilities, thus simulating a depleting resource. In line with the normative theory, mice showed greater patch residence times on patches with larger and more frequent reward deliveries ($p < 0.0001$ for both size and frequency, generalized linear mixed effects model, $n = 18$ mice). Despite substantial variance in waiting behavior across mice (s.d. = 4.6s), a 3-factor generalized linear mixed-effects model accurately predicted average waiting behavior. To investigate the algorithm mice employ to solve the patch leaving problem, we fit a series of ramping evidence accumulation models to their stay vs. leave decisions. We found that a model with gain-modulation on the rate of ramping based on an inferred latent motivational state best predicts patch residence times within and across sessions.
To probe the neural basis of this evidence accumulation strategy, we recorded activity of neurons in the medial prefrontal cortex (mPFC) using Neuropixels (3,683 neurons, 5 mice). We observed task-relevant dynamics throughout mPFC. The most prevalent pattern was a ramp in activity that increased as leave decisions grew more imminent. Ramps were shallower on patches with larger rewards and were inhibited by additional reward deliveries, with both features corresponding to patches with greater patch residence times. These reward-modulated ramps were apparent on individual trials and were well described by a recurrent linear dynamical model that ramped

gradually as time elapsed in the absence of reward deliveries. Ramps terminated after reaching a common firing rate prior to patch leave irrespective of reward size and frequency. Taken together, our results point to an evidence accumulation process in the mPFC that allows animals to solve the patch leaving problem in dynamic, naturalistic settings.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 234.27

Topic: H.03. Decision Making

Support: NIH grant 5R01DC017311-04

Title: A distributional code for learning across timescales in dopamine-based reinforcement learning

Authors: *P. MASSET[1], A. N. MALIK[2], H. R. KIM[3], P. BECH VILASECA[4], N. UCHIDA[1]; [1]Harvard Univ., Cambridge, MA; [2]Massachusetts Gen. Hosp., Boston, MA; [3]Sungkyunkwan Univ., Suwon, Korea, Republic of; [4]EPFL, Lausanne, Switzerland

Abstract: Temporal Difference Reinforcement Learning (TD-RL) has been extremely successful at characterizing the activity of dopaminergic (DA) neurons in the midbrain as signaling a reward prediction error (RPE). However, a number of recent experimental results from recordings in a wider set of tasks and anatomical locations have challenged this interpretation and showed a greater diversity of responses that cannot be explained by the canonical RPE framework. To reconcile these observations, we propose that DA neurons implement a distributed RL model along several dimensions. Specifically, we extend a recent proposal that DA neurons represent probability distributions of reward magnitude to another orthogonal dimension, learning across temporal horizons. We propose that distinct DA neurons have distinct discount factors and therefore compute RPEs at different temporal horizons. Such diversity of discount factors can be shown to be theoretically advantageous as it allows a distributed RL system to discount future rewards with complex temporal expectations rather than exponentially. We recorded the activity of optogenetically identified DA neurons in mice performing two behavioral tasks. A cued delayed reward task (n=47 neurons) and navigation in a 1-D virtual reality track (n=111 neurons). In the cued delayed reward task, the responses to odor cues decreased as a function of increasing delays. These neural discount functions were well-fit by an exponential discount function, allowing us to obtain a discount factor for each DA neuron. We further show that given the inferred discount factors, using a regularized decoder based on singular value decomposition (Tano et al., NeurIPS, 2020), we can decode the reward delay from

the population activity using a parameter-free approach. In the navigation task, we show that our model incorporating diverse discount factors across individual DA neurons can explain the qualitatively different ramping behavior of single neurons. Our experimental design allows us to estimate the discount factors for a subset of single neurons (n=40 neurons) in the two behavioral tasks. We show that inferred discount factors are correlated across tasks (r=0.42, p=0.0074) and that we can decode reward timing in the cued delayed reward task using parameters inferred in the VR task . These results suggest that individual DA neurons have their own discount factor that dictate specific manner they compute RPEs and regulate learning. Overall, our results provide evidence that DA neurons encode RPE at a diversity of timescales. This work opens new directions for efficient RL algorithms based on distributional coding along several dimensions.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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R01NS11675
Bipolar Seed grant-Harvard Brain Science Initiative
Simons Foundation

Title: The mechanistic to algorithmic link between tonic dopamine and biases in value learning

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Abstract: Various psychiatric disorders are characterized by abnormal future predictions. Positive or negative biases in future predictions can arise an from an imbalance in learning from positive versus negative outcomes. Although dopamine has been implicated in various psychiatric disorders, biological mechanisms through which abnormality in dopamine gives rise to biased future predictions remain elusive. Here we sought to elucidate such a mechanism using a reinforcement learning (RL) model incorporating recent biological findings. It is thought that value learning depends on plasticity in cortico-striatal synapses driven by dopamine reward prediction errors (RPEs) acting on D1 and D2 receptors (D1r, D2r) in medium spiny neurons (MSNs). At normal dopamine levels, D1r and D2r are mostly unoccupied and occupied by dopamine, making them sensitive to a phasic increase and decrease of dopamine, respectively. In support of this, recent studies have shown that synaptic potentiation in MSNs expressing D1r or D2r is triggered by a phasic increase or decrease of dopamine (Yagishita et al., 2018; Iino et al., 2020). Moreover, given different affinities (D2r is higher than D1r) and sigmoidal dose-

occupancy relationship of these receptors, a shift in dopamine baseline should change their sensitivity (the "slope" in the curve) to dopamine transients. We incorporated these biological features in an RL model. We first show that this model develops positive or negative biases in predictions of probabilistic rewards (hereafter, optimistic bias) if baseline dopamine is increased or decreased, respectively. Next, we examined whether this model can explain the behavioral and neuronal changes in mice with habenula (Hb) lesions (Tian and Uchida, 2015). Specifically, Hb lesions induced optimistic biases in reward-seeking behavior (anticipatory licking) and cue responses of dopamine neurons in a Pavlovian conditioning task. Based on the observed firing rates of dopamine neurons, we first predicted dopamine concentrations and receptor occupancies of D1r and D2r using a biophysical model (Dreyer et al., 2010). We then show that lesions led to an asymmetry in changes in receptor occupancies sufficient to explain optimistic biases in reward-seeking behavior and dopamine cue responses. Alternatively, the biases could arise from an imbalance in positive and negative RPE responses in dopamine neurons, but such imbalance was not observed. Together, our biologically inspired RL model identifies tonic dopamine as a key to understanding biased value predictions, which may underlie abnormal future predictions and risk sensitive behaviors in patients with various psychiatric disorders.

Disclosures: S.A. Romero Pinto: None. **N. Uchida:** None.

Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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National Science Foundation (CNS-1714623)

Title: Decision-making in marmosets: reward invigorates movements during foraging

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[2]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: To survive, animals must forage for food in environments that are often patchy. They spend time working to find food-rich patches, then harvest until resources are depleted. Because harvesting is often a costly act, a good policy is to begin a harvest only when the reward available at a patch justifies the effort required to acquire it. In the wild, marmosets search for trees that are rich in resins, and then harvest by creating incisions with their teeth and extract the food by using their long tongue. Here, in an experimental setting, we observed fascinating foraging dynamics when marmosets were head-fixed but allowed to freely work for food.

Marmosets performed visually guided saccades in return for food. However, food was delivered through tubes with diameters just large enough to allow the tongue to penetrate and extract the slurry mixture. To make the harvest an effortful act, we placed the tubes at an acute angle to the mouth, thus requiring bending of the tongue. Moreover, we delivered food for every successful trial, but only by very small amounts (0.015 mL). As a result, the animals naturally chose to not harvest after each successful trial, but rather work for 5-10 consecutive trials, allowing the food to accumulate before attempting to harvest. Each harvest period consisted of a sequence of licking movements, and each work period consisted of a sequence of visually guided saccades. At the start of the session when the animals were hungry, they worked for only a few trials before starting the harvest, but as the session continued and they ate, they worked for a longer period before commencing harvest. The first few licks in a harvesting bout were more vigorous than the last in the same bout. Lick vigor increased when the amount of reward that had accumulated was greater. Similarly, saccade vigor during the work period was greatest following the conclusion of the harvest, and then declined trial after trial until the start of the next harvest. Reward history had a clear effect on lick vigor: following a positive reward prediction error (unsuccessful lick followed by a successful lick), lick vigor increased. Following a negative reward prediction error (successful lick followed by an unsuccessful lick), lick vigor decreased. In summary, when the amount of reward was small and its acquisition was effortful, marmosets choose to work until the reward accumulated, then harvested with bouts of licking. Lick vigor depended on the magnitude of reward, and was affected by its history.

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Poster

234. Neural Mechanisms of Value Based Decision Making: Flies to Rodents

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Program #/Poster #: 234.30

Topic: H.03. Decision Making

Support: Howard Hughes Medical Institute
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Leon Levy Foundation
NIH Brain Initiative

Title: A rise-to-threshold signal for a relative value decision

Authors: *V. VIJAYAN[1], F. WANG[2], K. WANG[2], A. CHAKRAVORTY[1], A. ADACHI[1], H. AKHLAGHPOUR[1], B. J. DICKSON[2], G. MAIMON[1];
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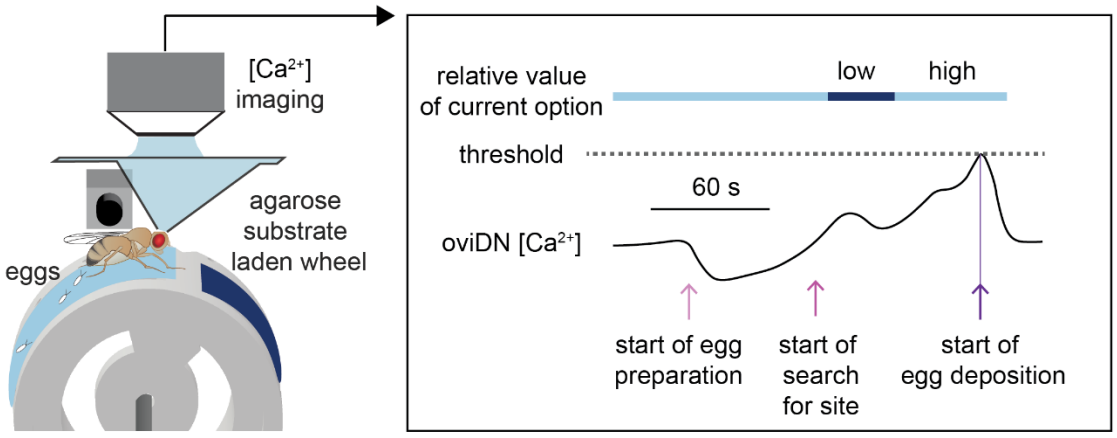
Abstract: Whereas progress has been made in identifying neural signals related to rapid, cued decisions, less is known about how brains guide and terminate more ethologically relevant deliberations, where an animal's own behavior governs the options experienced over minutes. *Drosophila* search for many seconds to minutes for egg-laying sites with high relative value (Yang et al., 2008) and recent work has discovered neurons, called *oviDNs*, whose activity fulfills the necessity and sufficiency criteria for initiating the egg-deposition motor program (Wang et al., 2020). We developed a head-fixed preparation for two-photon imaging, electrophysiology, and optogenetics in the brain of flies exploring options and laying eggs on a treadmill-like wheel. We find that oviDNs express a calcium signal that rises over seconds to minutes as a fly deliberates whether to lay an egg. The calcium signal dips when an egg is internally prepared (ovulated), rises at a rate related to the relative value of the current substrate being experienced, and reaches a consistent peak just prior to the abdomen bend for egg deposition. We use optogenetic-based activation to show that the egg-deposition motor program is initiated once the signal hits a threshold and gentle hyperpolarization to show that sub-threshold variation in the signal regulates the time spent deliberating and, ultimately, the option chosen. Finally, we identify a small recurrent circuit that feeds into oviDNs and show that activity in each of its constituent cell types is required for laying an egg. These results demonstrate that a rise-to-threshold signal regulates a relative-value, self-paced decision and provide initial insight into the underlying circuit mechanism.



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Poster

235. Behavioral and Neural Processes Associated with Executive Function and Inhibitory Control in Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 235.01

Topic: H.04. Executive Functions

Support: NIH Grant R01MH112267
NIH Grant R01NS119813
NIH Grant R21MH125107

Title: Interplay between the noradrenergic and cholinergic systems in a response inhibition task

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Abstract: Response inhibition is the ability to suppress actions that are detrimental or inappropriate in a given context. This inhibitory control involves multiple brain regions and is modulated by several neuromodulatory systems. However, the extent to which the noradrenergic and cholinergic systems and their interplay modulate response inhibition remains poorly understood. Using genetically encoded norepinephrine (NE) and acetylcholine (ACh) fluorescent biosensors, we simultaneously measured the NE and ACh dynamics in the parietal cortex of mice performing a response inhibition task. In the task, the water-deprived animal is trained to refrain from licking the water spout for 12 s. Early licking resulted in a mild punishment by a brief air puff to their face, while successful withholding of licks led to a water reward. Our data showed that there is a strong coherence between the NE and ACh dynamics over the frequency range of 0.5-1.5 Hz. However, the phase relationship between NE and ACh over the frequency range is still dynamic, with periods where the two systems were synchronous (i.e., coupled state) while in other periods the two systems were less stably coupled (i.e., oscillatory state). Our data further indicate that behavioral performance was strongly associated with the interplay between the noradrenergic and cholinergic systems. The number of switching from the coupled state to the oscillatory state between the noradrenergic and cholinergic systems prior to lick responses is significantly higher in failed trials compared to successful trials. Moreover, the phase relationship is more variant in successful trials than in failed trials. Taken together, our preliminary results suggest that the interplay between the noradrenergic and cholinergic systems plays an important role in modulating executive functions such as inhibitory control.

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Poster

235. Behavioral and Neural Processes Associated with Executive Function and Inhibitory Control in Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 235.02

Topic: H.04. Executive Functions

Support: NIH ZIA MH00295
NIH ZIC MH002952

Title: Optogenetic investigation of Galanin 1 receptors in fronto-temporal circuits that modulate impulse control

Authors: *K. MESSANVI[1], C. SENNECA[2], V. VISOCKIS[1], S. BRADLEY[1], Y. CHUDASAMA[3];
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Abstract: We have previously shown that the neuropeptide galanin differentially modulates ventral prefrontal cortex (vPFC) and ventral hippocampal (vHC) actions on impulse control mechanisms in rats (Messanvi et al. 2020, Psychopharm. 237:291-303). This modulation is mediated by galanin receptor 1 (Gal-R1) which is mostly expressed in glutamatergic neurons in both areas, and found in high levels in nor-adrenaline positive neurons of the locus coeruleus. To understand pathway specific functions of Gal-R1 expressing neurons in the vPFC and vHC regions, we first optically stimulated Gal-R1-expressing neurons of the vPFC or vHC of male Long-Evans rats while rats performed the 5-choice task using a touchscreen platform. In this task, rats must respond quickly and accurately to a brief light stimulus while maintaining stringent control of impulsive and compulsive responses. Bilateral photo-stimulation of the vPFC or vHC was applied during the interval prior to the light onset to specifically assess the animal's capacity to withhold their impulsive urge to respond. The results indicate that activation of Gal-R1 neurons in the vPFC (vPL and IL) and vHC (CA1, subiculum) triggered a reduction of premature responding, whereas optical inhibition of the vPFC caused an increase in impulse responses. These findings are in agreement with lesion studies (e.g., Chudasama et al., 2012, J. Neurosci. 32:10915-10924), and pharmacological studies that have targeted Gal-R1 neurons in the vPFC with agonists (Messanvi et al., 2020 Psychopharm. 237:291-303). Ongoing calcium activity recordings using fiber photometry during the 5-choice task will allow us to determine the profile of Gal-R1 expressing neurons activity in the vPFC and vHC during task performance. We will also determine whether the two regions are differently affected by norepinephrine release from the locus coeruleus.

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Poster

235. Behavioral and Neural Processes Associated with Executive Function and Inhibitory Control in Animal Models

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Topic: H.04. Executive Functions

Support: NIH Grant R01-MH55806
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NIH Grant P30-EY08126
Canadian Institutes of Health Research Postdoctoral Fellowship

Title: Absence of conflict signaling in midcingulate cortex: Neural spiking in macaques during a stop-signal task

Authors: *S. P. ERRINGTON[1], A. SAJAD[1], J. D. SCHALL[2];
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Abstract: Anterior cingulate cortex (ACC) is known as a hub for cognitive control. Convergent evidence across species and methodologies has supported its role in adapting behaviors and evaluating the outcome and value of actions. One prominent theory of ACC function is that it also contributes to the detection and resolution of conflict. Although evidence supporting this theory is prevalent in human literature, it has been notably less well supported from neurophysiological studies in non-human primates. In new data from two monkeys performing a saccade stop-signal task, we unbiasedly sampled local field potentials and identified 1645 units across 34 independent sites of ACC. Sites spanned an 8 mm rostro-caudal zone of the midcingulate cortex, and contacts captured the dorsal (area 6/32) and ventral (area 24c) banks of the anterior cingulate sulcus. In the stop-signal task, subjects are required to generate a saccade to a target on its appearance, but to inhibit this planned movement when an infrequent stop-signal appeared. On trials in which a movement is successfully inhibited, conflict arises due to the co-activation of two competing and mutually exclusive motor plans; this conflict is proportional to the probability of incorrectly generating a movement. At the single-neuron level, we previously described a population of neurons within the Supplementary Eye Field (SEF) that have activity scaling with putative conflict in this task. In our new data, we used a semi-supervised clustering algorithm to unveil common patterns of spiking activity differentiating between trials in which a movement was successfully generated, and those in which a movement was inhibited. Through this approach, we observed six clusters of ACC neurons modulating after stopping. Interestingly, some of these clusters showed differential activity prior to stop-signal and target onset, which we believe may reflect proactive control processes. However, we found no systematic variation in the activity of any populations of neurons with conflict. This preliminary work adds to a growing literature on the role of midcingulate cortex in cognitive control.

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Poster

235. Behavioral and Neural Processes Associated with Executive Function and Inhibitory Control in Animal Models

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 235.04

Topic: H.04. Executive Functions

Support: NIH Grant 5R01NS086104-09

Title: The role of primate prefrontal cortex in response inhibition and self control

Authors: *J. G. ELSEY[1], V. STUPHORN[2];
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Abstract: In everyday life, we must often interrupt or suppress a behavior in favor of another that is more appropriate in the current circumstances. Behavioral control is a fundamental component of executive control that is responsible for the suppression of actions, thoughts, and emotions. Two key aspects of behavioral control are response inhibition and self-control. Response inhibition is the ability to deliberately stop a prepared motor response. Self-control is the ability to inhibit self-defeating behavior in the face of temptation. Clinically, failures of response inhibition and self-control are commonly treated as signs of deficits in behavioral control. Currently, it is unknown whether the neural mechanisms that determine the success or failure of response inhibition or self-control are shared or distinct. Here, we trained macaque monkeys on saccade stop-signal (countermanding) and self-control tasks presented in a block-wise fashion. During the countermanding task, the monkey made a saccade to the peripheral target. On a subset of trials, a visual stop signal was presented after a variable stop-signal delay. During the self-control task, the monkey made a saccade to indicate their choice between a smaller, sooner (SS) and a larger, later (LL) reward. On a subset of trials, termed temptation trials, the unchosen option remained available at which point self-control must be exerted to the resist the suboptimal SS option in favor of the LL option. Temptation trials provide a clear behavioral marker for the level of self-control exerted on a trial-by-trial basis. The monkeys reliably switched between stop-signal and self-control tasks. Failures of self-control that led to increases in LL-SS switching on temptation trials and resulted in a leftwards shift of the choice function. Critically, in this situation of heightened need for self-control, the monkey was sometimes able to resist temptation and sometimes failed to do so. Supplementary eye field (SEF) generates proactive signals controlling saccade generation (Stuphorn et al., 2010). Further, preliminary data show that firing rates of a sub-population of SEF neurons predict when the monkey successfully exerts self-control compared to when they fail and give into temptation. DLPFC has a prominent role in the executive control of behavior and is known to encode abstract rules as well as long-term consequences of prior outcomes (Sakai, 2008). Currently, we are recording neuronal activity from SEF and DLPFC using multi-channel linear electrodes to investigate the role of the region in the inhibition of motor responses and self-defeating behavioral choices.

Disclosures: J.G. Elsey: None. **V. Stuphorn:** None.

Poster

235. Behavioral and Neural Processes Associated with Executive Function and Inhibitory Control in Animal Models

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Program #/Poster #: 235.05

Topic: H.04. Executive Functions

Support: Academy of Finland
Sigrid Juselius Foundation
University of Helsinki

Title: A transient beta oscillation occurs with high temporal regularity prior to stopping an ongoing movement

Authors: *J. F. DOUTEL FIGUEIRA[1], R. A. OJALA[1], D. VASILEV[1], R. IWAI[2], I. RAPOSO[2], N. SAFAEI[2], L. DE SARDENBERG SCHMID[2], N. K. TOTAH[1];
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Abstract: Beta (β) oscillations (~15-25 Hz) in the field potential are associated with immobility and stopping actions, but it is debated whether stopping involves a sustained increase in beta power or in the number of randomly timed 'bursts' (transient beta power increases). The role of β in movement control has been previously studied using the stop signal task, in which a cue instructs the subject to stop ongoing movement preparation. The time at which movement preparation stops is inferred from a 'race' model and provides an across-trial estimate of the movement preparation stop time. Thus, discrepancies about the role of β may be due to an inability to align brain activity with stopping on a trial-by-trial basis. Moreover, the causal role of β in stopping movements cannot be addressed without an observable stopping behavior. We used a new paradigm for head-fixed rats on a treadmill. The rats were trained to not run to a NoGo stimulus. On some trials, rats initiated a pre-potent running response but stopped before crossing a response threshold (distance) and returned to immobility. The peak velocity of these treadmill movements provides an unambiguous time when rats self-initiate stopping. We recorded 32-electrode EEG bilaterally over the entire cortex (39,366 trials, 306 sessions, 14 rats). Trial-averaged spectrograms centered on peak velocity revealed two distinct oscillations prior to stopping (9-12 Hz, α, and 15-25 Hz, β). Larger movements (and thus a greater need for stopping) were associated with greater α and β power prior to peak velocity. Moreover, α and β were apparent on more electrodes indicating greater spatial spread with greater need for stopping. These pre-stopping oscillations were observed globally and preceded initiation of stopping by 48 ms for β and 52 ms for α. Ongoing analyses are assessing Granger causality between EEG electrodes in the α and β bands and will report whether area 24a single unit spiking is phase locked to LFP oscillations prior to stopping. Our data show that, when field potentials can be aligned to overt acts of stopping, β power as well as α power are highly reliable predictors of the time of volitional stopping. JFDF and RAO are equal contributors and listed in alphabetical order.

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Poster

235. Behavioral and Neural Processes Associated with Executive Function and Inhibitory Control in Animal Models

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Program #/Poster #: 235.06

Topic: H.04. Executive Functions

Support: NIH grant MH053851

Title: Involvement of an orbito-striatal projection in motor impulsivity measured with the 1-choice serial reaction time test

Authors: *M. GIROTTI, M. G. BOEHMER, K. TUITE, D. A. MORILAK;
Pharmacol., UT Hlth. San Antonio, San Antonio, TX

Abstract: Motor impulsivity, or the inability to restrain a response, is a symptom shared by several psychiatric conditions such as bipolar disorder, ADHD, OCD and chronic substance abuse. Neuroanatomical regions involved in motor impulsivity have been defined with lesion studies, and using this approach a clear involvement of orbitofrontal cortex (OFC) and dorsomedial striatum (DMS) has been demonstrated. However, it is not known if changes in activity of OFC to DMS connectivity are involved in motor impulse control. Here, we address this question using a rodent test of motor impulsivity, the 1-choice serial reaction time test (1-CSRTT). Premature responses in this test signal the inability of the animal to "wait" for a signal to receive a reward. We tested two conditions: in the first, rats were trained to master a task with "wait" time of 5 seconds (intertrial interval, ITI 5), then were tested in the same conditions; in the second "challenge" condition, after rats mastered ITI 5, they were tested with a longer ITI (ITI 8 sec) that increases premature responses. In the first experiment we determined if an OFC to DMS projection was activated in the 1-CSRTT. Rats were injected with retrograde AAV-EGFP in the DMS, trained in the 1-CSRTT and then perfused 1 h after the end of an ITI 5, or ITI 8 test. A third group was similarly trained but did not undergo behavior before perfusion. OFC sections were subjected to double IHC using an anti-GFP and an anti-Fos antibody. We found that the proportion of activated OFC-DMS neurons increased at ITI 8. Additionally, non-DMS projecting neurons were also activated during this task. We then used a floxed-DREADD/CRE adenoviral approach to inhibit (hM4D-Gi) or activate (hM3D-Gq) the OFC-DMS projection during performance in the 1-CSRTT. A control group received floxed-mCherry. Activating OFC-DMS neurons reduced premature responses in the ITI 5 task, whereas inhibiting did not affect responding. We then tested if inhibition or activation changes the behavioral response to a first exposure to ITI 8, and then upon repeated days of exposure to measure the rate of adaptation to the new timing. Rats with inactivated OFC-DMS projection had lower premature responses on the first 2 days of testing compared to animals that received Gq. However, their ability to further improve their motor impulse control over repeated ITI 8 exposures was slower than that of the Gq and control group. These data suggest that activation of the OFC-DMS circuit improves impulse control in a mastered task. Conversely, inhibiting this circuit improves wait ability when conditions first change; however, it slows down the rate at which the new timing requirement is mastered.

Disclosures: **M. Girotti:** None. **M.G. Boehmer:** None. **K. Tuite:** None. **D.A. Morilak:** None.

Poster

235. Behavioral and Neural Processes Associated with Executive Function and Inhibitory Control in Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 235.07

Topic: H.04. Executive Functions

Support: NIH-NEIR00 EY025768
Brain & Behavior Research Foundation #28174
Alfred P. Sloan Foundation FG-2020-13415
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Title: Transcranial electrical stimulation for selective intervention in task-switching and performance monitoring in monkeys

Authors: *B. SAHOO[1], A. C. SNYDER[2];
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Abstract: Cognitive control is an essential brain function that involves two sub-processes: flexible selection of action plans based on context (task-switching) and suppression of habitual but inappropriate actions (response conflict/performance monitoring). These processes rely on different brain networks: a cingulo-opercular network important for performance monitoring, and a frontoparietal network important for task-switching. We hypothesized that targeted stimulation of these networks would specifically and selectively improve the function of associated cognitive control subprocesses in monkeys performing a task involving both performance monitoring and task switching.
To do so, we performed single pulse, short-duration, non-invasive, transcranial electrical stimulation (TES) in the range 1000-2000mA on monkeys while they were engaged in a cued-saccade task. The cue indicated whether to make a saccade towards a target, i.e. pro-saccade, or away from the target, i.e. anti-saccade. The anti-saccade is an unnatural behavior requiring response conflict management, while the randomly-cued nature of the task invokes task-switching. Animals flexibly switched between the two different actions for the same input target i.e. pro-saccade and anti-saccade. The reaction time in making a saccade reflected the commonly observed task-switching cost. TES differentially impacted the task switching process: saccade reaction time when switching to pro-cue trials increased with TES, while it decreased when switching to anti-cue trials with TES. This shows that TES can selectively intervene in cognitive control sub-processes depending on task contexts, supporting the potential for TES as a targeted therapeutic approach for disorders affecting cognition.

Disclosures: **B. Sahoo:** None. **A.C. Snyder:** None.

Poster

235. Behavioral and Neural Processes Associated with Executive Function and Inhibitory Control in Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 235.08

Topic: H.04. Executive Functions

Support: MICINN-FEDER PGC2018-099117-B-C21

Title: Neurobehavioral and emotional alterations in a preclinical model of compulsivity

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Abstract: Compulsivity has been proposed as a transdiagnostic trait presenting high comorbidity between compulsive behavior and other neuropsychiatric disorders such as obsessive-compulsive disorder, autism, and schizophrenia. The purpose of the present research was to assess behavior and emotional domains and brain correlates in the vulnerability to compulsion. Animals were characterized as high (HD) or low (LD) drinkers according to their compulsive behavior on Schedule-Induced Polydipsia (SIP). Then, we assessed cognitive inflexibility by Probabilistic Spatial Reversal Learning (PSRL), impulsivity by Variable Delay-to-Signal (VDS), decision making by Rodent Gambling Task (rGT), social dominance by Tube Test (TT) and emotional memory by Passive Avoidance (PA). Moreover, we used high-resolution magnetic resonance imaging to evaluate structural alterations. HD rats performed fewer reversals on PSRL, more premature responses on VDS and performed more disadvantageous choices on rGT. HD rats showed less victories against unknown competidor on TT and more latency of avoidance on the PA compared to LD rats. Voxel-based morphometry revealed that HD rats showed increased volume of white matter structures (Corpus Callosum and Anterior Commissure), cortical structures (Motor Cortex and dorsolateral Orbitofrontal Cortex), subcortical structures (Striatum, Preoptic Area, Amygdala, Dentate Gyrus, Subthalamic Nucleus, Periaqueductal Gray, Midbrain and Parasubiculum) and Cerebellum relative to LD animals. However, HD rats showed a decreased volume of mPFC compared to LD rats. No differences were observed between HD and LD groups in either the whole brain or in cerebrospinal fluid (CSF) volume. These results highlight and extend the knowledge about behavior and brain morphological alterations in the compulsive phenotype which may underlie the behavioral inhibition deficits observed.

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Poster

235. Behavioral and Neural Processes Associated with Executive Function and Inhibitory Control in Animal Models

Location: SDCC Halls B-H

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NIH R01ES032270 (C.V.V. and M.T.W.)

Title: Lphn3 Knockout Rats Exhibit Executive Function Deficits That Are Sometimes Sex Specific

Authors: M. S. CARBAJAL[1], A. J. C. BOUNMY[1], E. V. CULP[2], H. G. NOLEN[1], S. L. REGAN[3], M. T. WILLIAMS[4], C. V. VORHEES[4], *H. J. K. SABLE[1];
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Abstract: Deficits in executive function are common in attention deficit hyperactivity disorder (ADHD) and substance use disorder (SUD). Recent epidemiological data linked variants in the *Lphn3* (*Adgrl3*) gene to ADHD and SUD. To assess the impact of *Lphn3* deletion and sex on executive function, we compared *Lphn3*$^{-/-}$ (i.e., knockout, KO) rats to *Lphn3*$^{+/+}$ (i.e., wildtype, WT) rats of both sexes on tasks of impulsive action, impulsive choice, working memory, and cognitive flexibility. Impulsive action was measured via a differential reinforcement of low rates (DRL) task in which rats had to wait 15 s (i.e., DRL 15) between lever presses to earn a food reinforcer. Impulsive choice was measured using a delay discounting (DD) task where pressing one lever delivered a single food pellet immediately, while pressing the other lever delivered three pellets, but only after a delay (0, 4, 8, 12, or 16 sec). Working memory was assessed using a delayed spatial alternation (DSA) task that required rats to alternate lever presses from one trial to the next to earn the food reinforcer after random delays of 0, 5, 10, or 20 s were imposed between trials. Lastly, cognitive flexibility was assessed using a spatial reversal task (RL) in which both response levers were extended but only one was correct. Once reliably pressing on the correct lever, the response requirement was switched to the opposite side. This process was repeated four times. Compared with WT controls, the KO rats exhibited deficits on all four tasks, but in some cases, the deficits observed were sex-specific. For DRL 15 and DSA, a significant

effect of genotype was present that was not influenced by sex. KO rats exhibited decreases in the ratio of reinforced:non-reinforced responses and the percent correct for the two tasks, respectively. Male, but not female, KO rats demonstrated a problem with response acquisition during the first 5-day block of DD, eventually catching up to their WT counterparts by the last testing block (days 21-25). On the other hand, female (but not male) KOs demonstrated impairment during reversal learning. In particular, female KO rats made a higher number of incorrect responses per reversal, which required completion of a greater number of trials per reversal to exhibit performance similar to the female WTs. Results showed impulsive action and working memory are equally affected by the loss of *Lphn3* in male and female rats, while impulsive choice and cognitive flexibility show greater impairment in KO males and KO females, respectively. Overall, these results confirm *Lphn3* KO rats exhibit widespread executive dysfunction and suggest *Lphn3* deletion may play a role in ADHD and/or SUD where such dysfunction is prevalent.

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Poster

235. Behavioral and Neural Processes Associated with Executive Function and Inhibitory Control in Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 235.10

Topic: H.04. Executive Functions

Support: ANID Doctorado (PhD) Nacional Scolarship 21180727
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FONDECYT 1201848

Title: Evaluation of executive functions in a transgenic mouse model of OCD with increased forebrain expression of EAAT3

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Abstract: EAAT3 is a neuronal glutamate transporter expressed in glutamatergic synapses that regulates glutamate spillover and affects synaptic transmission. The *SLC1A1* gene encoding EAAT3 has been associated with obsessive-compulsive disorder (OCD). We previously generated and characterized a mouse model with increased forebrain expression of EAAT3

(EAAT3^{glo}/CaMKII) that displays increased anxiety and compulsive behavior, and deficits in long-term extinction of conditioned fear (Delgado-Acevedo et al, 2019 PMID: 30622300). Individuals with OCD can have impaired executive functioning, including cognitive flexibility and inhibitory control. It is currently unknown if EAAT3^{glo}/CaMKII mice have alterations in these cognitive domains. Here we aim to investigate if the EAAT3^{glo}/CaMKII mice have alterations in executive functions using operant learning tasks. For this, we evaluated executive functions using instrumental and visuospatial learning in EAAT3^{glo}/CaMKII mice. RoBucket operant conditioning chambers were used to determine operant learning, extinction, and reversal learning. Bussey-Saksida Touchscreen operant chambers were used to evaluate working memory using the trial unique non-matching to location task (TUNL) as well as impulsivity/compulsivity domains using the 5-choice serial reaction time task (5-CSRTT). Visuospatial memory, extinction and reversal learning were evaluated with the Morris water maze (MWM). EAAT3^{glo}/CaMKII mice were found to have impaired extinction of the operant learning and displayed perseverative behaviors. EAAT3^{glo}/CaMKII mice have impaired reversal learning in the MWM task, suggesting an alteration in executive functions in this model. Ongoing experiments using the 5-CSRTT as suggestive of deficits in inhibitory control in EAAT3^{glo}/CAMKII mice. Collectively, our results suggest that EAAT3^{glo}/CaMKII have impaired executive functioning, highlighting the relevance of this model for translational studies of OCD.

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Poster

235. Behavioral and Neural Processes Associated with Executive Function and Inhibitory Control in Animal Models

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Topic: H.04. Executive Functions

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Title: Cognitive rigidity and altered dopamine signalling in Alzheimer's disease mouse model

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Abstract: Apart from memory loss, one early symptom of Alzheimer's disease (AD) is a reduction in cognitive flexibility - the ability to make cognitive adaptations to changing environmental demands. However, cognitive rigidity in AD and its neurobiological underpinning have received little attention. Here, via a spatial discrimination reversal learning paradigm, we

detected this problem in 3xTg-AD mouse as early as at 3 month, before a clear deficit in memory function was found. At the same time, we found signs of degeneration of dopaminergic neurons in the ventral tegmental area of AD mice and diminished dopaminergic innervation in different target brain regions. Among these targets is the nucleus accumbens core, an area that has been implicated in cognitive flexibility. Intriguingly, administration of the dopamine precursor levodopda or optogenetic activation of the mesoaccumbens pathway rescued cognitive flexibility impairment in the AD mice. Therefore, early degeneration of the mesoaccumbens pathway may underlie cognitive rigidity observed in AD. Intervention of this system has the potential to ameliorate this cognitive deficit.

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Poster

235. Behavioral and Neural Processes Associated with Executive Function and Inhibitory Control in Animal Models

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Program #/Poster #: 235.12

Topic: H.04. Executive Functions

Support: CNRS
Aix-Marseille University
France Parkinson Association
Dhune ANR-11-IDEX-0001-02
H2020 Fetopen N°767092

Title: Contribution of cholinergic activity in the dorsomedial striatum to behavioural flexibility and impulsivity

Authors: J. LHOST, Jr, C. VIELLE, Jr, A.-M. OUAGAZZAL, Senior, M. LIBERGE, Senior, ***M. AMALRIC**;
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Abstract: A growing body of evidence suggests that behavioural flexibility relies on the integrity of frontostriatal circuits in which the dorsomedial striatum (DMS) is involved. Striatal cholinergic interneurons (CINs) take an important part in cognitive processes underlying flexible behavior, however, how and when modulation of CINs activity of the DMS affects flexibility is still a matter of debate. To address this question, we used an optogenetic approach to selectively inactivate CINs activity of the DMS in behaving mice during operant tasks requiring behavioural flexibility. We investigated the effects of CINs photoinhibition, in transgenic mice ($Rosa^{eNpHR/+}$ ::$ChAT^{cre/+}$) expressing halorhodopsin (eNpHR) in CINs, on reversal learning task performance. In operant chambers, mice were trained to nose poke into either a left or right nosepoke indicated by a light, to be rewarded by a sucrose pellet. When a baseline level of 70% correct responses was reached, the reinforced contingency was switched. CINs photoinhibition did not impact task

acquisition but impaired reversal learning as mice were unable to rapidly shift their pattern of response under changing task contingencies, thus increasing perseverative responses in the first sessions after reversal. This might reveal deficits of inhibitory control, and was further tested in a signaled nose poke task that provides independent measures of associative learning and motor impulsivity. Another group of mice was thus tested in a cued response inhibition task in which they needed to withhold a nose-poke response during presentation of a variable visual cue duration and respond to the correct nosepoke afterwhile. CINs photoinhibition induced an increase of premature responses, showing that flexibility disorders could be closely related to impulse control deficits. Dorsomedial striatal CINs may thus facilitate the expression of behavioral flexibility and regulate different aspects of behavioral inhibition. These results emphasize the critical involvement of cholinergic interneurons in the DMS in behavioral flexibility and impulsivity and in pathological disorders related to the loss of proactive inhibitory control.

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Poster

235. Behavioral and Neural Processes Associated with Executive Function and Inhibitory Control in Animal Models

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Program #/Poster #: 235.13

Topic: H.03. Decision Making

Support: NIH RF1 AG060778
McKnight Brain Research Foundation

Title: Effects of reproductive experience on cost-benefit decision making in females and males

Authors: ***M. FARAJI**[1], O. A. VIERA[1], B. SETLOW[1], J. L. BIZON[2];
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Abstract: The overwhelming majority of people experience some form of reproductive activity (sexual activity, pregnancy, and/or parenting) and it is well established that such experience, particularly pregnancy and childbirth, can have lasting effects on the brain including changes in risks of psychiatric disorders. Almost all preclinical research, however, employs reproductively naïve subjects. Research in rodents shows that pregnancy and parturition (reproductive experience; RE) can influence learning and memory functions supported by the hippocampus, but the influence of such experience on other aspects of cognition is not well understood. The goal of these experiments was to determine the long-term effects of RE on cost-benefit decision making. Female Long-Evans rats (n=16) were mated, gave birth, and nursed for 21 days until pup weaning (RE group) whereas reproductively naïve (RN, n=16) females were unmated. One

week after weaning, rats began behavioral testing in intertemporal choice and risky choice tasks in standard operant chambers. An additional cohort of males (n=8 mated, RE, n=8 unmated, RN) was tested alongside the females. In the intertemporal choice task (in which rats chose between a small immediate food reward and a large food reward delivered after a variable delay period), RE and RN females did not differ, whereas RE males chose the large, delayed reward more often than RN males at long delays (less impulsive choice). In contrast, in the risky choice task (in which rats chose between a small, "safe" food reward and a large food reward accompanied by variable probabilities of footshock), RE and RN males did not differ, whereas RE females chose the large, risky reward more often than RN females (greater risk-taking behavior). Additional experiments suggested that greater risk-taking behavior in RE females is not due to reduced cognitive flexibility, greater food motivation, or reduced shock reactivity. Considered together, the results show sex-specific effects of reproductive experience on cost-benefit decision making. Ongoing experiments are investigating the mechanisms that mediate these effects.

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Poster

236. Social Cognition and Motivation

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Program #/Poster #: 236.01

Topic: G.03. Motivation

Support: 5R01MH120638-03

Title: Focal pharmacological manipulation of serotonin signaling in the amygdala alters primate social behavior

Authors: *J. T. JACOBS[1], H. WAGUESPACK[1], R. S. MAIOR[2], C. CAMPOS RODRIGUEZ[3], L. MALKOVA[4], P. A. FORCELLI[5];
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Abstract: Previous research from our lab and others has implicated the amygdala as a critical node in the social network. We previously reported that reversible inactivation of the BLA in nonhuman primates resulted in an increase in social contact, solicitation of grooming and reception of grooming (Wellman et. al, 2016). Serotonin is also known to play a role in social behavior. Acute systemic administration of the selective serotonin reuptake inhibitor Fluoxetine decreases social interaction in rodents (Badgy et al., 2001; Payet et al., 2018). Very little research has examined for the effect of serotonin signaling in the amygdala specifically on social behavior, and no studies have addressed this topic in primates. To address this gap in the literature we acutely manipulated serotonin signaling in the amygdala in macaque monkeys via focal infusion of serotonergic drugs. Drugs included: Fluoxetine (SSRI), 8-OH-DPAT (5-HT_{1A}

agonist), (S)-WAY 100135 (5-HT_{1A} antagonist), TCB-2 (5-HT_{2A} agonist), R-96544 (5-HT_{2A} antagonist), m-CPBG (5-HT_3 agonist), and Palonosetron (5-HT_3 antagonist). We hypothesized that the 5-HT1A receptor agonist, 5-HT2A antagonist, and 5-HT3 antagonist would increase social behavior, while the 5-HT1A antagonist, 5-HT2A agonist, and 5-HT3 agonist would decrease social behavior. Subjects included 5 young-adult macaques (5 infused, 5 uninfused partners). Following drug infusion, the experimental animal was placed in an observation cage with a highly familiar non-injected partner and behavior was scored for social contact for 60 min. Preliminary results revealed a significant decrease in social contact following infusion of Fluoxetine at a lower, but not higher concentration. Additionally, there was a significant difference in social contact between 8-OH-DPAT vs. (S)-WAY 100135 infusion, with decreased contact after (S)-WAY 100135 infusion. Similar patterns of behavior were observed for total contact and reception of grooming. These results suggest differential contribution of particular serotonin receptors in the amygdala to social behavior in the nonhuman primate.

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Poster

236. Social Cognition and Motivation

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Program #/Poster #: 236.02

Topic: G.03. Motivation

Support: This project was supported by an Undergraduate Research Experience and Creative Activity (URECA) grant through the Office of Research and Sponsored Programs at Middle Tennessee State University.

Title: Oxytocin Receptor Activity in the Nucleus Accumbens Alters Social Reward in Male and Female C57BL/6J Mice

Authors: H. R. HUDSON, I. RAMOS, A. CAMPBELL, S. BROWN, A. KETEL, B. DONOVAN, K. HENSLEY, *T. D. ROGERS;
Middle Tennessee State Univ., Murfreesboro, TN

Abstract: While oxytocin is a neurotransmitter well-known for its role in social behavior, the specific mediating circuitry is not yet fully understood. Oxytocin receptors are found in the nucleus accumbens (NAc) on dopaminergic projections originating in the ventral tegmental area (VTA), an area associated with social reward. In the current study, intra-NAc infusions of either oxytocin, Atosiban, or phosphate buffered saline (PBS) were administered to behaving, adult C57BL/6J male and female mice to determine the effects of NAc oxytocin receptor activity on social reward. Social reward was measured by the social interaction conditioned place preference (siCPP) task one week after cannula implantation into the NAc of each mouse. The siCPP task conditions mice to associate specific bedding types (aspen or paper) with housing conditions

(grouped or isolated). Mice are placed in a two-chambered arena with one bedding type in each chamber to measure initial bedding preference. Mice were then placed in an isolated housing condition with one type of bedding (counterbalanced across trials) for 24 hours and then placed in a group housing condition with cagemates for 24 hours with the second bedding type. Mice were then allowed to freely explore the two-chambered arena again with one bedding type per chamber during an infusion of either PBS (0.2uL), oxytocin (0.1ug/0.2uL), or Atosiban (0.1ng/0.2uL) into the NAc. A multivariate ANOVA indicated that the main effects for sex were not significant (males n = 36, females n = 38; $p > .05$) and no sex by drug interactions were found ($p > .05$). Due to this, groups were collapsed across sex for all subsequent analyses. In contrast to previous research indicating oxytocin's role in prosocial behavior, the results indicated a significant decrease in duration for exploring the bedding type associated with the group housing condition when administered oxytocin (n = 24; $p = .007$) as compared to PBS (n = 24). Mice administered an intra-NAc infusion of Atosiban (n = 26) displayed a significant decrease in frequency of chamber entry for both the group-housed bedding type ($p = .001$) and the isolation-housed bedding type ($p = .006$) and a decrease in distance traveled ($p = 0.002$) as compared to PBS. Duration for exploring each bedding type did not differ between Atosiban and PBS ($p > .05$). Our findings suggest that oxytocin receptor activation in the NAc is associated with decreased social reward as measured by the siCPP in both males and females. While oxytocin mediates many social behaviors, the current findings highlight the need to elucidate region-specific effects of this neurotransmitter in various subtypes of social behavior including social reward.

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Poster

236. Social Cognition and Motivation

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Program #/Poster #: 236.03

Topic: G.03. Motivation

Support: NSERC
CIHR

Title: Tipping the Motivational Scale: Examining the Role of AgRP in Ghrelin-Driven Feeding and Foraging

Authors: *F. SHERRATT, L. HYLAND, A. SMITH, B. MACAULAY, A. TELFER, K. JOY, A. ABIZAID;
Carleton Univ. Neurosci., Ottawa, ON, Canada

Abstract: A growing body of literature suggests that ghrelin not only increases hunger, but also initiates a spectrum of feeding-related responses. Indeed, foraging requires both hunger and a

suppression of fear of potential threats. The exact mechanism by which ghrelin alters motivational states, however, has yet to be fully elucidated. We hypothesized that these effects are realized through ghrelin's actions on agouti-related peptide (AgRP) neurons of the arcuate nucleus. To test this, we used the novelty suppressed feeding test to determine if exogenous ghrelin injected peripherally would facilitate approach to a palatable snack (cookie dough), and to see if Melanotan II (MTII), a synthetic analogue of α-melanocyte-stimulating hormone, would block these effects. Intraperitoneal injections of either 0.1 mg/mouse MTII and 0.1 mL of 0.9% saline, MTII and 20μg/mouse ghrelin, saline and ghrelin, or saline and saline, were given to groups of male and female mice at times of the estrous cycle when estradiol concentrations were either high or low (n=18 mice/group). 30 Minutes post-injection, we recorded the latency to approach food, the amount of time spent in the corners of the open field box, and amount of food eaten for a period of 10 minutes. Data were also analyzed using a recently developed pose estimation software to determine the amount of time spent in the center of the open field box and the locomotor activity of the mice. Results indicate that while ghrelin increased the amount of time spent eating and the amount of food eaten, it did not significantly decrease the amount of time spent in the corners of the arena. Further, animals treated with saline and MTII showed significantly less locomotor activity than any other treatment group. No main effects of sex nor stage of the estrous cycle were found for any of the outcome measures. In sum, these findings suggest that ghrelin acts on AgRP neurons to facilitate food intake, but that it is not the sole factor involved in facilitating foraging behaviours.

Disclosures: F. Sherratt: None. **L. Hyland:** None. **A. Smith:** None. **B. MacAulay:** None. **A. Telfer:** None. **K. Joy:** None. **A. Abizaid:** None.

Poster

236. Social Cognition and Motivation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 236.04

Topic: G.03. Motivation

Title: Evaluating the role of the medial prefrontal cortex in social and nonsocial reward behavior using a novel operant paradigm

Authors: *J. ISAAC[1], M. MURUGAN[2];
[2]Biol., [1]Emory Univ., Atlanta, GA

Abstract: Animals navigate a world filled with complex stimuli that they must process and respond to accordingly. These responses are shaped by the drive to seek reward, such as food and social interactions. While there is extensive literature documenting the neural circuitry underlying nonsocial reward processing, less is known about social reward processing. The medial prefrontal cortex (mPFC) has been implicated in driving both social and nonsocial reward-related behaviors. It is unclear if overlapping or distinct subsets of neurons in the mPFC mediate these divergent reward-related behaviors. To directly compare the neural representations

underlying social and nonsocial reward, we developed a novel (two-choice) operant paradigm. In the two-choice paradigm, trained mice can choose between two ports in a self-paced task. Choosing one port gives the mouse access to a conspecific, while selecting the other port results in a sucrose reward. We found that access to a social target can drive similar levels of positive reinforcement as sucrose reward in trained mice (n = 29). To determine how the mPFC represents social and nonsocial reward, we performed cellular resolution calcium imaging of mPFC neurons in mice during the two-choice operant task. We found that distinct subsets of mPFC neurons are modulated by social and sucrose reward. A large fraction of mPFC neurons exclusively increase their activity in response to social but not sucrose reward (134/459 neurons). Another fraction of mPFC neurons is activated by social reward but is inhibited during sucrose reward (57/459 neurons). A smaller fraction is activated by both sucrose and social reward (31/459 neurons). We found that these mPFC reward representations changed with motivational state. With water deprivation, fewer mPFC neurons responded to social reward (34/444 neurons) and more neurons responded to sucrose reward (225/444 neurons) compared to control conditions. By tracking the same neurons across water access conditions, we found that the increase in sucrose reward-responsive neurons was driven by the recruitment of neurons that were previously reward-unresponsive. In addition, we found that both optogenetic inhibition (NpHR n = 9 mice, control n = 5 mice) and excitation (ChR2 n = 6 mice, control n = 4 mice) of mPFC neurons disrupted performance on the task. Overall, our results suggest that mPFC neurons differentially encode social and nonsocial reward and that these neural representations are modulated with changing motivational states. Furthermore, the activity of these neurons is necessary for mice to flexibly choose between social and nonsocial reward in this novel behavioral assay.

Disclosures: **J. Isaac:** None. **M. Murugan:** None.

Poster

236. Social Cognition and Motivation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 236.05

Topic: G.03. Motivation

Title: A friend in need, investigating empathy and prosocial behavior utilizing rat models

Authors: *T. ST VINCENT, D. ROEVER, C. PETERSON, S. PECK, J. ARNT, J. J. CORTRIGHT;
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Abstract: Empathy, commonly understood as the ability to understand and share the emotions of others, is an important factor in social interactions. Being an internal cognitive process that is not under direct conscious control makes it a concept that is difficult to research, especially with non-human participants. Animal models are often the most effective way to conduct research, however, animal models in this area are limited. This study seeks to test and expand upon this

limited research in various ways. In this study, long evans rats were housed and tested in groups. Training sessions took place over 15 days, during which one rat was placed in a restraint tube in the middle of an arena with a free roaming rat from its group. The tube was designed so the free rat could open it, releasing the trapped rat. After the training period, each rat was tested in six conditions that were conducted three times each in a larger arena. Condition one was the same as the training condition, condition two involved adding a second tube containing treats, condition three added a light over the tubes, condition four was a control condition in which a single empty tube was placed in the arena, condition five placed a stuffed rat in the tube as another control and, finally, condition six was a repeat of condition three after a short period of food restriction. After finishing all trials, animals were deeply anesthetized, transcardially perfused with saline, and their brains were placed in a Golgi cox solution for subsequent dendritic spine density analysis. We expect most animals will free their trapped counterparts and share treats during test conditions; but that when the light is added, they will cease these behaviors. Currently, our first group of seven animals have completed testing and are behaving in line with expectations, with a minimum of 20 more subjects projected to be complete in time to conduct behavioral analysis within the next few months. Since many of the world's problems arise due to conflicts between people, learning more about the mechanisms which produce empathy, and how empathy motivates prosocial behaviors, will provide knowledge that can be utilized to benefit both individuals and society, which could be invaluable to mental health professionals. Future dendritic spine analysis may provide insight into the physical causes for mental disorders and may provide additional knowledge that can be implemented to help those suffering from mental disorders or promote empathetically driven prosocial behavior in society.

Disclosures: T. St Vincent: None. **D. Roever:** None. **C. Peterson:** None. **S. Peck:** None. **J. Arnt:** None. **J.J. Cortright:** None.

Poster

236. Social Cognition and Motivation

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 236.06

Topic: G.03. Motivation

Support: NIMH110212
NIMH122622
GSU Brains & Behavior Fellowship

Title: Winning increases tonic and phasic DA release in the NAc while losing increases phasic DA release but reduces overall DA tone in the NAc in Syrian hamsters

Authors: *E. A. CROSS[1], K. L. HUHMAN[2], H. E. ALBERS[3];
[1]Georgia State Univ., Atlanta, GA; [2]Neurosci. Inst., Neurosci. Institute/Georgia State Univ., Atlanta, GA; [3]Georgia State Univ. Neurosci. Inst., Atlanta, GA

Abstract: Social interactions are highly salient regardless of their valence (i.e., whether they are rewarding or aversive). Social stress is one of the most salient stressors across taxa, but it is less clear how winning versus losing an agonistic encounter might impact the rewarding or aversive properties of these interactions. Recent data suggest that the mesolimbic dopamine system (MDS), which includes the ventral tegmental area (VTA) and its dopamine (DA) projections to the nucleus accumbens (NAc) encodes reward as well as aversion based on activity of specific subregions in the MDS. Axonal projections from the VTA into the NAc core release dopamine in a phasic manner, with sharp spikes functioning as a salience cue, "stamping in" important information associated with intense stimuli so that individuals can appropriately respond to future stimuli of any valence. Release of DA in the NAc shell occurs in a tonic manner with changes occurring over a longer time scale than in the core. We have begun to test the hypothesis that the NAc core encodes the intensity or salience of social stimuli while the NAc shell encodes the valence, whether it is rewarding or aversive. In male and female Syrian hamsters, we are measuring DA release in the NAc during social interactions using in vivo amperometry. We have obtained DA measurements in the NAc core and shell in awake, behaving hamsters during socially rewarding interactions wherein the subject "wins" (i.e., becomes dominant over a nonaggressive intruder) and during aversive interactions wherein the subject "loses" (i.e., is defeated by to a larger, resident aggressor). In support of the hypothesis, we found that similar bursting occurred in the NAc core during social interactions, suggesting that agonistic encounters are associated with similar DA activity regardless of outcome. By contrast, tonic DA in the NAc shell was found to be higher in the rewarding social context and lower in the aversive social context. Baselines recorded before and after social sessions indicate that these DA changes are tied to the social experiences, and spiking in the NAc core is time-locked to attacks. Electrodes outside the NAc (anatomical controls) did not show the same trends, suggesting that the signals recorded in the core and shell are specific to DA efflux in those NAc subregions. In these experiments, winning (rewarding) is salient (spiking in core) and has positive valence (tonic increase in shell), whereas losing (aversive) is salient (spiking in core) but has a negative valence (tonic decrease in shell), indicating that that NAc subregions (core v shell) and their characteristic DA release (phasic v tonic) are encoding valence and salience, respectively.

Disclosures: **E.A. Cross:** None. **K.L. Huhman:** None. **H.E. Albers:** None.

Poster

236. Social Cognition and Motivation

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Program #/Poster #: 236.07

Topic: G.03. Motivation

Support: NIH R01 MH114994
NIH T32 MH093311

Title: Behavior and brain response to social odor in juvenile mice

Authors: *J. BROWN[1], I. BAYRAMOV[2], E. A. HAMMOCK[1];
[1]Program in Neurosci. & Dept. of Psychology, [2]Dept. of Psychology, Florida State Univ., Tallahassee, FL

Abstract: Social behavior impairments are present in many psychiatric disorders, including anxiety, depression, and autism spectrum disorder. Oxytocin and its receptor (OXTR) play a role in adult social behavior. The contribution to juvenile social behavior is less well understood. In order to examine the role of *Oxtr* in social motivation during the adolescent critical period, male and female $Oxtr^{+/+}$, $Oxtr^{+/-}$, or $Oxtr^{-/-}$ peri-adolescent mice were exposed to the soiled bedding of same-sex adult or a clean nesting material control in a social odor investigation between-subjects design. Bedding stimuli were equally balanced across groups (sex and genotype). Animals were allowed 30 minutes to interact with the social stimuli in a social odor arena. Behaviors associated with social approach (olfactory investigation) and orienting toward a stimulus from a distance were quantified. The preliminary between-subjects factors analyzed were sex (male and female) and bedding type (social and non-social). Results indicate that juvenile mice investigate soiled adult bedding to a greater degree, compared to clean control bedding. Female juvenile mice oriented to a greater degree than males, and mice demonstrated more frequent orienting towards clean control bedding. Additionally, future analyses will explore genotype effects and neuronal activation via c-Fos analysis.

Disclosures: J. Brown: None. **E.A. Hammock:** None.

Poster

236. Social Cognition and Motivation

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Program #/Poster #: 236.08

Topic: G.03. Motivation

Support: NRF-2017M3C7A1048089
22-BR-04-03

Title: Ventral tegmental area to nucleus accumbens dopaminergic circuit is responsible for effort-based social decision making behavior

Authors: *T.-E. KIM, J. KIM, J. KOO;
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Abstract: Motivation is an essential part of an animal's goal-oriented behaviors, making animals overcome many adversities. For highly social animals like humans and some rodents, social interaction per se is a powerful driving force to endure effortful conditions. However, the underlying mechanism of motivation for social rewards has not yet been well studied. Effort-based social decision-making (ESDM) task was designed for this study and it was suitable for evaluating the social motivation levels. With this behavior paradigm, we analyzed the effort-

based choice behaviors of male mice to meet female. When the interaction time with the female was given as a freely accessible social reward, the male mice chose to meet the female (EFBX). Interestingly, we observed that the male mice chose to meet female even if they had to climb the barrier (EFBO) more frequently than the EFBX group on the last day of the task. To explain these phenomena, we first investigated gene expression levels of dopamine receptor D1 gene (*Drd1a*) and D2 gene (*Drd2*) in the nucleus accumbens (NAc), the key brain region that mainly receives dopaminergic projections, by quantitative PCR. As a result, *Drd1a* gene expression, but not *Drd2*, was significantly higher in the EFBO group than in other groups. To confirm the role of the D1 receptor in triggering social motivation, we infused D1R antagonist SCH-23390 directly into the NAc and found that effort-related choice level was decreased in the EFBO group. Since the ventral tegmental area (VTA) is the principal region for releasing dopamine, we manipulated the VTA-to-NAc circuit during the decision-making. Optogenetic inhibition reduced the effort-related choice level in the EFBO group. Conversely, activation on the second training day increased the level. Taken together, these data suggest that NAc D1-cells receiving signals from VTA are possibly involved in effort-based decision-making for the social reward.

Disclosures: **T. Kim:** None. **J. Kim:** None. **J. Koo:** None.

Poster

236. Social Cognition and Motivation

Location: SDCC Halls B-H

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Program #/Poster #: 236.09

Topic: G.03. Motivation

Title: Interpersonal competition and reward-processing: a neural explanation for the effects of perceived competence on intrinsic motivation

Authors: *J. C. BARCH, J. M. CARLSON, E. NIEMAN, R. MILLER, J. FOLEY, H. GABOURY, J. LAWRENCE, H. SNYDER, A. VASSALLO, A. NAGY, N. MARION, S. HOUPT;
Psychological Sci., Northern Michigan Univ., Marquette, MI

Abstract: Decades of Self-Determination Theory research demonstrates that supporting or thwarting perceived competence enhances or impairs intrinsic motivation, respectively. Less clear however, are the neural processes underlying these effects. Recent EEG studies examining needs for autonomy and optimal challenge have demonstrated the feedback-related negativity difference wave (dFRN), which represents a difference in participants' neuro-cognitive response to win and loss feedback, can serve as a neural representation of task engagement. Here, we used two studies where participants engaged in competitive reaction time trials with a confederate while EEG data was collected. The task was to stop a clock after a precise number of seconds with the target clock time masked during the final second. Ostensibly, whoever was closer to the exact second count won the trial. In reality, feedback was a predetermined bright green 'WIN' or a bright red 'LOSE' shown on the screen. After the EEG task, participants completed a self-

report measure of psychological need satisfaction (autonomy/competence/relatedness) and self-reported intrinsic motivation (interest/enjoyment). In the first study (N = 48), the data suggest effective manipulation of competence perceptions, which predict interest and enjoyment as expected. Moreover, we found increased dFRN ($F = 16.83$, $p < .001$) for participants whose perceptions of competence were threatened ($n = 25$; $M = -2.34$, $SE = 0.37$) compared to those with enhanced competence ($n = 23$, $M = -0.16$, $SE = 0.38$). A second study (N = 31) was designed to replicate those results and gather pre/post-competition dFRN data as well. The competitive trial data replicated ($F = 6.72$, $p = .015$) with larger dFRN for incompetent ($n = 14$, $M = -2.37$, $SE = 0.46$) than competent ($n = 17$, $M = -0.75$, $SE = 0.42$). Further, there was a significant condition by time interaction ($F = 3.87$, $p = .029$) where, the dFRN compared between groups in pre-competition trials was not different. But, during competitive trials as noted previously, dFRN was larger for the competence thwarted group. Then, as predicted by Self-Determination Theory, the post-competition dFRN for those with supported competence shows enhancement of reward processing, while post-competition dFRN for those with thwarted competence does not. In both studies, analysis of P2 and P3 ERP components demonstrate theoretically expected results showing feedback salience and odd-ball effects respectively. Importantly, the P2 & P3 results by condition and across time points differ from the dFRN findings, which provides additional support for our Self-Determination Theory driven conclusions.

Disclosures: **J.C. Barch:** None. **J.M. Carlson:** None. **E. Nieman:** None. **R. Miller:** None. **J. Foley:** None. **H. Gaboury:** None. **J. Lawrence:** None. **H. Snyder:** None. **A. Vassallo:** None. **A. Nagy:** None. **N. Marion:** None. **S. Houpt:** None.

Poster

236. Social Cognition and Motivation

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Program #/Poster #: 236.10

Topic: G.03. Motivation

Support: NIH grant R01 HD100007-01

Title: Efficacy of Bremelanotide (Vyleesi) and melanocortin 4 receptors to enhance sexual motivation in female Syrian hamsters

Authors: *J. M. BORLAND, A. L. KOHUT-JACKSON, A. C. PEYLA, M. A. L. HALL, P. G. MERMELSTEIN, R. L. MEISEL;
Univ. of Minnesota, Minneapolis, MN

Abstract: For men, sexual dysfunction is typically represented by performance issues (e.g., erectile problems); whereas for women, low levels of sexual desire and interest are primarily reported. Characterized by diminished interest in sex, disinclination to initiate sex, and a loss of pleasure during sex; disorders of sexual desire among women are not only poorly understood

from a psychological perspective, but also in terms of their underlying neurobiology. This loss of sexual desire is a source of distress for many reproductive age women resulting in issues of low self-esteem and relationship conflict. This led to approval of the drug Bremelanotide, trade name Vyleesi, to treat hyposexual desire disorder in women. However, despite approve, very few clinical trials have been performed and almost nothing is known about its potential mechanism of action. Bremelanotide is a melanocrotin 4 receptor (MC4R) agonist; the melanocortin system is involved in the regulation of energy homeostasis and satiety. Thus, the following study investigated the role of Bremelanotide on sexual reward in female Syrian hamsters and the role of melanocortin receptors in the striatum and ventral tegmental area (VTA). Female Syrian hamsters experienced five, two or zero 10-min sexual interactions (paired with an adult male) in the conditioned place preference (CPP) arena. Female hamsters that experienced two 10-min interactions were also subdivided into groups that received i.p. saline, 50 ug/kg or 200 ug/kg Bremelanotide 30-min prior to sexual experiences. Two (n=7) or five (n=6) days of sexual experience resulted in an increase in social preference ($p = 0.017$ and $p < 0.001$). However, neither a low (50ug, n=7) nor a high (200ug, n=7) dose of Bremelanotide (systemic) enhanced sexual preference ($p > 0.050$). Bremelanotide also failed to enhance sexual behavior, as there were no changes in the display of the lordosis posture ($p > 0.050$). Following assessment of sexual motivation, brains were collected for assessment of melanocortin 4 receptor expression in the striatum and melanocortin 3 and 4 receptor expression in the VTA. mRNA expression of dopamine 1 and 2 receptor, tyrosine hydroxylase and melanocortin 3 and 4 receptor were carried out using Syrian hamster customized RNAscope probes. Bremelanotide treatment had no effect on melanocortin 4 receptor mRNA expression in the striatum and no effect on melanocortin 3 and 4 receptor mRNA expression in the VTA ($p > 0.050$). Collectively, these studies support the clinical ineffectiveness of Bremelanotide to enhance sexual motivation in women.

Disclosures: **J.M. Borland:** None. **A.L. Kohut-Jackson:** None. **A.C. Peyla:** None. **M.A.L. Hall:** None. **P.G. Mermelstein:** None. **R.L. Meisel:** None.

Poster

236. Social Cognition and Motivation

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Program #/Poster #: 236.11

Topic: G.03. Motivation

Support: NIH RO1 MH117778
EMBO Longterm Fellowship

Title: Social vocalizations excite posterior insula neurons in courting mice

Authors: *T. POMBERGER[1], R. CARTER[1], B. J. SLATER[2], R. MOONEY[1];
[2]Neurobio., [1]Duke Univ., Durham, NC

Abstract: The insular cortex is implicated in many different functions, including interoception, risk estimation, decision making, processing of emotion, and combining sensory cues with emotional valence - just to name a few. In addition to processing sensory signals from within the body (interoception), the insula is also involved in processing and integrating a wide range of sensory cues from the environment. In monkeys, a part of the insular cortex contains neurons that respond to conspecific vocalizations and a region in the posterior insula of rodents has been shown to respond to pure tones. Here, we seek to characterize the insula auditory field in mice and explore its role in vocal communication during courtship. First, we used intersectional labelling methods to map afferent and efferent connections to the insula auditory cortical field. We then used multi-electrode recordings in awake, head-fixed mice to establish that certain neurons in the auditory insula respond to vocal playback. To investigate the role of the posterior insula in freely courting mice, we used miniature microscopes to monitor calcium-related activity during vocal interactions. We found vocalization correlated calcium activity in both females who interact with vocalizing males during courtship as well as in males eavesdropping on other males vocalizing to females. Our experiments indicate that the auditory insula of the mouse encodes information about socially and sexually salient vocalizations during courtship interactions.

Disclosures: **T. Pomberger:** None. **R. Carter:** None. **B.J. Slater:** None. **R. Mooney:** None.

Poster

236. Social Cognition and Motivation

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Program #/Poster #: 236.12

Topic: G.03. Motivation

Support: Seaver Foundation

Title: The impact of social isolation on social reward behavior and mesoaccumbens circuitry

Authors: *M. KIM, M. BARBIER, K. THIRTAMARA RAJAMANI, H. HARONY-NICOLAS;
Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Social interactions during development are crucial for establishing adult social behavior. An important facet of social behavior is the rewarding properties of social interaction, and in both humans and animals, social reward undergoes a shift in salience during adolescence. However, there is a gap in understanding how juvenile social isolation (jSI) may impact social reward processing in adulthood as well as neural circuitry mediating reward. At weaning age, rats are assigned to either jSI or group-housing for 3 weeks. At P42, rats are re-housed and re-socialized with a novel age and sex-matched rat until adulthood (P60). Once rats reach adulthood, we use a battery of behavioral assays to assess locomotor activity, anxiety-like behavior, social recognition memory, and social reward processing and motivation. We

investigate social reward processing and assess the rewarding value of social interaction through a task presenting a rat with both a novel social stimulus along with a competing non-social reward (food). During this task, we also record *in vivo* neural activity of dopaminergic neurons in the ventral tegmental area (VTA), one of the core nodes of the mesoaccumbens pathway, which is comprised of dopaminergic neurons projecting from the VTA to the nucleus accumbens (NAc), and is a main pathway known to mediate reward-seeking and processing behavior. We found male rats raised in jSI (n = 13) show a lower preference for social interaction compared to group-housed male rats (n = 12) but only when a social stimulus was presented along with a competing non-social reward (unpaired t-test, p = 0.06). Our results show impaired social reward-seeking in male rats raised in jSI when presented with competing stimuli, suggesting a possible link between jSI and impaired social reward behavior, and more precisely, impairments in processing the value of social interaction when presented with a competing stimulus. Ongoing *in vivo* fiber photometry recording experiments during social interaction will also shed light on the impact of jSI on neural activity of VTA-DA neurons and DA neurotransmission in the NAc and how changes in neural activity may underlie impairments in processing social reward.

Disclosures: **M. Kim:** None. **M. Barbier:** None. **K. Thirtamara Rajamani:** None. **H. Harony-Nicolas:** None.

Poster

236. Social Cognition and Motivation

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Program #/Poster #: 236.13

Topic: G.03. Motivation

Support: MEXT 17H07032
MEXT 17H06060
MEXT 17K19922
MEXT 19K16899
MEXT 19H03539

Title: We investigated effects of repeated social defeat stress on effort-based decision making test, and found that dopamine receptor type I expressing cells in the nucleus accumbnes is one of the candidates causing the decline of motivation in effort-based decision making behavior induced by social defeat stress.

Authors: *M. NISHI, N. ENDO;
Nara Med. Univ., Nara Med. Univ., Nara, Japan

Abstract: Stress is a major risk factor for the development of psychiatric disorders such asdepression, anxiety, and post-traumatic stress disorder. Repeated social defeat stress(RSDS) is commonly employed as an ethologically relevant stressor in rodents.Recently, many studies have shown that the RSDS affects depressive- and anxiety-likebehaviours that strongly affect

motivation. We examined motivation related to rewardacquisition in RSDS mice by using our original effort-based decision-making test basedon the concept of high-reward/high-cost choice versus low-reward/low-cost choice.Adult male C57BL/6N mice were repeatedly exposed to ICR mice for 10min/day for10days. RSDS mice that exhibited 40% or more social avoidance ratio were employedin subsequent behavioral tests. In the effort-based decision-making test, chocolate as ahigh-reward and food pellet as a low-reward were placed in each side of theexperimental box. A high wall was set upped as a high-cost in front of the chocolate,which was variable from 5-20cm, while a low wall was set upped as a low-cost in frontof the food pellet, which was fixed at 5cm. Mice were allowed to freely select eitherhigh-reward/high-cost or low-reward/low-cost option. Control mice mostly selectedhigh-reward/high-cost option. Contrary, RSDS mice were clearly divided into twogroups; i) RSDS mice that chose the comparable percent of high-reward/high-costoption as with control mice and ii) RSDS mice that chose only low-reward/low-costoption. Importantly, even RSDS mice that only chose low-reward/low-cost optionremained ability to overcome the high wall, showing an intact locomotor activity. Then,we investigated the gene expression level in the reward-related brain regions by usingRNA-Seq and found that the expression level of *Drd1* gene was significantly reduced inthe nucleus accumbens (NAc) of the group choosing only low-reward/low-cost optionas compared with high-reward/high-cost option group. These findings suggest thatdopamine receptor type I (DRD1) expressing neurons in the NAc are involved in thedecline of motivation due to RSDS. We are now investigating effects of DRD1 agonistand antagonist on effort-based decision-making behaviour.

Disclosures: M. Nishi: None. **N. Endo:** None.

Poster

236. Social Cognition and Motivation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 236.14

Topic: G.03. Motivation

Title: Pharmacological attenuation of distress reduces altruistic behavior in the trapped rat paradigm

Authors: E. SARACENO[1], G. RESTIFO-BERNSTEIN[1], M. WYSE[1], F. HOUGH[2], ***H. LOPEZ**[2];
[1]Neurosci., [2]Psychology, Skidmore Col., Saratoga Spgs, NY

Abstract: The developmental, social and neurobiological factors regulating altruistic behavior can be studied with animal models such as the trapped rat paradigm. This paradigm has previously been used to demonstrate that free rats are willing to liberate a trapped cagemate, even if they do not subsequently receive social stimulation. Additionally, treating the free rat with an anxiolytic reduces helping behavior, which suggests that altruistic behavior is motivated by a shared emotional state of distress. The present study was designed to further investigate the effects of pharmacological manipulation of distress on altruistic behavior in the trapped rat

paradigm. We hypothesized that treating the trapped rat with the benzodiazepine anxiolytic, midazolam, would decrease helping behavior from the free rat. Pairs of female Sprague-Dawley rats completed once/daily 40-minute trials for 12 days. During an individual trial, the designated trapped rat was placed in a restrainer in the center of an open field chamber, while its cagemate, the designated free rat, could move around freely. Trapped rats received an intraperitoneal injection of either physiological saline (control) or midazolam (1.5 mg/kg) 30 minutes prior to the start of each trial. The dependent variables measured were: 1) whether the free rat successfully opened the door within the allotted time, and 2) the latency to open the door. Consistent with our hypothesis, free rats in the midazolam condition took longer to learn to release the trapped rat and were consistently slower to open the door during each trial after day 3. These results suggest that midazolam administration decreased the motivation of free rats to liberate their cagemate, possibly due to a decrease in the emotional transfer of distress.

Disclosures: E. Saraceno: None. **G. Restifo-Bernstein:** None. **M. Wyse:** None. **F. Hough:** None. **H. Lopez:** None.

Poster

236. Social Cognition and Motivation

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Program #/Poster #: 236.15

Topic: G.03. Motivation

Support: German Sugar Association Grant

Title: Neural correlates of being a sweetie: experiencing sweet taste promotes social behavior

Authors: C. DENKE[1], M. GÄRTNER[2], A. KÜHNEL[2], F. SCHWEITZER[2], F. RUMPEL[3], *M. SCHAEFER[1,2];
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Abstract: Taste may be the first sense that emerged in evolution. Taste is also a very important sense since it signals potential beneficial or dangerous effects of foods. Given this fundamental role of taste in our lives, it is not surprising that taste also affects our psychological perception and thinking. Previous research demonstrated remarkable psychological effects of sweet taste experiences, suggesting that sweetness may be a source domain for prosocial functioning. For example, recent research reports that briefly experiencing sweet taste made participants more helpful in their intentions and behavior. The current study aims to further test this hypothesis and to examine the neural underpinnings of this effect by using an fMRI approach. Participants were asked to taste sweet, salty, and neutral taste while lying in the fMRI scanner. Subsequently their prosocial behavior was tested by playing the dictator game, a measure of prosocial behavior. Results showed that sweet taste made participants to behave more socially compared with

previously experiencing salty or neutral taste but did not affect control stimuli ratings. FMRI results revealed a modulation of the anterior cingulate cortex associated with this sweetness effect. This brain area is known to play a central role for monitoring conflicts and decisions, which seems to be affected by taste. The results demonstrate that sweet taste has complex psychological effects including positive and socially desirable outcomes. We discuss the results with other studies on psychological sweetness effects and suggest possible implications of these findings.

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Poster

236. Social Cognition and Motivation

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 236.16

Topic: G.03. Motivation

Support: MICINN (Spanish Science Ministry)

Title: Study of the dopaminergic system of the Cntnap2 knockout model of autism

Authors: *T. SIERRA-ARREGUI, M. PRIETO-PIZARRO, O. PENAGARIKANO; Pharmacol., Univ. of Basque Country, Leioa, Spain

Abstract: Alterations in social interaction are one of the core deficits in Autism Spectrum Disorder (ASD). Decades of research have revealed the basic neural circuits underlying social behavior. Among the vast and complex neural networks involved in social behaviors is the mesolimbic circuit, traditionally linked to reward and motivation processing. It is composed by dopaminergic neurons in the Ventral Tegmetal Area (VTA), in the midbrain, that project to the Nucleus Accumbens (NAc) of the Ventral Striatum, in the forebrain. Specifically, previous studies have reported that activation of VTA-NAc projections are enough to increase social interaction in mice. In this work, we use a mouse model of autism, knockout for the Cntnap2 gene, which present the core ASD symptoms, including social behavior alterations. Previous studies in our laboratory have shown an impaired dopamine release in NAc during social behavior in this model, which is likely due to alterations in the VTA-NAc circuit. In order to test the role of this pathway in the social behavior deficits found in the model, we chemogenetically activated VTA dopaminergic neurons with DREADDs and performed a three chamber social task. Our results showed an improvement in social behavior in the VTA activated KO mice, comparing with the controls. Further, to find out if the alterations in the VTA-NAc pathway are associated with structural alterations we analyzed the number of DA expressing neurons in the VTA and their NAc projections by viral tracing. Our results did not show any significant differences between WT and Cntnap2 KO, which suggests that the alterations found are of functional nature. Taken together, these results indicate a functional impairment in the VTA-

NAc pathway in the Cntnap2-KO model of autism upon social stimuli. Further studies are needed to understand the specific circuit impairments and their implications, in order to identify drug targets to alleviate the social symptoms of the disorder

Disclosures: T. Sierra-Arregui: None. **M. Prieto-Pizarro:** None. **O. Penagarikano:** None.

Poster

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Topic: G.03. Motivation

Support: NIDA Grant DA047678
NIDA Grant DA041482

Title: Dopamine control of social novelty preference is constrained by an interpeduncular tegmentum circuit

Authors: *S. MOLAS, T. G. FREELS, R. ZHAO-SHEA, M. BARBINI, A. R. TAPPER; Dep. Neurobiology, Brudnick Neuropsychiatric Res. Inst., UMass Chan Med. Sch., Worcester, MA

Abstract: Social behaviors represent evolutionary conserved innate motivational drives critical for survival. Animal species highly react to social novelty cues as compared to familiar as an action selection mode that adjust behavior in ever-changing environments. The behavioral and circuit bases underlying social novelty responses and novelty preference (NP) are unclear. Here, we combined calcium and neurotransmitter sensor expression with fiber photometry recordings integrated with optogenetic photostimulation, to investigate the precise behavioral patterns and the circuitry dynamics underlying social novelty exploration and NP in mice. We reveal that mesolimbic dopamine (DA) neurotransmission is strongly and predominantly engaged by social novelty with prolonged DAergic activity sustaining motivation for novel social investigations. DA responses exhibit neuronal drift, activating prior to social interaction and with reduced magnitude, when social novelty transitions to familiarity upon repetition. In addition, we show social familiarity activates interpeduncular nucleus (IPN) GABAergic neurons thereby constraining social NP via regulation of mesolimbic DA neurotransmission through the laterodorsal tegmental area (LDTg). Our results provide an unprecedented, detailed analysis of DA activity dynamic patterns underlying social novelty and NP while also functionally identifying a new circuit that may be implicated in numerous disorders associated with impaired social novelty responses.

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Poster

236. Social Cognition and Motivation

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Program #/Poster #: 236.18

Topic: G.03. Motivation

Title: Prepubescent Vaporized Nicotine Exposure Disrupts Sexual Motivation and Pubertal Onset in Female but not Male Long-Evans Rats.

Authors: *F. A. GUARRACI[1], J. B. BELFIELD[2], K. M. CARTER[3], M. KELLY[2], N. WILLIAMS[2], A. GALE[2], L. YEPEZ[2], L. AVENDANO[1], C. ESTOESTRA[2], H. VALDIVIA[2], B. SENCHEREY[2], J. A. STOKES[4];
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Abstract: The present experiment was designed to determine the effects of prepubescent vaporized nicotine exposure on pubertal onset and the subsequent development of sexual behavior in female and male rats. Long-Evans rats received four daily, 10-minute e-cigarette vape sessions, using Juul 5% Virginia Tobacco Pods in a whole-body exposure chamber or air on consecutive days (post-natal day (PD) 28-31). Puberty was monitored daily starting on PD 31 until vaginal opening or preputial separation was observed in female and male rats respectively. Two weeks after the last vape session on PD 45, the rats were tested for sexual motivation using the partner preference paradigm, whereby subjects were given the opportunity to approach either a sexual receptive, opposite-sex stimulus or a same-sex stimulus. The first phase of the partner preference test restricted physical contact between the subject and the stimulus animals, whereas the second phase permitted unrestricted physical contact (included sexual contact). Four weeks later, we tested partner preference again 10-15 min after a single 10-min vape session. We found that prepubescent vaporized nicotine disrupted puberty and sexual motivation in female rats. Specifically, the day of vaginal opening was delayed in nicotine-exposed female rats compared to air controls. Furthermore, nicotine-exposed female rats spent less time with the male stimulus but more time with the female stimulus when physical contact was restricted compared to air controls. In contrast, no effects of nicotine vapor were observed on pubertal onset or on any measures of copulatory behavior or sexual motivation in male rats. When we exposed the same rats to nicotine vapor or air again, no effect of acute nicotine was observed on sexual motivation in either female or male rats. In conclusion, prepubescent vaporized nicotine exposure affects the development of reproductive physiology and behavior in female rats but not in male rats, whereas acute nicotine vapor had no direct effects on sexual behavior in either female or male adult rat.

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Poster

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Topic: G.03. Motivation

Support: R01 MH121603
F31 MH125659

Title: Optogenetic Activation and Inhibition of Extended Amygdala Vasopressin Neurons Modulates Sex-Specific Social Approach and Communication in Mice

Authors: *N. RIGNEY[1], S. BELKASIM[1], S. SINGH[2], R. BEAUMONT[2], G. J. DE VRIES[1], A. PETRULIS[1];
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Abstract: The neuropeptide arginine-vasopressin (AVP) has long been implicated in the regulation of social behavior and communication, often sex-specifically, but the source of AVP release relevant for behavior has not been precisely determined. AVP cells in the bed nucleus of the stria terminalis (BNST) are a major source of sex-different AVP expression in brain regions associated with social behavior. Consequently, to define the behavior-relevant sources, we bilaterally injected AAVs that express Cre-dependent channelrhodopsin-2 (EF1a-DIO-hChR2(H134R)-YFP) for cell excitation, Cre-dependent soma-targeted Guillardia theta anion-conducting channelrhodopsin (hSyn1-SIO-stGtACR2-FusionRed) for cell inhibition, or Cre-dependent fluorescent label only (YFP; EF1a-DIO-YFP) as a control, into the BNST of adult AVP-iCre+ male and female mice. We next tested BNST AVP projections to the lateral septum (LS) by targeting BNST-LS AVP terminals. After recovery, subjects underwent a total of four tests for social communication (scent marking, ultrasonic vocalizations) and social investigation in a three-chamber apparatus. Each subject received two test days with light stimulation and two test days without light stimulation with each stimulus type (male and female conspecifics). We also tested whether BNST AVP neurons affect reward or aversion with a real-time place preference (RTPP) assay. Finally, mice were tested on an elevated-zero maze (EZM) for anxiety-like behavior. Preliminary results indicate that, in male mice, stimulation of BNST AVP-expressing neurons increases social investigation of male and female conspecifics, while inhibition of these neurons decreases social investigation to males, specifically. In females, stimulation of BNST AVP-expressing neurons increases social investigation of male conspecifics. When BNST AVP neurons were inhibited, only females displayed significant photostimulation-side preference during the RTPP relative to controls. Future work will test if the BNST-LS AVP pathway is involved in modulating these results. Overall, these results point to differential involvement of BNST AVP neurons in social behavior and communication. Similar sex differences in the neurochemical underpinnings of behavior may contribute to sex differences in disorders of social behavior and communication.

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Poster

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Title: Neurogranin regulates blood-brain barrier permeability and accumbal reward response

Authors: *A. AKANDE, H. W. NAM;
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Abstract: Disruption of the BBB in the NAc has been linked to susceptibility to social defeat-induced stress and depression; however, its molecular mechanism remains unclear. Neurogranin (Ng), a calcium-calmodulin regulating protein, is highly expressed in the neuron and regulates reward response in rodents. In humans, Ng dysregulation has been associated with neurological diseases such as autism spectrum disorder, Alzheimer's disease, addiction, and schizophrenia. Interestingly, we identified that Ng is also expressed in the BBB. Therefore, we hypothesize that Ng expression in the BBB of NAc contributes to reward response. First, we assessed BBB permeability using Evans blue extravasation assay and found increased apical-basolateral permeability from blood to brain tissue in Ng null mice. Then, fluorescein-labeled dextran dye was injected bilaterally into the ventricles to measure basolateral-apical permeability. Ng null mice showed significantly increased levels of blood dextran dye compared to their wild-type littermates. We found that Ng null mice show considerably decreased claudin-5 and occludin expression in the NAc of Ng knockout mice which may contribute to increased BBB permeability. Finally, label-free proteomic analysis of NAc and endothelial-specific Ng null mice identified Akt-mediated gene expression change consistent with conventional Ng null mice. We found that depletion of Ng expression in the NAc decreases the sociability in mice. In conclusion, Ng depletion in the neurovascular unit leads to a loss of BBB integrity, as evidenced by increased BBB permeability and a decrease in the expression of crucial tight junctional proteins in the NAc. This may contribute to neurological disease phenotypes of altered reward response against natural reward and hedonic stimuli.

Disclosures: A. Akande: None. **H.W. Nam:** None.

Poster

236. Social Cognition and Motivation

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Title: Mapping experience-dependent change in a macro-circuit for aggression

Authors: *J. M. IRAVEDRA GARCÍA, E. M. GUTHMAN, A. L. FALKNER;
Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: Prior social experience shapes future social decision-making. In many species, the experience of winning fights can lead to several behavioral changes, including increased aggression. However, the extent of these changes and what neural mechanisms instantiate them remain elusive. Here, we paired unsupervised behavioral classification methods with large-scale multi-region calcium imaging to map experience-dependent changes in a neural macro-circuit. First, CD1 mice (male and female, at least 8 weeks old) were given aggression experience in a resident-intruder assay across multiple consecutive days. We derived posture dynamics from each session via a deep neural network-based strategy (SLEAP), and used these to parcellate behavior into clusters via a recently developed unsupervised technique (Willmore et al. 2022). Our clustering approach revealed over 20 distinguishable behavioral states, which included multiple modes of asocial, agonistic, and non-agonistic behaviors. Changes in the occupancy and transitions of these states varied across experience and from animal to animal, with different animals displaying peak agonistic behavior levels during different days of training. We next sought to map the precise timing of these behavior changes to changes in neural activity. We targeted changes in the social decision-making network, a highly conserved network of regions broadly involved in social behavior. These include nucleus accumbens (NAc), lateral habenula (LHb), lateral septum, preoptic area, bed nucleus of the stria terminalis, anterior and ventromedial hypothalamus, ventral premammillary nucleus, medial and posterior amygdala, and periaqueductal gray. To record from this macro-circuit, we built a fiber photometry system which enabled precise targeting of each region, as well as simultaneous recording of putative excitatory and inhibitory cell populations using a dual-color strategy (vgat+ and vgat-). Our recordings reveal that, as animals become more task engaged, so too do a majority of excitatory and inhibitory nodes across the network. Moreover, aggression experienced animals demonstrate selective increases in cross-regional coactivity mapped to social behavior, along with changes in the lead-lag relationship of cross-region activity mapped to certain nodes like NAc and LHb. Moreover, using site-specific perturbation (activating each site and recording from the rest), we tested whether aggression experience changes plasticity in the functional macro-circuit. Altogether, our results provide a framework through which experience-dependent changes in macro-circuit activity guide and stabilize social behavior.

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Poster

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NIGMS Postdoctoral Research Associate Training Program

Title: Aggression priming is regulated by diverging medial amygdala pathways

Authors: *S. SKINNER[1], C. J. BARTSCH[1], X. MA[2], Q. GU[2], A. V. KRAVITZ[3], H. LI[4], M. POTEGAL[5], Z. LI[6], J. C. NORDMAN[7];
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Abstract: Recurring and excessive violent aggression is a serious concern for society and is a symptom of many psychiatric diseases such as PTSD. It is estimated that over 20 million violent crimes are committed in America each year, costing the taxpayers 2.2 trillion dollars annually. Unfortunately, few clinical options are available to treat excessive and recurring aggression and those that do exist are largely ineffective. A better understanding of the neurocircuitry of aggression will be essential in developing new and better therapies. The medial amygdala (MeA) is a key area in violent aggression and is a successful target for neurosurgical interventions to treat uncontrollable escalated aggression. Recently we showed that prior attack experience primes aggression in mice by strengthening synapses between the MeA and the ventromedial hypothalamus (VmH) and bed nucleus of the stria terminalis (BNST). Furthermore, we show that optogenetically weakening these pathways can suppress aggression priming while optogenetically strengthening these pathways can simulate aggression priming in a pathway-dependent manner. To further examine the role of these MeA pathways in aggression, we conducted electrophysiological recordings of VmH and BNST neurons in mice during an interaction with a conspecific. We found an increase in VmH and BNST activity during an attack bout. These changes correlated with the potentiation of the MeA pathways that drive aggression priming. These results indicate a previously unknown role for the VmH and BNST in regulating specific stages of an attack, suggesting new potential drug targets to treat excessive and recurring violent aggression.

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Poster

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R01AA026256

Title: Stress biases information routing through circuits implicated in valence processing

Authors: *J. L. MAGUIRE, B. T. STONE, P. L. COLMERS, A. EVANS-STRONG, N. L. WALTON, P. ANTONOUDIOU;
Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Chronic stress is a major risk factor for psychiatric illnesses, including anxiety and depression; however, the pathophysiological mechanisms whereby stress leads to mood disorders remain unclear. Communication within and between the medial prefrontal cortex (mPFC) and basolateral amygdala (BLA) has been shown to play a critical role in both positive and negative valence processing. We recently demonstrated that chronic unpredictable stress (CUS) induces long-term changes in the network communication between the mPFC and BLA associated with protracted behavioral deficits. However, the cellular mechanisms mediating the CUS-induced altered network and behavioral states remains unclear. Using slice electrophysiology, we demonstrate an increase variance in the properties of principal neurons in the BLA following CUS, leading to subsets of neurons with increased excitability and others with decreased excitability. Using retrograde tract tracing approaches, we were able to map these changes in intrinsic excitability following CUS to projection-specific subsets of principal neurons in the BLA. Specifically, we found that following CUS principal neurons projecting to the bed nucleus of the stria terminalis (BnST) are facilitated whereas those projecting to the nucleus accumbens (NAcc) are suppressed. Moreover, using in vivo calcium imaging, we found that the activity of principal neurons in the BLA, which were subjected to the CUS paradigm, fell into three distinct clusters that responded different to stress. One population increased their activity, one population decreased their activity, and one population remained unchanged. Consistent with a role of these changes in driving altered behavioral states, chemogenetic activation of the BnST-projecting neurons and simultaneous suppression of NAcc-projecting principal neurons in the BLA was sufficient to increase negative valence processing in naïve mice; whereas, chemogenetic activation of NAcc-projecting and simultaneous suppression of BnST-projecting BLA principal neurons prevented the behavioral deficits observed following CUS. These data demonstrate that chronic stress differentially alters the activity of specific subsets of principal neurons in the BLA, facilitating specific outputs and suppressing others. These findings suggest that prolonged exposure to stressful stimuli alters information routing through the BLA that is sufficient to control/alter behavioral states.

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Poster

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Support: NIDA R00DA045662
NIDA P30DA048736
NIDA T32 DA 007278

Title: Classification of social choice behavior in male and female mice during operant chronic social defeat stress reveals classical social stress phenotypes.

Authors: *K. N. SCHNEIDER[1,2], J. NAVARRETE[1,2,3], B. M. SMITH[1], Y. ZHANG[1,2], E. GROSS[1], S. A. GOLDEN[1,2];
[1]Dept. of Biol. Structure, [2]Ctr. for Excellence in Neurobio. of Addiction, Pain and Emotion (NAPE), Univ. of Washington, Seattle, WA; [3]Univ. of Washington Grad. Program in Neurosci., Seattle, WA

Abstract: Background: Chronic social defeat stress (CSDS) is commonly used to induce depression-like behavior in mice, marked by presence or absence of anhedonia and social avoidance in stress susceptible (S) or resilient (R) populations, respectively. We recently introduced an operant (oCSDS) version of the CSDS procedure that allows for tractable behavioral readouts during the development of these phenotypes. While traditional approaches have relied on exploratory affiliative behavior during social interaction tests to determine susceptibility, here we present a computational approach combining dimensionality reduction (PCA) with supervised classification techniques to integrate multimodal prosocial behaviors observed during oCSDS. **Methods:** Experimental male and female C57BL/6J mice underwent the oCSDS procedure (see associated poster). Briefly, mice were trained to socially self-administer (SA) conspecific partners and then exposed to repeated social stress. Following the social stress phase, they were tested for socially non-reinforced (extinction test) and reinforced (progressive ratio test, PR) social seeking, and classical social interaction (SI). Data from each phase of oCSDS were combined into a feature space that was normalized and reduced via PCA before clustering analysis. Results were then retroactively labeled based on classification, in order to examine behavioral differences between clusters. **Results:** PCs were most influenced by a combination of social stress, post-defeat non-reinforced social seeking and reinforced PR metrics. Consistent with previous CSDS findings, classification of social reward-seeking measures in oCSDS revealed R and S populations of male mice. Male S mice showed reduced motivation for affiliative interaction compared to control animals, which in turn were not

significantly different from R mice. Unexpectedly, while females did not differ in SA compared to controls across social stress, they exhibited higher social reward seeking during post-defeat tests. On SI tests, males exhibited social behavior consistent with their operant results, while females showed no differences in social avoidance. **Conclusions:** Cluster analysis reveals that males classified as S or R also show social behavioral patterns throughout oCSDS that correlate with traditional assessments. These results provide further evidence for the tractability of oCSDS as an alternative to classical CSDS procedures. Future studies aim to combine oCSDS with detailed behavioral tracking and large-scale recordings, in order to investigate behavioral and neural dynamics underlying development of social stress phenotypes during CSDS.

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Poster

236. Social Cognition and Motivation

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Program #/Poster #: 236.25

Topic: G.03. Motivation

Support: KAKEN 22K07336

Title: Can sexual experience enhance male attractiveness in mice?

Authors: *Y. N. OHNISHI, Y. KAWAHARA, Y. H. OHNISHI, A. NISHI;
Kurume Univ. Sch. of Med., Kurume Univ. Sch. of Med., Kurume, Japan

Abstract: We have established the methods to detect the most attractive male mouse for female mice among four male mice and reported them in the past SfN (2017, 2018). This attractiveness, demonstrated by the behavior of female mice, was independent of the female's estrous cycle, genetic background, age, sexual experience, and history of childbearing. At SfN 2019, we reported that the hairstyles of skinheads on attractive mice do not affect mouse attractiveness. We also recently attempted to define which color of mouse is more attractive: white or black mice. The results showed no conclusive difference. However, when male mice were hidden by four layers of breathable filters, female mice were unable to select attractive male mice. Furthermore, genetically blinded female mice showed very different preferences for the same male mice. This indicates that factors other than obvious differences in appearance to humans may be one of the major factors in male attractiveness. In this study, we examined the effects of sexual experience on male attractiveness. First, we prepared five male mice of the same litter; one male mouse, A, was placed in a cage with five female mice, and the other 4 mice were placed in a separate cage. Male preference tests revealed significant differences in male attractiveness in the order B > C, D, E. After three weeks, male attractiveness was examined under conditions in which A, who had repeated sexual experiences, and E, who did not, were exchanged, and A showed extremely high male attractiveness. Next, we put male mouse E in the

cage of five female mice, and the rest of A, B, C and D in a same cage. After three weeks, we performed male attractive test with male mice A, B, C, E. However, there is no significant difference of male attractiveness among them, suggesting that sexual activity does not increase the attractiveness of all male mice, and that the increased attractiveness might not be sustained in the absence of females. Therefore, we are trying to find out whether chemical activation of brain regions after sexual experiences enhances male attractiveness, and to explore the conditions and expiration date of the male sexual experience effect.

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Poster

236. Social Cognition and Motivation

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Topic: G.03. Motivation

Support: NOMIS Foundation
Simons Foundation

Title: Neural Control of the Homeostatic Need for Social Interactions

Authors: D. LIU, M. RAHMAN, A. JOHNSON, C. DULAC;
Harvard, Cambridge, MA

Abstract: Social interactions are central to both human and animal lives. In animals, social grouping decreases the risk of predation, reduces energy consumption and leads to mutually beneficial behaviors such as parenting and group foraging. By contrast, social isolation leads to a wide range of mental and physical problems in both human and animals, suggesting that social interaction is an evolutionarily selected and conserved fundamental need. However, how this instinctive social need is generated and regulated by specific neurons and neuronal circuits remain unknown.
In this study, we report novel populations of hypothalamic neurons in mice that are activated by either social isolation or social reunion, providing promising candidates as well as genetic tools to investigate neural circuits encoding social need and social satiety. The functional contributions of these neurons to social need were causally assessed by optogenetic approaches. Moreover, cell type-specific viral-mediated neural tracing uncovered the connectivity between these neuronal populations and brain areas associated with emotional state, social reward as well as metabolism, offering an overarching understanding of the generation and regulation of social need. Finally, we identified social touch as an important sensory input modulating social need and social satiety.
This study provides novel mechanistic insights into the nature and function of neuronal circuits associated with instinctive social need and suggests new avenues for the understanding and treatment of mental disorders that are induced or exacerbated by social isolation.

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Poster

236. Social Cognition and Motivation

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Topic: G.03. Motivation

Title: The amplitude of the P300 component is significantly modulated by the Proteus effect in VR

Authors: *M. IWASAKI[1,2], Y. YOKOTA[1], Y. NARUSE[1];
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Abstract: Virtual reality (VR) and metaverse technologies provide us an avatar which is an electronic own alter ego. In these environments, a psychological effect of the avatar's appearance on user's behavior and cognition is called the Proteus effect. However, few studies on the Proteus effect on electroencephalogram (EEG) activities have been reported. The present study investigated the modulation of event-related potentials' (ERPs) amplitude while participants performed a gambling task using two different avatars. We hypothesized that the appearance of the avatars during the gambling task in VR space change participants' behavioral choice about the task and modulate the cognitive processing of outcome of the task.Twenty-one individuals participated in this study. In the study, participants performed a simple gambling task in a VR casino space. In a single trial, participants were given two choices and asked to choose between one of them. Each of the choice was labeled with a reward probability and a gain point, and a penalty probability and a loss point, respectively. The two choices had different risk appetite; one of which was high-risk-high-return choice and the other was low-risk-low-return. After that, the outcome of the participants' choices was presented. Participants performed 150 trials in the gambling task with avatars dressed in two different outfits to produce different Proteus Effect; one of avatar was a formal outfit (formal avatar) and the other was a casual outfit (casual avatar). EEG were measured during the tasks. After the tasks, participants answered the subjective questionnaire regarding each avatar.The questionnaire showed that participants were significantly larger exuberance in formal avatar condition than in the casual avatar ($p = 0.0167$, $t = -2.6121$). We did not observe significant difference in risk appetite for the choice of the task due to the use of a particular avatar ($p = 0.2211$, $t = -1.2629$). In contrast, P300 component — one of ERP component that related to the engagement of attention — showed significantly larger amplitudes in formal avatar condition than in the casual avatar, regardless of outcome valence ($p = 0.0089$, $Chisq = 6.8374$). These results suggest that the increase of attentional resources to the outcome of the task was enhanced by the participants' exuberance from wearing the formal avatar. This study showed the possibility of objectively evaluating changes in attentional resources in the Proteus effect by using P300. In the future, it will be possible to evaluate a participant's attentional state from P300 and suggest an appropriate avatar for the VR situation to enhance their exuberance.

Disclosures: **M. Iwasaki:** None. **Y. Yokota:** None. **Y. Naruse:** None.

Poster

236. Social Cognition and Motivation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 236.28

Topic: G.03. Motivation

Support: NIH P60 AA010760
R25 DA050727
I01 BX004699
U01 AA013519
T32 AA007468

Title: Acute and chronic wheel-running behaviors in High Drinking in the Dark and Heterogeneous Stock/Northport Mice

Authors: K. GRIGSBY, ***Z. USMANI**, J. ANDERSON, A. OZBURN;
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Abstract: Maintaining healthy and consistent levels of physical activity (PA) is a clinically proven and low-cost means of reducing the onset of >40-chronic diseases and may provide an excellent strategy for managing and treating harmful alcohol use. Population-based studies indicate a positive relationship between PA levels and alcohol intake across moderate drinkers, while non-drinkers and heavy drinkers tend to be less physically active. This suggests healthy PA levels may help promote moderate alcohol intake and offer a path to harm reduction. Previous human and animal studies demonstrate that there is a strong genetic contribution to both PA and alcohol use. Wheel-running (WR) – a well-characterized rodent model of voluntary PA – is known to reduce self-administration and craving for many drugs of abuse; however, its effects on alcohol intake are mixed. Here, we test whether differences exist in acute (presumably aversive) and chronic (presumably reinforcing) wheel-running behavior in two genetically unique mouse lines: inbred High Drinking in the Dark line 1 (iHDID-1; selectively bred to drink alcohol to intoxication and then inbred to maintain phenotype) and Heterogeneous Stock/Northport (HS/Npt; genetically heterogeneous line derived from an 8-way intercross). Adult male and female mice of both lines (n=10/sex/line) were given wheel access for 28-days, whereby wheel revolutions were continuously recorded (every 6-minutes, used to calculate distance/minute). Weekly averages of distance revealed that male iHDID-1 mice run ~2-fold further during week 2 than do female iHDID-1 or HS/Npt (of both sexes; main effect of time [$F(3,68) = 12.42$; $p < 0.001$)] and time x genotype interaction [$F(3,24) = 2.89$; $p < 0.05$]). iHDID-1 mice are less sensitive to the aversive effects of alcohol (compared to HS/Npt), and our data suggest they may be less sensitive to the acute aversive effects of WR. The earlier escalation of WR observed in male iHDID-1 mice may also reflect a quicker transition to the reinforcement of WR. As a proxy for measuring their hedonic state, mice were provided 2d of access to

saccharin (0.4% w/v) solution vs. tap water in a two-bottle choice preference test to assess potential line differences in consumption of this highly palatable, non-caloric, solution after chronic WR. No differences in saccharin intake or preference were observed at this time point. Ongoing work is being carried out to measure saccharin intake at the 2-week WR time point as a proxy for measuring their hedonic state at a time when WR is potentially physiologically stressful for one line, but not the other. Future studies will measure the effects of acute and chronic WR on binge-like ethanol drinking.

Disclosures: K. Grigsby: None. **Z. Usmani:** None. **J. Anderson:** None. **A. Ozburn:** None.

Poster

236. Social Cognition and Motivation

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Program #/Poster #: 236.29

Topic: G.03. Motivation

Support: NIDA 1R01DA053472

Title: Sated vs Starved: Sex Differences in Highly Palatable Food Demand

Authors: *K. OGUNSEYE[1], E. J. GARCIA[2], H. SUN[2], J. KASPER[2], J. HOMMEL[2];
[1]Human Pathophysiology and Translational Medicine, Ctr. for Addiction Res., [2]Pharmacol. and Toxicology, Ctr. for Addiction Res., Univ. of Texas Med. Br., Galveston, TX

Abstract: Obesity is a growing global public health issue and one of the leading contributors to preventable death in the United States. Obesity is a complex and multifactorial disease in which aberrant behavior, like the overconsumption of highly palatable food, is a major determinant of disease. Highly palatable food is known to be reinforcing in both humans and in rodents. Women and men tend to experience weight gain and weight loss differently, and the underpinnings of motivated feeding behavior in different sexes is largely unclear. Utilizing a within-session behavior economic paradigm, we took concurrent measurements of hedonic set point (Q0) and demand elasticity (α) which allowed for the determination of P Max (defense of elasticity), and essential value (the strength of the reinforcer) for high fat food in four different feeding conditions in both male and female C57BL6 mice. We observed that during hedonic feeding conditions, high fat food demand is primarily driven by interactions between sex and background diet of either standard food or high fat food. When these animals are under mild food restriction conditions, palatable food demand is influenced by background diet. These findings provide evidence of distinct differences in several aspects of motivational behavior for highly palatable foods and indicate important interactions between sex, feeding context, and diet.

Disclosures: K. Ogunseye: None. **E.J. Garcia:** None. **H. Sun:** None. **J. Kasper:** None. **J. Hommel:** None.

Poster

236. Social Cognition and Motivation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 236.30

Topic: G.03. Motivation

Support: Takeda Pharmaceutical Company Limited

Title: The effect of the antidepressant ketamine in effort-based decision making task in rats

Authors: *Y. YAMAMOTO, M. NAKASHIMA, K. SUZUKI;
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Abstract: Anhedonia is a core feature symptom of major depressive disorder (MDD), which is thought to be driven by reduced anticipation and motivation; however, its behavioral and neurobiological mechanism has not yet been fully understood. Clinical studies using translational measurement of reward motivation, such as the effort-based decision-making task, have demonstrated that MDD patients were less willing to expend effort for rewards than controls, suggesting that anhedonia may reflect the impairments in reward motivation. Translating behavioral tasks from animals to humans and assessing the subcomponent relevant to anhedonia is important as it provides objective biomarkers in the development of new antidepressants. Ketamine, an N-methyl-$_D$-aspartate receptor antagonist, has emerged as a rapid-acting and potent antidepressant. Although some clinical studies suggest that ketamine has efficacy in reward processing, the effort-based decision making task may help elucidate more precisely the mechanisms underlying its efficacy. The present study is aimed at investigating the effect of ketamine on reward motivation in the rat effort-based decision-making task. This task allows rats to choose between preferred reinforcers that require high effort to obtain and low-effort/low reward options under an operant conditioning paradigm. Tetrabenazine (TBZ), a vesicular monoamine transport type-2 inhibitor that blocks dopamine storage and depletes dopamine, is known to induce depressive-like symptoms such as motivational impairments in rodents and humans; TBZ has been shown reliably to induce a low-effort bias. Indeed, intraperitoneal (i.p.) administration of TBZ at 0.75 mg/kg to Wistar rats caused a significant decrease in the number of lever presses for reward (sucrose pellet) and increased chow consumption without a robust increase in the number of non-active lever presses and a significant change in the preference to sucrose pellet, suggesting impairment of reward motivation. We confirmed that this impairment by TBZ was improved by i.p. administration of bupropion at 20 mg/kg, which has been reported to have some efficacy in patients with depression. Under these experimental conditions, when i.p. administration of ketamine at 30 mg/kg was conducted 24 hours prior to the treatment with TBZ to avoid an acute effect of psychotomimetic-like behavior, TBZ-induced impairment in reward motivation was prevented; pretreatment with ketamine increased the number of lever presses for reward and decreased chow consumption. These results support the idea that ketamine has the potential to ameliorate impairment of reward motivation relevant to anhedonia.

Disclosures: **Y. Yamamoto:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **M. Nakashima:** A. Employment/Salary (full or part-time):;

Takeda Pharmaceutical Company Limited. **K. Suzuki:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited.

Poster

237. Learning and Memory

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 237.01

Topic: H.09. Spatial Navigation

Support: Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, project no. NC17062.0
Consejo Nacional de Ciencia y Tecnología, project no. 285181

Title: Blockade of androgen receptors impairs spatial memory in aged male rats

Authors: *G. JIMÉNEZ RUBIO[1], J. J. HERRERA PÉREZ[1], O. T. HERNÁNDEZ HERNÁNDEZ[2], L. MARTÍNEZ MOTA[1];
[1]Inst. Nacional de Psiquiatría Ramón de la Fuente Muñiz, Ciudad de México, Mexico; [2]Consejo Nacional de Ciencia y Tecnología, Ciudad de México, Mexico

Abstract: Spatial memory declines with age, this deficit may be associated with the age-related decrease in testosterone levels. Signaling of androgen receptors participate in testosterone-mediated cognitive effects; however, the role of androgen receptors in the spatial memory of aged males is complex and require further investigation. Our study aimed to evaluate the effect of androgen receptor blockade on the performance of aged male rats in a spatial memory task. Aged (21 months old) Wistar rats were assigned to one of three independent groups: control, vehicle or flutamide (an antagonist of androgen receptors) treated (10 mg/kg, sc, for 14 days). Spatial memory was evaluated using the Barnes maze from day 8 to 14 of flutamide administration. The acquisition (4 daily trials/4 days) and retention (1 trial/day, 3 days after acquisition) phases of spatial memory were evaluated. We found that the rats that received flutamide showed a delay in learning of the task because the latency to find the goal and distance traveled decreased until the third day of the acquisition phase, in relation with the control and vehicle groups that showed such improvement since the second day. The blockade of androgen receptors also impaired the memory consolidation evaluated at the first trial of every day during acquisition phase. Control and vehicle groups decreased the latency to find the goal from day 3, and the vehicle group reduced the number errors since the second day of evaluation. Interestingly, flutamide-treated males decreased the latency to find the goal until the last day of evaluation and did not decrease the number of errors from days 2 to 4. No statistical differences were observed in the retention phase among groups. Present findings show that flutamide impaired the performance of aged male rats in the Barnes maze and suggest that androgen receptor signaling is important for spatial memory learning in aged individuals.

Disclosures: **G. Jiménez Rubio:** None. **J.J. Herrera Pérez:** None. **O.T. Hernández Hernández:** None. **L. Martínez Mota:** None.

Poster

237. Learning and Memory

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 237.02

Topic: H.09. Spatial Navigation

Title: Caffeine and camellia sinensis enhanced cognition and increased acetylcholine-esterase activity in scopolamine-induced memory loss in female swiss mice

Authors: *A. O. IMAM-FULANI[1], L. O. OLAJIDE[1], T. A. ADEDEJI[1], O. J. OGUNGBEMI[2]; [1]Physiol., Univ. of Ilorin, Ilorin, Nigeria; [2]Biochem., Univ. of Ibadan, Ibadan, Nigeria

Abstract: Caffeine and *Camellia sinensis* (green tea) has been known to have positive effect on memory. The present study investigated the possible effect of caffeine and green tea co-administration on spatial memory, acetylcholine esterase activity, inflammatory marker and oxidative stress markers in scopolamine-induced memory loss in female swiss mice. Spatial memory tests using Y-maze and Morris water maze was carried out, followed by acetylcholine esterase activity, Tumor Necrosis Factor (TNF) alpha and oxidative stress biomarkers evaluation from the mice brain tissues after caffeine and green tea administration. Scopolamine administered intraperitoneally at a dose of 1mg/kg BW for 7 days significantly reduced the percent alternation of the mice in Y-maze thus, increased acetylcholinesterase activity and increased TNF alpha level. However, caffeine administered orally at a dose 50mg/kg BW and green tea administered orally at a dose of 60mg/kg BW increased the percent alternation significantly, reduced acetylcholine esterase activity and reduced the TNF alpha level significantly. Treatment with caffeine and green tea shows no significant effects on malondialdehyde (MDA) and glutathione (GSH) level compared to control and scopolamine-induced memory loss group. This findings shows scopolamine has a deteriorating effect on cognition by increasing acetylcholinesterase activities thus mopping out acetylcholine at a faster rate. However, caffeine and green tea singly and in combination restored cognition, reduced acetylcholinesterase activity and restored TNF alpha level. The neuroprotective effect of caffeine and green tea was compared to that of Donepezil, an established cognition enhancing drug and the effect was agonistic. The ability of caffeine and green tea to reduce acetylcholinesterase activity could be the mechanism for its ability to enhance memory. The ability of these compounds in restoring TNF alpha level further potentiates its neuroprotective capability.

Disclosures: **A.O. Imam-Fulani:** None. **L.O. Olajide:** None. **T.A. Adedeji:** None. **O.J. Ogungbemi:** None.

Poster

237. Learning and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 237.03

Topic: H.09. Spatial Navigation

Title: The impact of hormonal contraceptives on spatial navigation in the virtual Hex maze task.

Authors: *J. M. LACASSE[1], S. DEVINE[1], M. PROFITT[1], T. D. FERGUSON[3], B. EPPINGER[1], W. G. BRAKE[2];
[2]Ctr. for Studies in Behav Neuro, [1]Concordia Univ., Montreal, QC, Canada; [3]Univ. of Victoria, Victoria, BC, Canada

Abstract: When navigating an environment, humans can navigate according to an internal "cognitive map", a specific environmental cue, or motoric body-turns learned over time. Women using androgenic forms of hormonal contraceptives (HC) and men show shorter latencies to complete a spatial navigation task compared naturally cycling women. However, no studies have examined the impact of hormonal contraceptives on the navigation strategy used in a spatial navigation task. We recruited naturally cycling women (NC; n=55), women using androgenic forms of HC (n=56), and men (M; n=56) and assessed spatial navigation using a virtual Hex maze task. We found that men have shorter latencies compared to cycling women and those taking hormonal contraceptives. However, we observed no differences in navigation strategy among these groups. Contraceptive type impacted latency during spatial navigation. Specifically, those using 3rd generation progestins had latencies similar to those observed in men which were significantly shorter than those observed in users of 2nd generation progestins. There was a small negative correlation (R^2 = .19) which indicated that increasing doses of ethinyl estradiol used in contraceptive formulations predicted shorter latencies in the Hex maze. Our results replicate previous findings that men, on average, have shorter latencies in spatial navigation tasks. Further, in hormonal contraceptive users, progestin generation and dose impact latency during spatial navigation.

Disclosures: J.M. Lacasse: None. **S. Devine:** None. **M. Profitt:** None. **T.D. Ferguson:** None. **B. Eppinger:** None. **W.G. Brake:** None.

Poster

237. Learning and Memory

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Program #/Poster #: 237.04

Topic: H.09. Spatial Navigation

Support: NIDA R01DA043535

Title: Chronic tetrahydrocannabinol attenuates exploratory behavior abnormalities in the DAT knockdown mouse model of bipolar mania

Authors: *M. NOBACK[1], P. PHAM[1], S. FODOR[1], M. FLESHER[1], M. R. BUELL[1], A. L. HALBERSTADT[1], A. MINASSIAN[1], W. PERRY[1], J. W. YOUNG[3], M. A. GEYER[2]; [1]Univ. of California San Diego, San Diego, CA; [2]Psychiatry, Univ. of California San Diego, La Jolla, CA; [3]Dept Psychiatry, UCSD, La Jolla, CA

Abstract: Bipolar disorder (BD) is a common psychiatric illness affecting 2-5% of the population, yet developing targeted treatments has proven difficult. Mania - the defining feature of BD - is likely driven by dysregulated dopaminergic system. Lowered function and/or expression of dopamine transporter (DAT) are observed in people with BD, implicating elevated dopamine signaling in the disease state. DAT knockdown (KD) mice recreate a myriad of BD-related behaviors including risky decision-making and hyperexploratory behavior in the behavioral pattern monitor (BPM), and may be useful to test potential treatments. Cannabis use is common among people with BD, at rates higher rate than the general population. The primary psychoactive ingredient of cannabis is tetrahydrocannabinol (THC), which decreases dopamine levels over time, and thus has potential as a treatment for BD mania. We tested whether the hyperexploratory behavior of DAT KD mice would be attenuated after chronic (14 days) THC treatment in the BPM. 45 mice (51% DAT KD, 22% male) were tested in the BPM after daily intraperitoneal injection of either THC (3 mg/kg) or saline. THC attenuated several behaviors of the DAT KD mice, including rearing (p=0.055), holepoking (p = 0.08), and spatial d (p= 0.079). THC did not affect the activity of the wildtype littermate mice. Thus, people with BD may be using THC to self-medicate as chronic treatment attenuated some of the hyperexploratory behaviors of DAT KD mice. Given that these effects were specific to the DAT KD animals suggests that chronic THC may address symptoms of BD mania without affecting healthy dopaminergic system function. Additional mice will be tested to meet *a priori* proposed sample sizes. Brains have been removed for analysis for potential mechanistic changes in dopamine receptor availability.

Disclosures: **M. Noback:** None. **P. Pham:** None. **S. Fodor:** None. **M. Flesher:** None. **M.R. Buell:** None. **A.L. Halberstadt:** None. **A. Minassian:** None. **W. Perry:** None. **J.W. Young:** None. **M.A. Geyer:** None.

Poster

237. Learning and Memory

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Program #/Poster #: 237.05

Topic: H.09. Spatial Navigation

Support: Krembil Foundation Grant
CIHR

Title: Mice rely on path-integration to create a cognitive map during navigation

Authors: *J. XU, J.-C. BEIQUE, L. MALER;
Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Navigation is an essential ability that allows us to travel between remembered spatial locations. Navigation can be based on external sensory cues (allothetic) or rather on path integration using self-motion cues (idiothetic). Mice are frequently used to study the neural basis of spatial learning, yet much is unknown about how they plan routes in unconstrained, goal-based navigation. Here, we designed a novel maze which can disambiguate navigation strategies that rely on allothetic versus idiothetic cues. In our "Hidden Food Maze" (HFM), mice are trained to search for food hidden in the floor of a circular arena. The mouse enters from one of four possible, permutable entrances. Crucially, the arena is rotationally symmetrical and appears identical from all entrances, so mice can only learn the food's location by orienting to experimenter-introduced cues if their entrances change, or internal cues if their entrance does not move. Notably, the mouse's home cage is attached to the arena so there is no handling required to start the task. Performance in the HFM is assessed using the distance mice traveled to find the target, search bias in the target location before and after training, and trajectory analysis of search behaviour.
We found that mice (N = 8, C57Bl/6 wild-type male, 8 weeks old) did not significantly improve their performance when images on the arena walls were available to them (Early vs. Late Distance ANOVA p = n.s., search bias in correct area = 28.8%, no optimization of search path). However, mice showed a dramatic improvement in HFM performance when the position of their entrance and the food was fixed, thereby allowing them to rely on path-integration strategy (Early vs. Late Distance ANOVA p < 0.001, search bias in correct area = 44.3%, search path optimized). When mice learned two locations via path integration from the entrance, they followed a novel route to check both entrances during probe trials (distance untrained vs trained p < 0.05).
These results demonstrate that mice strongly rely on path-integration as an orienting system for navigation. Their ability to compute novel routes show mice can create a cognitive map of their environment using path-integration alone.

Disclosures: **J. Xu:** None. **J. Beique:** None. **L. Maler:** None.

Poster

237. Learning and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 237.06

Topic: H.09. Spatial Navigation

Support: Simons Collaboration on the Global Brain, 543015
Simons Collaboration on the Global Brain, 543025

Title: Mice in Manhattan: efficient exploration and automated theory testing in a rapidly reconfigurable maze

Authors: *J. ZHENG[1], R. GUIMARÃES[1], P. PERONA[2], M. MEISTER[1];
[1]Div. of Biol. and Biol. Engin., [2]Div. of Engin. and Applied Sci., California Inst. Technol., Pasadena, CA

Abstract: In the wild, mice need to rapidly navigate between home and resources to forage and escape efficiently. Our previous study showed that mice can learn 10-bit choices in a complex labyrinth after only 10 reward experiences - a learning rate 1000-fold higher than in prevalent two-alternative-forced-choice (2AFC) tasks. Here we further explore and model their learning capacity using the "Manhattan maze" - a novel, three-dimensional maze that can be easily reconfigured to up to 2^{121} graph permutations. We trained the animals in complete darkness to traverse a maze that requires 21 decisions at different turning locations. The mice solved this task and developed error-free trajectories after a few hours. Surprisingly, the animals could learn and remember more than three different maps. Over days, new configurations were solved much faster than the first, potentially via an updated exploration strategy. When switched back to a previously-experienced configuration, the mice solved the maze faster and quickly re-developed error-free trajectories. We then compared the exploration strategies of animals with zero- and first- order Markov models of a random walk, and found that for certain maze configurations the animals were much more efficient than the random walkers. Drawing upon the flexibility and complexity of the Manhattan maze framework, we designed computational methods to automatically generate configurations that test alternate hypotheses about mouse exploration. Given two hypothetical exploration strategies, one can optimize the maze to best differentiate among them, by maximizing the difference in navigation performance under the two policies. This framework could automate experimental design and allow quantitative evaluation of many domains of learning and memory.

Disclosures: J. Zheng: None. **R. Guimarães:** None. **P. Perona:** None. **M. Meister:** None.

Poster

237. Learning and Memory

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Program #/Poster #: 237.07

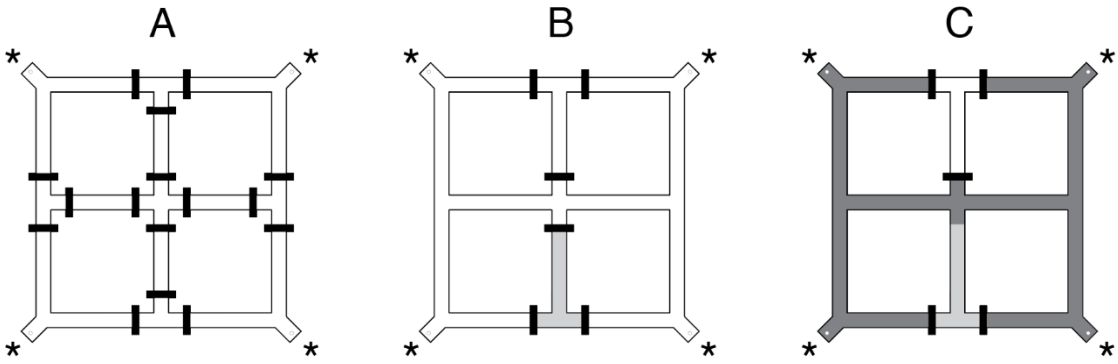
Topic: H.09. Spatial Navigation

Support: NSF1707408

Title: The corner-seeking task: A novel behavior paradigm for investigating spatial memory and navigational decision making

Authors: *R. GRGURICH[1], H. T. BLAIR, IV[2];
[1]UCLA, [2]Dept Psychology, UCLA, Los Angeles, CA

Abstract: Here we describe a novel corner-seeking task designed to investigate path integration, working memory, and trajectory planning in freely behaving rodents. The task is administered on a maze apparatus consisting of a standard plus maze centered inside a 2x2 m square track (A). All track segments are 10 cm wide. The rat's access to choice points is regulated by 16 automatic doors (bold lines in 'A'): 4 surrounding the central choice point, and 3 surrounding each of the four peripheral choice points where the plus bisects an edge of the square. Liquid reward can be delivered at any of the four maze corners (asterisks). On each day of training, one of the four corners is randomly assigned as the only rewarded location on that day. To begin a trial, rats are confined to one of the four inner plus maze segments (B). The center door then lowers to provide access to an H-shaped region (C) where each of the four reward sites is accessible via a different sequence of two choices (LL,LR,RL,RR) at T-intersections beginning from the start arm. The rat freely wanders the H region until it finds the assigned reward site, triggering delivery of 100-300 ul (depending on weight) sweetened condensed milk. Doors are then reconfigured to guide the rat to a new pseudorandomly assigned start location selected from among the 4 inner plus maze segments (B). A new trial then begins, during which the rat must return to the same rewarded location from the new starting point. We trained different groups of rats to perform the corner-seeking task either in the light (with overhead orienting cues) or complete darkness, allowing up to 48 trials or 48 min (whichever comes first) per daily session. Rats took 4-21 days to reach a criterion of 70% correct choices on >30 trials for two days in a row.



Disclosures: **R. Grgurich:** None. **H.T. Blair:** None.

Poster

237. Learning and Memory

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Topic: H.09. Spatial Navigation

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T2 CRC in Neural Circuits of Cognition and Control
CFI – John R. Evans Leaders' Fund

Djawad Mowafaghian Centre for Brain Health Alzheimer's Disease Research Grant

Title: A novel maze apparatus that enables dynamically configurable routes and visual cues

Authors: *A. W. LESTER[1], A. G. MOMBEINI[1], H. C. NGUYEN[1], M. HABIB[2], R. ALAIN[1], M. S. MADHAV[1];
[1]Sch. of Biomed. Engin., [2]Integrated Engin., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: We have developed a novel physical maze that enables highly flexible experimental control comparable to that afforded by VR systems but in the context of unconstrained real-world rodent behaviour. The maze is composed of a 2.1 x 2.1 m platform with 228 independently movable wall segments that can be programmatically reconfigured to generate unique routes. The use of octagonal geometry accommodates both orthogonal and diagonal path trajectories, enabling path junctions with 45°, 135° and 315° turns in addition to the 90°, 180° and 270° turns that a city-block maze would permit. Four projectors arrayed around the perimeter of the maze can display distinct visual cues on any subset of raised walls. The system also incorporates high-speed 3D tracking of rat position and orientation for closed-loop control of the available paths based on real-time behaviour. Additionally, we developed a custom reward delivery system to provide food-based reinforcement anywhere in the maze. The maze apparatus allows us to dynamically present paths that can be parameterized as graphs - i.e. edges (paths) connecting nodes (decision points). This abstraction allows us to systematically scale up the complexity of existing navigational paradigms (e.g., T-maze, radial arm maze) or implement new task designs. We will use the maze apparatus to electrophysiologically investigate the neural correlates of increasing task complexity in the cognitive map of rodents. In addition to rats, the scale of the maze accommodates a robotic cart operated by human participants using a virtual reality (VR) headset and joystick. Consequently, both rats and humans can be exposed to near identical task conditions, allowing for cross-species approaches that leverage the advantages of each. We are currently piloting the use of the maze with both rodent and human participants and have also developed a purely virtual version of the maze that can be navigated by human participants through a VR interface. The maze was designed from the ground up to use affordable, non-proprietary, open-source hardware and software, with an eye toward accessible fabrication and assembly that will aid replicability by other investigators.

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Poster

237. Learning and Memory

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Program #/Poster #: 237.09

Title: WITHDRAWN

Poster

237. Learning and Memory

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Topic: H.09. Spatial Navigation

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Howard Hughes Medical Institute
Japan Society for the Promotion of Science

Title: A cognitive process occurring during sleep revealed by rapid eye movements

Authors: *Y. SENZAI, M. SCANZIANI;
Univ. of California, San Francisco, Univ. of California, San Francisco, San Francisco, CA

Abstract: Since the discovery of REM sleep, the nature of the rapid eye movements that characterize this sleep phase has remained elusive. Do they reveal gaze shifts in the virtual environment of dreams or simply reflect random brainstem activity? We harnessed the head direction (HD) system of the mouse thalamus, a neuronal population whose activity reports, in awake mice, their actual HD as they explore their environment and, in sleeping mice, their virtual HD. We discovered that the direction and amplitude of rapid eye movements during REM sleep reveal the direction and amplitude of the ongoing changes in virtual HD. Thus, rapid eye movements disclose gaze shifts in the virtual world of REM sleep, thereby providing a window in the cognitive processes of the sleeping brain.

Disclosures: Y. Senzai: None. **M. Scanziani:** None.

Poster

237. Learning and Memory

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 237.11

Topic: H.09. Spatial Navigation

Support: Marga und Walter Boll-Stiftung grant 210-05. 01-21

Title: Role of task difficulty for decision making in wayfinding tasks

Authors: *O. L. BOCK[1], J.-Y. HUANG[1], Ö. A. ONUR[2], D. MEMMERT[1];
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Abstract: To follow a route through a city or building, we can memorize a sequence of directions (e.g., "at the first intersection turn left, at the second walk straight on, …"; serial order strategy = SO), or we can memorize associations between directions and visual cues along the way (e.g., "at the playground turn left, then at the post office turn right, …"; associative cue strategy = AC). It has been hypothesized that AC is more efficient than SO when the task is difficult (Hamburger, 2020). To scrutinize this view, we asked participants to repeatedly pass through a virtual maze; depending on the participant group, task difficulty was either low (12 intersections) or high (18 intersections), and the available strategy was either SO (all intersection looked alike) or AC (visual cues whose order varied between repetitions). The first trial was experimenter-guided, and the remaining five trials were self-guided with immediate error feedback. We found that in all groups, decision accuracy was above chance level already on the first self-guided trial, and increased thereafter. ANOVA of the dependent variable 'accuracy' yielded significance for the factors 'trial' and 'difficulty' (both $p < 0.001$), but not for 'strategy', 'gender', or any interaction term (all $p > 0.05$). Primacy, recency and landmark similarity effects emerged. Participants' accuracy at successive intersections was more consistent under SO than under AC. We conclude that in our study, decision making at intersections depended on task difficulty in a similar fashion for both strategies, which provides no support for the above hypothesis.

Disclosures: O.L. Bock: None. **J. Huang:** None. **Ö.A. Onur:** None. **D. Memmert:** None.

Poster

237. Learning and Memory

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 237.12

Topic: H.09. Spatial Navigation

Title: A method to study whether goal representations generalize

Authors: E. HAPGOOD[1], **K. G. CASTANEDA CARRERA**[1], A. M. METOYER[1], A. E. SHAN[1], J. L. GAUTHIER[2];
[2]Biol., [1]Swarthmore Col., Swarthmore, PA

Abstract: The hippocampus has been implicated in goal-oriented navigational behaviors, but the definition of a goal can be very broad. For example, the Morris water maze involves avoiding an aversive experience, while a reward-based task involves seeking an appetitive stimulus. Despite the plethora of possible goals, most previous studies have employed only one type of goal due to methodological constraints, making it difficult to draw conclusions about how hippocampal activity represents goals per se. To overcome this limitation, we developed an aversion-

avoidance virtual reality task for mice that can be interleaved with a traditional water-reward task. By switching between these two tasks within a session, this behavior is compatible with head-fixed recording techniques, such as two-photon imaging or intracellular recording, allowing measurements of how hippocampal activity generalizes across different goal types. In the aversion-avoidance task, air puffs are delivered at all maze locations except in a designated safe zone, conceptually similar to a water maze, and mice trained on this task learned to seek the safe zone. Mice were also able to learn a more difficult version of the task, in which they needed to wait in the safe zone before airpuffs ceased. This demonstrated that they had learned the virtual reality location rather than simply stopping when airpuffs were no longer present. Future studies will be able to use this task to study how neural activity in the hippocampus, such as putative "goal cells", generalizes across different goal types.

Disclosures: E. Hapgood: None. **K.G. Castaneda Carrera:** None. **A.M. Metoyer:** None. **A.E. Shan:** None. **J.L. Gauthier:** None.

Poster

237. Learning and Memory

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 237.13

Topic: H.09. Spatial Navigation

Support: Northern Norwegian Regional Health Authority (Helse Nord) SFP1 165-14

Title: Effects of growth hormone on hippocampal CA1 place cells

Authors: *K. G. HAUGLAND[1], S. V. JORDBRAK[1], K. B. KJELSTRUP[1,2], V. H. BRUN[1,2]; [1]UiT - Arctic Univ. of Norway, Tromsø, Norway; [2]Univ. Hosp. North Norway, Tromsø, Norway

Abstract: The hippocampal network is sensitive to environmental changes as well as neuromodulation. A common approach to investigate hippocampal plasticity is to compare the neural activity of place cells during exposures to different environments. When the environment changes, the place cells change their firing properties in a process known as remapping. In our study, we modulated the hippocampal plasticity by increasing the levels of growth hormone (GH) or blocking the GH receptor using antagonizing GH (aGH). This was achieved by injecting recombinant adeno-associated viruses to either over-express GH or aGH in the dorsal hippocampus of male Long Evans rats. We found that all groups exhibited place field and that the spatial correlation was high between the sessions of the familiar environments with no differences between the groups ($p = 0.9195$). However, when comparing the novel and familiar environments, the place cells of the GH animals displayed no spatial correlation ($r = -0.036$), while the aGH and control animals exhibited some spatial correlation (aGH $r = 0.211$, control $r = 0.263$) with a significant difference between GH and control ($p = 0.0012$). Only the place cells of the control group displayed rate remapping ($p = 0.0258$). Moreover, in the novel environment, the aGH place cells exhibited enhanced average firing rate ($p = 0.0146$), peak rate ($p = 0.0007$)

and information ($p < 0.0001$) and reduced sparseness ($p < 0.0001$). Since global remapping is suggested to be a mechanism of organising experiences in separate spatial maps, GH might favour this approach to optimize the hippocampal network to detect smaller changes in each environment. Lastly, we found that GH overexpression increased sharp wave ripple rate after exploration of the novel environment ($p = 0.048$), indicating a role of GH in memory consolidation. Our findings are of importance as GH seems to be an understudied neuromodulator in the hippocampus. We show that GH affects place cell activity and could therefore influence significantly on the hippocampal memory system.

Disclosures: **K.G. Haugland:** None. **S.V. Jordbrak:** None. **K.B. Kjelstrup:** None. **V.H. Brun:** None.

Poster

237. Learning and Memory

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 237.14

Topic: H.09. Spatial Navigation

Support: 5R01GM118801

Title: Midazolam suppresses place cell formation and spatial engrams at doses that suppress contextual memory

Authors: *R. LENNERTZ, M. ZHU, M. G. PERKINS, R. A. PEARCE;
Univ. of Wisconsin, Madison, Madison, WI

Abstract: Midazolam is a benzodiazepine with widespread use for procedural amnesia and anxiolysis. It is a positive allosteric modulator that enhances the effects of γ-amino butyric acid (GABA) at $GABA_A$ receptors. We have previously shown that midazolam causes a dose-dependent reduction in contextual fear conditioning in mice. Since place cells and spatial engrams are increasingly regarded as neural correlates of contextual memory, we tested whether doses of midazolam that impair contextual memory also suppress place cells and spatial engrams. All experiments were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin, Madison. Adult male C57BL6/J mice ($n = 3$-4) were injected in the CA1 region of the hippocampus with adenovirus expressing GCaMP6f under the CaMKIIα promoter and a miniature endoscopic lens was implanted. Pyramidal neuron activity was recorded using the Inscopix nVoke system. Movement and neuronal activity were recorded as mice explored a novel context following an injection of either saline or midazolam (0.25, 1.25 or 2.5 mg/kg). After 4 hours, these recordings were repeated as mice were returned to the same context. Place cells and spatial engrams, quantified by rate-map (RM) and population-vector (PV) correlations, were quantified as previously described ($n \geq 500$ cells for each condition). Data were analyzed using linear mixed effects models in R Studio and are presented as mean ± standard deviation. Midazolam reduced exploratory activity in a dose-dependent manner from 78

± 2% following saline to 12 ± 2% following 2.5 mg/kg ($p < 0.0001$), but had no effect on the frequency of neuronal activity (event rate 0.14-0.15 s^{-1}, all groups; $p = 0.12$). Midazolam strongly suppressed the proportion of place cells that formed, even at doses of 0.25 mg/kg (5 ± 3%, versus 27 ± 8% following saline; $p < 0.0001$). Similarly, midazolam suppressed spatial engrams as measured by RM correlation ($p < 0.0001$) and PV correlation ($p < 0.0001$). A lack of exploratory activity following 2.5 mg/kg midazolam precluded an analysis of place cells and spatial engrams for this dose.
We conclude that midazolam suppresses place cell formation and spatial engrams at doses that suppress contextual memory. These neural correlates of memory can be measured without the painful stimulus required for traditional fear conditioning tests, allowing serial assessments to be performed in the same mice. Thus, place cells and spatial engrams may provide a useful and efficient measure of the amnestic effects of medications and other experimental manipulations.

Disclosures: **R. Lennertz:** None. **M. Zhu:** None. **M.G. Perkins:** None. **R.A. Pearce:** None.

Poster

237. Learning and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 237.15

Topic: H.08. Learning and Memory

Title: Study of the effect of REM sleep deprivation and chronic REM sleep restriction on memory and learning in male Wistar rats.

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Abstract: Sleep is a fundamental physiological state for humans and several animal species, as it regulates various metabolic, immunological and cognitive processes, among others. Many studies have shown that sleep is involved in the consolidation and retrieval of certain types of memory: procedural, declarative or working memory. However, we do not know the differential effect of selective REM sleep deprivation (REMSD), chronic REM sleep restriction (REMSR) and retrieval period on declarative memory. Therefore, the objective of this study was to evaluate the effect of REMSD and REMSR on learning and episodic memory, as well as during the recovery period of REMSD through the novel object recognition test (NOR test). Twenty male Wistar rats were used and randomly placed in the following groups (n=6): 1. Control, 2. REMSD x 72 h, 3. REMSD x 72 h + 8 days of recovery, 4. REMSR x 11 days. REMSD and REMSR were performed using the multi-platform technique. After the sleep deprivation, restriction and recovery periods, the animals were evaluated in the NOR behavioral test. The results obtained showed that sleep deficit produced a decrease in NOR test performance. The REMSD x 72 h was the condition that caused the greatest alterations affecting performance in the NOR task and the 8-day sleep recovery of the animals was insufficient to adequately restore the adverse effects of

sleep loss generated by the REMSD. From the results obtained, we suggest that differential sleep loss has an impact in the correct learning and produces degradation in declarative memory.
1

Disclosures: G. Montero-Mendez: None. **A. Jimenez-Anguiano:** None.

Poster

237. Learning and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 237.16

Topic: H.08. Learning and Memory

Title: The Impact of sleep deprivation on human cortical excitability, LTP/LTD like neuroplasticity, and cognitive functions in humans

Authors: *M. SALEHINEJAD, E. GHANAVATI, M.-F. KUO, M. A. NITSCHE; Psychology and Neurosciences, Leibniz Res. Ctr. for Working Envrn. and Human Factors, Dortmund, Germany

Abstract: Sleep strongly affects synaptic strength, making it critical for cognition, especially learning, and memory formation. Whether and *how* sleep deprivation modulates human brain physiology and specifically the parameters that are relevant for human cognition and also cognitive functions is not well understood. Here we examined how overnight sleep deprivation vs overnight sufficient sleep affects (a) cortical excitability, measured by different protocols of transcranial magnetic stimulation (TMS), (b) inducibility of LTP- and-LTD-like plasticity via transcranial direct current stimulation (tDCS), and (c) learning, memory and attention. The results suggest that sleep deprivation upscales cortical excitability due to enhanced glutamate-related cortical facilitation and decreased and/or reversed GABAergic cortical inhibition. Furthermore, tDCS-induced LTP-like plasticity is abolished while the inhibitory LTD-like plasticity is converted to excitatory LTP-like plasticity under sleep deprivation. This is associated with increased EEG theta oscillations due to sleep pressure. Finally, we show that learning and memory formation, behavioral correlates of plasticity, and working memory and attention, which are associated with cortical excitability, are impaired during sleep deprivation. Our data suggest that upscaled brain excitability, and altered plasticity, due to sleep deprivation, are associated with impaired cognitive performance.

Disclosures: M. Salehinejad: None. **E. Ghanavati:** None. **M. Kuo:** None. **M.A. Nitsche:** F. Consulting Fees (e.g., advisory boards); Neuroelectrics, NeuroDevice.

Poster

237. Learning and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 237.17

Topic: H.08. Learning and Memory

Title: Changes in cognitive memory associated with prolonged consumption of non-nutritive sweeteners in a murine model

Authors: *J. A. ESTRADA[1], M. NAVA-GONZALEZ[1], L. A. ZAPI-COLIN[1], M. LOPEZ-MEZA[2], I. CONTRERAS[3];
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Abstract: Consumption of non-nutritive sweeteners (NNS) is common worldwide. The possible effects frequent intake of these compounds may have on cognitive performance have not been well studied. The aim of this study was to determine the presence of changes in cognitive performance related to prolonged dietary supplementation with sucralose and steviol glycosides in a murine model. Sixty-four eight-week-old BALB/c mice were divided into 4 study groups, each one consisting of eight male and eight female animals. The control group received no NNS supplementation; study groups were supplemented with solutions of either 10% commercial sucrose, 1% sucralose or 1% steviol glycosides in drinking water, respectively. After six weeks, behavioral tests were performed using the Barnes maze model. Changes in body weight, feeding behavior food, short-term memory, and long-term memory were evaluated throughout the study period. Preliminary results show that males from the sucralose (3.05±1.26 g) and steviol glycosides (2.85±0.79 g) groups gained less weight compared to the control (4.03±0.21 g) and sucrose (5.40±0.34 g) groups at the end of the study. Likewise, females in the steviol glycosides (1.15±0.24 g) group gained less weight compared to the control group (1.30±0.18 g). Accordingly, males and females from the steviol glycosides group consumed less food and drink compared to the control group. Finally, males from the steviol glycosides group had longer latency periods in the short-term (44.01±11.71 s) and long-term (66.25±12.03 s) memory tests in the Barnes maze model compared to controls (28.33±6.11 s and 6.04±2.17 s, respectively). Our preliminary results suggest that prolonged consumption of sucralose and steviol glycosides promote changes in weight gain, feeding behavior, and cognitive memory in male and female BALB/c mice.

Disclosures: J.A. Estrada: None. **M. Nava-Gonzalez:** None. **L.A. Zapi-Colin:** None. **M. Lopez-Meza:** None. **I. Contreras:** None.

Poster

237. Learning and Memory

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Program #/Poster #: 237.18

Topic: H.08. Learning and Memory

Title: Effect of pre- and postnatal exposure to a Western diet on spatial learning/memory and hippocampal phosphorylated tau protein as indicators of cognitive impairment in offspring of CD-1 mice

Authors: *R. PEDRAZA-MEDINA[1], S. REBECA[2], M. ORTIZ-VALLADARES[3], M. F. PINTO-GONZÁLEZ[4], J. GUZMÁN-MUÑIZ[5], O. GONZALEZ-PEREZ[7], N. A. MOY-LOPEZ[6]; [1]Univ. De Colima, Colima, Mexico; [2]Lab. de neurociencias, [3]Facultad de Medicina, Univ. de Colima, Colima, Mexico; [4]Lab. of Neurosciences, Univ. of Colima, COLIMA, Mexico; [6]Neurosci., [5]Univ. of Colima, Colima, Mexico; [7]Psicologia/University of Colima, Colima, Mexico

Abstract: Introduction: Consumption of unhealthy diets during sensitive periods, such as pregnancy and lactation, could affect the hippocampal development of offspring, processes associated with learning, spatial memory, and cognitive impairment. **Objective:** To evaluate the effect of exposure to a Western diet in pre- and postnatal periods on spatial learning/memory and hippocampal concentration of phosphorylated tau protein as a marker of cognitive impairment in the progeny of CD-1 mice. **Study design:** 54 offspring were used: 27 from mothers with a western diet and 27 from mothers with a balanced diet during gestation and lactation. On postnatal day (PD) four, weight monitoring began. At PD 90, 180, and 360, learning, memory, and mobility were assessed using the Morris Water Maze (MWM) and Open Field (OF). Nine mice per group were euthanized in each of the behavioral evaluations, the hippocampus was removed, and the levels of phosphorylated tau protein (Ser199) were evaluated by the ELISA technique. **Results:** consumption of a Western diet during prenatal and postnatal periods caused an increase in weight at birth [t(52)=-7.711, $p<0.001$, two-tailed] and after weaning [t(40)=-2.903, $p=0.006$, two-tailed], however, these changes disappeared once adolescence began, causing a decrease in weight towards the end of life [t(14)=2.250, $p=0.041$, two-tailed]. At PD90, no modifications were found in learning [t(51)=-0.372, $p=0.711$, two-tailed], but there were significant differences in spatial memory [t(52)=2.191, $p=0.033$, two-tailed], At DP180 and 360, no significant differences were found in learning [t(34)=-1.014, $p=0.318$, two-tailed], [t(15)=1.063, $p=0.304$, two-tailed], or spatial memory [t(34)=0.505, $p=0.617$, two-tailed], [t(15)=1.028, $p=0.320$, two-tailed]. Finally, significant differences were identified in the concentration of tau pS199 at DP180 ($p=0.018$) and 360 ($p=0.015$) compared to DP 90 in the control group [F(2)=6.115, $p=0.007$], but not in the experimental group [F(2)=1.340, $p=0.282$]. **Conclusions:** It is proposed that the Western diet in the pre- and postnatal periods causes a decrease in long-term memory capacity during early adulthood and a weight loss at the end of life.

Disclosures: R. Pedraza-Medina: None. **S. Rebeca:** None. **M. Ortiz-Valladares:** None. **M.F. Pinto-González:** None. **J. Guzmán-Muñiz:** None. **O. Gonzalez-Perez:** None. **N.A. Moy-Lopez:** None.

Poster

238. Hippocampus Physiology

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 238.01

Topic: H.09. Spatial Navigation

Support: ISF #638/16
CIHR-IDRC-ISF #2558/18
CRCNS BSF#2015577
ERC #679253

Title: Local activation generates transient theta phase precession in CA1 pyramidal neurons

Authors: *H. E. SLOIN, A. LEVI, S. SOMECK, L. SPIVAK, R. GATTEGNO, E. STARK; Sagol Sch. of Neurosci. and Dept. of Physiol. and Pharmacology, Sackler Fac. of Medic, Tel Aviv Univ., Tel Aviv, Israel

Abstract: Multiple studies demonstrated theta phase precession as a spatial code and as a mechanism generating multi-neuronal sequences. However, the components contributing to precession and the locus of generation remain unclear. Here, we tested the hypothesis that given ongoing theta, a transient position-dependent rate increase of CA1 pyramidal neurons (PYR) is sufficient for generating precession. We implanted high-density silicon probes equipped with miniature light sources in hippocampal region CA1 of four mice expressing ChR2 in PYR. As the mice traversed a 150 cm linear track, we used closed-loop illumination to emulate spontaneous place fields. Light and no-light control trials were interleaved. In two-thirds of the PYR, firing rates within the illuminated region increased during light compared to control trials. Although illumination was indifferent to ongoing theta, a third of the activated PYR exhibited induced precession in either the spatial or temporal domain. Thus, local activation of PYR induces de novo precession of spikes. Local activation may induce precession by simply adding spikes to preexisting precession of subthreshold membrane potentials. To disambiguate between de novo precession and exposure of preexisting subthreshold dynamics, we compared the properties of induced and spontaneous precession. Compared to spontaneous precession, induced precession was faster. When illumination overlapped with preexisting precession of spikes, PYR activation did not quicken but rather slowed the spontaneous precession. To determine whether induced precession depends on local theta, we repeated experiments in the parietal cortex of two mice, where PYR are paced by volume-conducted theta. Neocortical PYR exhibited spontaneous place fields and increased firing rates during illumination, but did not exhibit spontaneous or induced precession in neither the spatial nor the temporal domain. The absence of precession in the neocortex suggests that volume-conducted theta is insufficient for precession. Thus, phase precession can be generated locally in CA1. The local generation of precession is inconsistent with inheritance from CA3 or other upstream regions. Our findings support the hypothesis that precession is generated by an intra-CA1 generator, requiring a local firing rate increase and local theta oscillations.

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Poster

238. Hippocampus Physiology

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Program #/Poster #: 238.02

Topic: H.09. Spatial Navigation

Support: NIH U19 NS104590

Title: Probing the functional organization of CA3 recurrent circuits with 3-photon imaging and single-cell optogenetic stimulations

Authors: *T. GEILLER, A. LOSONCZY;
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Abstract: Recurrent connectivity in the brain is thought to support a plethora of higher cortical functions from sensory perception to learning and memory. In the hippocampus, the initial and rapid encoding of contextual representations is thought to be implemented via excitatory synaptic plasticity at recurrent synapses between pyramidal cells. These nascent representations are subsequently consolidated during sharp-wave ripples, a local synchronous population event within CA3 during which a compressed version of the memory traces is thought to be reactivated and transferred to the neocortex for long-term storage. However, our current knowledge of CA3 circuits' functional organization supporting these processes is largely limited. For instance, it is still unknown how activity propagates among recurrently connected neurons, what connectivity rules orchestrate the activation or suppression of different ensembles, and whether these computations are different along the proximodistal axis.
To address these questions, we first characterized the long-term dynamics of CA3 place cells during a spatial navigation task in mice. While numerous studies reported anatomical and computational differences along the proximodistal axis of CA3, the deepest portions (CA3c) near the dentate gyrus have remained inaccessible with traditional 2-photon (2P) microscopy and have thus lacked proper characterization with optical techniques. Here, we performed fast 3-photon (3P) functional imaging in both proximal and distal compartments of the CA3 region and report differences in terms of activity and coding properties.Second, we developed an all-optical method based on two-photon acousto-optic deflection to image and stimulate single CA3 pyramidal neurons scattered in three dimensions. The aim is to test whether photoactivating a fraction of neurons which belong to a functionally-identified ensemble is sufficient to drive pattern completion. We are also currently performing repeated population activations of randomly chosen neurons to examine whether it produces a lasting imprinted pattern, as was previously demonstrated in the neocortex.
Together, these results will uncover the functionally structured organization that coordinates operations and plasticity in hippocampal recurrent circuits in awake behaving animals.

Disclosures: T. Geiller: None. **A. Losonczy:** None.

Poster

238. Hippocampus Physiology

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Program #/Poster #: 238.03

Topic: H.09. Spatial Navigation

Support: ANR Hippocomp
USIAS 2020 Fellowship -D Battaglia

Title: Hippocampal gamma oscillations form complex ensembles modulated by behavior and learning

Authors: *R. GOUTAGNY[1], V. DOUCHAMPS[2], M. DI VOLO[3], A. TORCINI[3], D. BATTAGLIA[4,5];
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Abstract: The hippocampus and the entorhinal cortex display a rich oscillatory activity, believed to support neural information processing in key cognitive functions. In the hippocampal region CA1, a “slow gamma” rhythm (30-80 Hz) generated in CA3 would support memory retrieval whereas a “medium gamma” rhythm (60-120 Hz) generated in the entorhinal cortex would support memory encoding. However, descriptions involving discrete gamma sub-bands can only partially account for the haphazard diversity of oscillatory behaviors observed in individual recordings during spatial navigation behavior, challenging thus functional interpretations based on a massive averaging over time and trials. Here, we stress that transient gamma oscillatory episodes at any frequency or phase relative to the ongoing theta (4-12 Hz) rhythm can be recorded at any layer within CA1. Eventually, the commonly reported averages are dominated by a minority of very strong power events overshadowing gamma heterogeneity. Nevertheless, we show that such gamma diversity can be naturally explained by a simple mechanistic model, and that behavior-related information (position within a maze) can be decoded from most individual gamma events, despite their low power and erratic-like nature. Our results indicate that behavior specifically shapes ensembles of irregular hippocampal gamma oscillations, in a way which evolves with learning, depends on the hippocampal layer and is hard to reconcile with the hypothesis of rigid, narrowly tuned gamma sub-bands. Beyond randomness, the pervasive gamma diversity may thus reflect complexity at the edge-of-synchrony, likely functional but invisible to classic average-based analyses.

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Poster

238. Hippocampus Physiology

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Topic: H.09. Spatial Navigation

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Title: Hippocampal representation of complementary positions of stable and moving visual objects

Authors: *D. LEVCIK[1], T. DVORAKOVA[1], N. AHUJA[1], S. DÍEZ-HERMANO[2], P. MANUBENS-CODA[2], A. SANCHEZ-JIMENEZ[2], J. VILLACORTA-ATIENZA[2], A. STUCHLIK[1];
[1]Inst. of Physiol. CAS, Prague, Czech Republic; [2]Complutense Univ. of Madrid, Madrid, Spain

Abstract: For survival in the real world, animals must internally represent positions of both stable (e.g. a nest) and moving (e.g. a predator) objects in space. Moreover, animals receive critical spatial information from the part of the environment that can be only visually explored. The hippocampus is a key structure in spatial cognition, however, there is a lack of understanding of the neural mechanisms that mediate the information processing of stable and moving visual objects' positions. It is also unclear whether spatial representations of stable and moving objects share mutual features or if they belong to separated processing domains. We trained male Long-Evans rats (N=7) to discriminate a particular reward static position of a circle displayed on a distant computer screen and a complementary reward dynamic scene consisting of two circles approaching but not reaching the same static position. We observed that the rats learned to discriminate dynamic objects' positions faster than static ones. Once the rats mastered the task, we presented them with a set of novel dynamic stimuli to decipher whether they could transfer the acquired knowledge about the previously discriminated static and dynamic spatial scenes into new dynamic spatial scenes. Our data showed that the rats could effectively generalize the objects' dynamics and preferred the novel dynamic scene in which two circles approached the familiar reward static position. Next, we implanted the rats with an array of tetrodes and recorded the activity of dorsal hippocampal CA1 neurons during the task. A general linear mixed-effects model showed that the firing rate of individual hippocampal neurons could be influenced by particular positions of visual objects in our discrimination task. Preliminary analysis suggests that hippocampal neurons can encode information about learned complementary static and dynamic objects' positions jointly or separately. Further analysis is currently underway to examine whether this positional information can be encoded using a similar mechanism.

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Poster

238. Hippocampus Physiology

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Program #/Poster #: 238.05

Topic: H.09. Spatial Navigation

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Title: Inactivation of the intermediate hippocampus impairs value-based navigation in a virtual environment more severely compared to the dorsal hippocampal inactivation

Authors: *H. HWANG, S.-W. JIN, I. LEE;
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Abstract: During goal-directed navigation, the hippocampus may process value information of the goal location along with its spatial information. However, the neural mechanisms underlying the hippocampal integration of place and value representations are poorly understood. To dissociate the dorsal and intermediate regions of the hippocampus, we inactivated the dorsal hippocampus (dHP) and the intermediate hippocampus (iHP) in a place-preference task by using a VR navigation system. Body-fixed rats (n=7) were trained to run on a ball-shaped treadmill to find two unmarked reward zones, each associated with a different amount of honey water at a six-to-one ratio in a circular VR arena. Once trained, rats showed a significant preference for the high-reward zone. Inactivation of either dHP or iHP bilaterally in the same rats increased the travel distance to the goal zone significantly compared to control conditions ($p<0.01$ for dHP, $p<0.0001$ for iHP; one-way ANOVA). However, only iHP-inactivated rats were significantly impaired in finding the high-value zone by showing decreased place preference ($p<0.0001$). The accuracy and effectiveness of wayfinding behavior, measured by both the departing angle at the start location and the arriving angle at the arena boundary in a given trial, were more severely disrupted with the iHP inactivation than with the dHP inactivation compared to control conditions. Specifically, dHP-inactivated rats showed less accurate wayfinding by showing less optimal orientations when departing the start point and also when arriving at the boundary of the arena ($p<0.01$ at departure, $p<0.0001$ at arrival; Watson-Williams test). However, the dHP-inactivated rats still demonstrated effective wayfinding behavior on average (*n.s.* when comparing the mean vector lengths for the angular deviations measured at both departure and

arrival; one-way repeated measures ANOVA). In contrast, iHP-inactivated rats were severely disrupted in both measures (p's<0.0001 for angular deviation at both departure and arrival; $p<0.01$ and $p<0.0001$ for the mean vector lengths for the angular deviations measured at departure and arrival, respectively). Direct comparisons between the inactivation conditions for the dHP and iHP also showed that the iHP inactivation caused more severe impairment than dHP inactivation (p's<0.05 for departure and p's<0.01 for arrival for both angular deviation and mean vector length). Overall, our findings suggest that the iHP is essential for accurate and effective goal-directed navigation, whereas the dHP is more important for precise spatial targeting during navigation.

Disclosures: H. Hwang: None. **S. Jin:** None. **I. Lee:** None.

Poster

238. Hippocampus Physiology

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 238.06

Topic: H.09. Spatial Navigation

Support: NIH Grant 1R01EB026955-01

Title: News without the buzz: recovering information from weak theta rhythms in the hippocampus

Authors: *G. AGARWAL[1], B. R. LUSTIG[2], E. PASTALKOVA[2], A. K. LEE[2], F. T. SOMMER[3];
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Abstract: Oscillations in the local field potential (LFP) reflect the synchronized activity of large neuronal populations. Particularly studied is the ~5-11 Hz theta rhythm, thought to coordinate neuronal spiking in the hippocampus and widespread cortical regions. During locomotion, theta is highly spatially coherent: principal component analysis identifies a single oscillatory component ('principal' or 'p-theta') that explains >90% of the observed variance across electrodes in hippocampal region CA1 and is likely generated by rhythmic input from the medial septum. Previously we showed that we could decode the animals' position from multi-electrode patterns of LFP phase modulation relative to p-theta (i.e., by 'demodulating' the LFP), matching the accuracy of traditional spike rate-based decoding. However, the demodulation approach becomes far less accurate during immobility, a behavioral condition in which theta power decreases. One explanation for this decrease in accuracy is that a strong p-theta rhythm is required to synchronize place cells and generate informative LFP patterns ("pacemaker model"). Alternatively, it is possible that place cell populations can still synchronize locally even when p-theta is weak ("assembly model"). In this case, the theta demodulation approach would fail, but local oscillatory phase patterns could still carry behavioral information. To test the assembly

model, we have designed a weakly-supervised neural network with a single layer of complex-valued hidden units ('FieldNet') that can learn to extract behaviorally tuned weak oscillatory patterns ('informative' or 'i-theta'). We find that FieldNet can recover position accurately even when p-theta is weak and the demodulation-based approach fails. Our findings support a 'dual oscillator' model in which hippocampal cell assemblies can coordinate rhythmically in the absence of theta input from the medial septum. Further, FieldNet may offer a general tool for recovering information from weakly rhythmic LFPs, which are not only observed in the hippocampus, but across many different brain regions and behavioral contexts.

Disclosures: G. Agarwal: None. **B.R. Lustig:** None. **E. Pastalkova:** None. **A.K. Lee:** None. **F.T. Sommer:** None.

Poster

238. Hippocampus Physiology

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Program #/Poster #: 238.07

Topic: H.09. Spatial Navigation

Support: Kavli IRG 2020
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AFOSR

Title: Subiculum directional activity provides link for different perspective-driven spatial representations

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Abstract: An often-experienced demand in spatial cognition is to relate locations observed from a top-down (TD) view with those observed from a first-person (FP) view. Recent computational models predict that two different representations of environmental location would be required to translate between FP and TD views[1]. Yet, the ability to coordinate dual spatial representations across such different perspectives has not been examined in neurophysiological recordings of the 'cognitive map' in rodents.
To test whether the connecting viewpoint changes follow current models, we developed a task that requires rats to relate overlapping locations between maze levels that differ in structure and environmental vantage point. We recorded neural activity in hippocampal (HPC) output regions, CA1 and Subiculum (SUB), as rats traversed between levels of a two-story maze. On each trial, rats first searched a top-level plexiglass open-field for a reward, which, once found, served to cue the rat towards the matching position on a complex-grid maze visible directly underfoot. Rats then descend a ramp, connecting the levels, before ultimately running maze segments that most-efficiently lead to the cued position observed from above.
Recordings reveal that HPC generates separate maps of upper and lower-level spaces, with many

neurons in CA1 exhibiting “place-specific” firing to a single level. As in CA1, some SUB neurons exhibit “place-specific” tuning for either level, such that the correlation between maps for upper and lower levels is weak. A sub-population of neurons fires to head orientation, with many neurons exhibiting tuning to multiple directions, consistent with axis tuning[2]. Orientation tuning in SUB was better maintained across maze levels. Thus, CA1 and SUB are dissociable in both the tuning most robustly observed (spatial versus directional) and in the extent to which tuning persists across upper and lower levels.
Although CA1 has been found to generate 3D activity fields to instances that afford travel along three axes[3], we find that spatial firing patterns do not transfer to environments separated along the vertical dimension. SUB directional responses can be engaged amongst wholly different CA1 assemblies, suggesting CA1 place cells are not solely driving SUB directional tuning.
Xing et al. (2022). In review at PNAS.Olson et al. (2017). Nat. Neuro. 20(2).170-172.Grieves et al. (2020). Nat. Com. 11(789).1-13.

Disclosures: **R. Place:** None. **S. Kim:** None. **D.A. Nitz:** None.

Poster

238. Hippocampus Physiology

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Program #/Poster #: 238.08

Topic: H.09. Spatial Navigation

Support: 1R15 AG060461-(01)

Title: Application of nonlinear modeling algorithms finds novel relationships between juvenile hippocampal local field potentials and maze location

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Abstract: Complex and coordinated neuronal activity within the hippocampus enables spatial learning and memory. While other cortical areas are associated with decision making in spatial navigation tasks, the hippocampus provides the spatial context and is involved in the vicarious trial and error processes that precedes spatial trajectory choices. Exploration of novel environments and navigation to known goal locations produce synchronous activity of hippocampus neurons resulting in rhythmic oscillation events in local networks. Power of specific oscillatory frequencies and numbers of these events recorded in local field potentials correlate with distinct cognitive states during the spatial navigation process. Traditionally, oscillatory power has been analyzed with Fourier transforms, which involve assumptions about the signal that may be wrong as these signals are naturally quasi-periodic. Diffusion mapped delay coordinates (DMDC) may serve as a tool for signal analysis that avoids limitations seen in traditional methods by decomposing the signal into values of Dimensional and Volume. We

applied DMDC to unfiltered and filtered signals recorded from area CA1 of the hippocampus in juvenile rats treated with a positive AMPA receptor modulator as they freely explored a Y maze. Signal epochs preceding arm entry choices at the maze center and elsewhere in the maze were analyzed and comparisons were made across age, drug, and position in the maze. Our current study shows that DMDC replicates outcomes seen in previous work on hippocampal development in relation to maturation of spatial navigation and reveals position effects in network oscillations that traditional analyses are unable to detect. Thus, DMDC may serve as a suitable complement to traditional analyses of oscillatory activities recorded from brains of behaving subjects.

Disclosures: D.A. Gonzalez: None. **J.H. Peel:** None. **T.C. Dumas:** None. **J.R. Cressman:** None.

Poster

238. Hippocampus Physiology

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Program #/Poster #: 238.09

Topic: H.09. Spatial Navigation

Title: Characterization of inhibitory interneuron dynamics during remapping in medial entorhinal cortex

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Abstract: The medial entorhinal cortex (MEC) is critical for spatial navigation. It contains two distinct spatial-coding cell types known as the ‘grid cells’ and the ‘non-grid spatial cells’. In an open environment, grid cells fire regularly at specific locations arranged in a hexagonal ‘grid’ pattern, while non-grid spatial cells fire selectively at one or two locations and do not display spatial periodicity. Interestingly, when an animal navigates across a distinct environment, grid cell populations coherently translate their periodic firing fields to ‘realign’ with the novel environment, while non-grid spatial cells randomly rearrange firing fields independent of each other. It is unclear what circuit mechanisms lead to their distinct responses to novel environments. Previous work showed that inhibition of MEC parvalbumin (PV)-expressing interneurons disrupted the spatial selectivity of grid cells, but not non-grid spatial cells. Conversely, inhibition of somatostatin (SOM)-expressing interneurons exclusively disrupted the spatial selectivity of non-grid spatial cells but not grid cells. We propose that MEC contains two parallel circuits: 1) a structured network consists of PV+ interneurons and grid cells with rigid synaptic connections; 2) a more flexible network consists of SOM+ interneurons and non-grid spatial cells that is suited for plasticity changes when experiencing novelty. If so, how will PV+ vs. SOM+ interneurons respond to novel environments? We hypothesize that PV+ interneurons might display stable and coherent population activity during remapping, while the activity of

SOM+ interneurons may be more heterogeneous. Using 2-photon Ca2+ imaging combined with transgenic mouse strains, we can specifically record the activity of genetically defined interneuron types. Using navigation tasks in virtual reality, we can change environments instantaneously and capture the immediate neural activity changes. We found that, for both SOM+ and PV+ interneurons, many individual cells displayed significant increases or decreases in activity transiently during initial exposure to novel environments. However, the mean population activity of PV+ interneurons is elevated during the ~10 mins interval navigating in novel environments, while the mean population activity of SOM+ interneurons did not change. These results reveal the heterogeneity within both genetically defined interneuron types and suggest that PV+ interneurons may receive distinct neuromodulatory inputs in novel environments compared to SOM+ interneurons. Together, these findings will help uncover the MEC circuit mechanisms responsible for navigation in novel environments.

Disclosures: J. shi: None. **J.G. Heys:** None.

Poster

238. Hippocampus Physiology

Location: SDCC Halls B-H

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Program #/Poster #: 238.10

Topic: H.09. Spatial Navigation

Support: Simons Foundation

Title: Sharp wave-ripple dynamics encode the motivation for physical activity

Authors: *C. BUHLER[1], A. SMITH[1], K. C. ARNDT[1], E. GILBERT[1], J. C. BASSO[2], D. F. ENGLISH[1];
[1]Sch. of Neurosci., [2]Human Nutrition, Foods & Exercise, Virginia Tech., Blacksburg, VA

Abstract: Exercise produces robust modifications to hippocampal structure and function including improvements in learning and memory. Despite such widely reported effects of exercise on memory, underlying mechanisms are largely unknown, with previous work focused on changes within the dentate gyrus. However, hippocampal-cortical dialogue, which relies on outputs from CA1, is essential for memory and has not been investigated in the context of exercise. It is also unknown how changes in hippocampal circuits induced by exercise relate to exercise motivation. Hippocampal-cortical oscillatory biomarkers for learning and memory, such as sharp wave-ripples (SWR) and theta oscillations are entrained by respiratory rate, which is elevated in response to exercise. Theta occurs during ambulatory behaviors and REM sleep, when large populations of CA1 neurons become dynamically entrained to the rhythm. SWRs occur during consummatory behaviors, immobile wakefulness, and NREM sleep, during which CA1 neurons encoding place become activated outside of their receptive fields in temporal sequences representing replay of the physical experience of moving through space. We hypothesize that exercise enhances learning and memory by modifying hippocampal circuits in

ways that change SWR and theta dynamics. Here, we recorded hippocampal LFP and running behavior across days under a voluntary running protocol (sleep-run-sleep; SRS) designed to examine the relationship between acute exercise experience, theta and SWRs across sleep/wake states. To accomplish this, 64-channel linear silicon probes were chronically implanted across CA1-dentate gyrus to facilitate anatomically precise recordings. SRS began with 90-minutes of sleep in the home cage, followed by 2-hours of running wheel access in a novel cage, ending with another 90-minutes of sleep in the home cage. We collected 7 days of consecutive SRS recordings to examine effects across time, followed by exercise deprivation for 5-7 days and subsequent re-exposure to exercise under the SRS protocol. Allowing us to examine the evolution of changes in theta and SWRs across the acquisition of running as a motivated behavior, the latter evidenced by increased time spent running over days, and the highest time being during rebound days after deprivation. In n=4 female FVBN/C57B6J mice we found significant correlations between the rate and duration of SWRs in NREM sleep and the total time spent running each day. Additionally, features of SWRs in NREM sleep preceding anticipated running experience correlated with the amount of subsequent time spent running, suggesting that the motivation for exercise may be encoded in SWR dynamics.

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Poster

238. Hippocampus Physiology

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Program #/Poster #: 238.11

Topic: H.09. Spatial Navigation

Support: R00MH118423

Title: Catecholamine signaling in CA1 correlates with movement, novelty and sleep state

Authors: *I. PIMENTEL[1], T. N. DONALDSON[2], M. KAKANI[3], S. A. MCKENZIE[4]; [1]Univ. of New Mexico Dept. of Neurosciences, Albuquerque, NM; [2]Dept. Of Psychology, Univ. of New Mexico, Albuquerque, NM; [3]Virginia Commonwealth Univ., Richmond, VA; [4]Neurosciences, UNM HSC, Albuquerque, NM

Abstract: Norepinephrine (NE) and dopamine (DA) signaling in the hippocampus are required for normal memory function. Prior studies have used microdialysis to demonstrate that exposure to novel stimuli is associated with an increase in catecholamines which promote the synaptic plasticity associated with new learning. In other brain regions, catecholamine release is also known to occur around movement onset, as well as during transitions from sleep to wakefulness. Due to the coarse temporal resolution of microdialysis, it is unknown to what extent catecholamine release in the hippocampus is related to novelty *per se*, or to the movement and arousal changes that accompany exposure to novel stimuli. To better understand the causes of

DA and NE release in the hippocampus, we monitored transmitter binding with virally delivered genetically encoded fluorescent indicators GRAB-NE and GRAB-DA as mice engaged in various behavioral tasks. To test signaling related to novelty, mice were presented with objects, conspecifics, and environments as well as novel object-context pairings and unexpected rewards. To dissociate catecholamine signaling related to movement versus that related to novelty, two strategies were taken. As a first approach, movement and arousal correlates were captured in the home cage and during running on a familiar track. These correlates were then used to factor out motor-related signaling. For the second approach, mice were trained to run for water on a familiar track and on surprise trials, novel objects were presented, thus causing mice to stop to explore, rather than initiate movement. We found a strong and reliable DA and NE response to new objects and contexts that decayed as a function of experience. Repeated samplings of the same objects, in which motor output was held constant, were associated with diminishing catecholamine release, supporting our hypothesis that novelty detection contributes to transmitter release. Additionally, we observed a strong DA and NE release upon bouts of movement not associated with novelty sampling, as well as during the transition from slow-wave sleep to waking (REM sleep was associated with low DA). We conclude that movement, novelty, and arousal from sleep all contribute to hippocampal catecholamine signaling.

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Poster

238. Hippocampus Physiology

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Program #/Poster #: 238.12

Topic: H.09. Spatial Navigation

Support: R00MH118423

Title: Plasticity in feedback inhibition affects CA1 assembly membership

Authors: Z. SACCOMANO[1], P. PAUDEL[2], *D. ENGLISH[3], **S. A. MCKENZIE**[4];
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Abstract: Memories are believed to be stored in the pattern of synaptic connectivity between neurons. Coincident neural activity between connected cells is known to change synaptic strength, but the rules for how activity affects connectivity *in vivo* are largely unknown. Area CA1 of the hippocampus has been the focus of most *in vivo* investigations of hippocampal function, but excitatory neurons in this region primarily influence each other indirectly through inhibitory interneurons. Therefore, we tested the plasticity rules governing the excitatory synaptic strength onto local inhibitory cells. The red-shifted opsin ChrimsonR was expressed

under the parvalbumin (PV) promoter to target fast-spiking interneurons, and Channelrhodopsin 2 (Chr2) was expressed under the CaMKIIα promoter to target primarily pyramidal cells (PYR). Theta bust stimulation (TBS) was used to induce plasticity; red (635 nm) and blue (473 nm) light were pulsed at 8 Hz either simultaneously, to pair PYR and PV+ INT activity, or asynchronously to uncouple PYR and PV+ INT. Paired TBS caused an increase in responsiveness of PYR to ChR2 stimulation and an increase in ripple recruitment. Unpaired TBS did not affect the responsiveness of PYR to ChR2 stimulation; firing during spontaneous ripples was decreased. In experiments where one population of PYR was given TBS with PV+ interneurons and another was driven asynchronously, the activity of the two PYR populations became decorrelated from one another. In a second experiment, units were detected online and cross-correlations between spike trains were calculated to identify putatively connected pairs. After a baseline recording, firing of the presynaptic PYR was used to trigger 10 ms of red light to activate ChrimsonR in PV+ interneurons. In a subset of putatively connected pairs, this protocol strongly increased the correlation in firing between the pre- and post-synaptic cell pairs. The increase in coupling strength correlated with the efficacy of the closed-loop protocol in driving short-time scale spike-spike correlations, as expected from a Hebbian learning rule. Together, our findings show the potential plasticity at the excitatory to inhibitory synapse in area CA1 of the hippocampus in controlling neural excitability and assembly membership.

Disclosures: Z. Saccomano: None. **P. Paudel:** None. **D. English:** None. **S.A. McKenzie:** None.

Poster

238. Hippocampus Physiology

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Program #/Poster #: 238.13

Topic: H.09. Spatial Navigation

Title: Circuit dynamics of high frequency oscillations in the granular retrosplenial cortex

Authors: *K. ARNDT[1], E. GILBERT[1], L. M. KLAVER[1], C. BUHLER[1], J. C. BASSO[1], S. A. MCKENZIE[2], D. F. ENGLISH[1];
[1]Virginia Tech., Blacksburg, VA; [2]Neurosciences, UNM HSC, Albuquerque, NM

Abstract: Dialogue between the hippocampus and cortex is essential for memory formation and is supported by hippocampal theta oscillations during encoding and sharp wave-ripples (SWR) during consolidation. Elucidating the mechanisms [AK1] by which cortex reads hippocampal information during these states is essential to a mechanistic understanding of memory. One way in which cortex may read out such information is by engaging in oscillatory activity in concert with hippocampal rhythms. It has recently been found that several cortical areas exhibit high-frequency oscillations (HFO) similar to the SWR frequency (~150Hz) at the time of hippocampal SWRs, which may support this function. The granular retrosplenial cortex (gRSC) is downstream of the hippocampus, required for consolidation of spatial memories, and exhibits

such HFOs. gRSC HFOs occurring during SWRs are localized to superficial layers and are driven by inputs from bursting cells in the subiculum. However, HFO-like activity has also been observed in the gRSC during theta. It is currently unclear if gRSC HFOs in theta and SWRs share similar functions or mechanisms. Specifically, it is not known if HFOs in theta and SWRs occur in the same gRSC layers, or how gRSC neurons in different layers support and participate in HFOs in these distinct states. This is critical to understanding HFO function as different layers of the gRSC receive region-specific inputs and theta and SWRs support different aspects of behavior. Here, we used dense (20 μm site spacing) local field potential (LFP) and single unit recordings across all layers of the gRSC concurrent with CA1 LFP recordings in behaving mice to investigate circuit activity of HFOs in theta and SWRs. By comparing HFOs occurring at times of SWRs (+/-50 ms) or during theta, we found using current-source density analysis that HFOs are uniformly localized to layer 2/3, and that HFO power (but not frequency or duration) was higher during SWR-associated events. Additionally, in ensemble recordings of gRSC neurons, subsets of both excitatory and inhibitory neurons had different activity during each type of HFO. These findings suggest that gRSC HFOs may have distinct functions and mechanisms across different brain states and behaviors. [AK1]Mechanisms and mechanistic in the same sentence is redundant

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Poster

238. Hippocampus Physiology

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Program #/Poster #: 238.14

Topic: H.09. Spatial Navigation

Support: Simons Foundation

Title: Ca1 pyramidal neurons control the participation of axo-axonic interneurons in sharp wave-ripples

Authors: *E. GILBERT[1], L. M. KLAVER[1], K. ARNDT[1], C. BUHLER[1], J. C. BASSO[1], S. A. MCKENZIE[2], D. F. ENGLISH[1];
[1]Virginia Tech., Blacksburg, VA; [2]Neurosciences, UNM HSC, Albuquerque, NM

Abstract: Memory-guided behaviors require the coordinated spike timing of hippocampal CA1 pyramidal neurons (PYR) during sharp wave-ripples (SWRs). PYR spike timing is controlled by GABAergic interneurons (INTs), of which there are over 20 physiologically distinct types. Understanding how different INTs control PYR spiking in SWRs is thus essential to a mechanistic understanding of memory. Among CA1 INTs, axo-axonic cells (AACs; homologous to neocortical chandelier cells) are thought to have the most precise control over PYR spike timing because they hyperpolarize the axon initial segment and veto spike initiation, even at

times of somato-dendritic depolarization. This suggests that AACs may be important for controlling CA1 network activity in SWRs, when temporal precision of PYR spiking is essential. However, AAC activity in SWRs and its underlying mechanisms are not well understood.AACs were long thought to be silent in SWRs. However, using advanced methods including an AAC-specific Cre mouse, we and others recently found that ~50% of AACs robustly participate in SWR events. The mechanisms controlling this participation are unknown. To address this question, we identified AACs in ensemble recordings of CA1 neurons in behaving mice, using an AAC-specific Cre transgenic mouse combined with virally delivered opsins to opto-tag AACs. Using these data, we quantified AAC activity and interactions with local PYR. For each AAC we examined the spiking autocorrelation, activity in theta, gamma and SWRs and soma location relative to the pyramidal layer. We then determined the number and strength of monosynaptic inputs from CA1 PYR to each AAC and the spiking autocorrelation of each presynaptic PYR. We found that the participation of AACs in SWRs was related most to their preferred theta phase and inputs from PYR. SWR-active AACs tended to fire at the trough of the theta oscillation (the time of highest PYR spiking), while SWR-silent AACs tended to fire closer to the theta peak. SWR-active AACs also received more and stronger inputs from PYR and these PYR had a higher burst spiking propensity than those connected to SWR-silent AACs. These results suggest that CA1 PYR control the activity of AACs in SWRs.

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Poster

239. Hippocampal Physiology I

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Program #/Poster #: 239.01

Topic: H.08. Learning and Memory

Support: ZIA DA000587

Title: Hippocampus is necessary for dopamine neurons to compute reward prediction errors when states are partially observable

Authors: *Z. ZHANG[1], Y. K. TAKAHASHI[1], A. LANGDON[2], G. SCHOENBAUM[1]; [1]NIDA Intramural Res. Program, Baltimore, MD; [2]NIMH Intramural Res. Program, Bethesda, MD

Abstract: Dopamine (DA) neurons in the ventral tegmental area signal reward prediction errors (RPEs). These RPEs depend critically on how a task is represented, a function likely dependent on orbitofrontal (OFC) and hippocampal (HC) regions. We have previously demonstrated that OFC supports RPE signaling by DA neurons when those errors depend on hidden or inferred information. Here we applied a similar methodology to examine the contribution of the HC to dopaminergic prediction errors in the VTA. VTA DA neurons were recorded in rats with sham or

neurotoxic lesions of the ipsilateral dorsal and ventral HC during the same odor-guided choice task used in our prior study. In this task, odors signaled left and right choices for rewards that changed in either their timing or number across blocks of trials to induce errors in reward prediction. As expected, dopamine neurons recorded from sham rats showed a phasic increase in firing to unexpected rewards, a phasic decrease in firing to the omission of expected rewards, and also adjusted their activity to the cues to reflect their current expected value. By contrast, dopamine neurons recorded from HC lesioned rats showed intact responses to the odor cues, but no longer signaled RPEs to the delivery and omission of rewards. To explain these phenomena, we developed a temporal difference reinforcement learning model in a semi-Markov framework with partial observability, in which the transition structure of the task was learned through experience. In the full model, positive and negative RPEs occurred to unexpected reward delivery and omission at the start of each block, when the learned transition probabilities did not match the unexpectedly altered reward contingencies. These RPE signals diminished with learning while expected value signals to the odor cues evolved to reflect their updated value, as observed in the DA responses. Within this model, we were able to mimic the effects of HC lesions by preventing the update of transition probabilities between hidden states of the task. Without this critical aspect of the task representation, the model showed a loss of RPE signals to reward delivery and omission after block changes. Updating the value of the cues was still accomplished via a retrospective revaluation during the inter-trial interval. These effects are consistent with the hypothesis that HC helps construct a cognitive map of task states, particularly when these are partially observable, and show that this function critically impacts the computation of RPEs by DA neurons in the VTA.

Disclosures: **Z. Zhang:** None. **Y.K. Takahashi:** None. **A. Langdon:** None. **G. Schoenbaum:** None.

Poster

239. Hippocampal Physiology I

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Program #/Poster #: 239.02

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Biotechnology and Biological Sciences Research Council Research grant (BB/T005475/1)

Title: How the past influences the present- temporal dynamics of hippocampal encoding in multiple environments

Authors: *M. TIROLE, D. A. BENDOR;
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Abstract: The hippocampus plays a central role in encoding new behavioural episodes as well as discriminating between environments. When rodents explore new environments, place cells in the hippocampus typically globally remap to create a new representation that is distinct from previous environments. Place cells remap by altering the firing rate and/or location of their place field, with the possibility of some cells not forming a place field in the new environment. While remapping is a well-known phenomenon, it is unknown whether and how place cells' activity in previous environments affect the encoding of new environments. To address this question, we recorded single units in the dorsal CA1 region of the hippocampus of rats each day running along three novel linear tracks each day. Place cells active on earlier tracks formed active place fields in the very first moments of exploration of subsequent novel tracks. However, these cells were either rapidly pruned from, or integrated and reorganised into the new representation over the timescale of a few lapruns along the linear track. Based on the firing rate of these cells during the very first lap, we could predict whether they would eventually be pruned or integrated. Furthermore, while awake replay on the track was initially evenly split between local and remote replay (i.e. replay of the current and previous tracks) that proportion rapidly changed, with remote replay becoming more infrequent and local replay becoming more prevalent. This increase in the proportion of local replay on the track mirrored a similar increase in the spatial stabilisation of place fields. Finally, cells that were spatially active in previous environments immediately participated equally in both local and remote replay. In contrast, newly emerging place cells that were previously silent on the earlier track, increased their participation in local replay events more slowly, as their place field stabilised. Together, these findings suggest that past experiences may influence novel ones during a temporally defined window and highlight a potential role for local and remote replay in the discrimination and generalization of experiences.

Disclosures: M. Tirole: None. **D.A. Bendor:** None.

Poster

239. Hippocampal Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 239.03

Topic: H.08. Learning and Memory

Support: Fi2 GM133534
ZIA DA000587

Title: Calcium activity is a degraded estimate of spikes

Authors: *E. E. HART[1], M. P. GARDNER[2], T. KAHNT[3], G. SCHOENBAUM[4];
[1]NIDA IRP, NIH, NIDA IRP, Baltimore, MD; [2]NIDA IRP, [3]NIDA Intramural Res. Program, [4]NIDA IRP, Baltimore, MD

Abstract: Many principles of neural function were discovered by recording action potentials extracellularly during behavior. Calcium flux has become a commonly used proxy for action potentials. However, it is unclear whether calcium flux matches the dynamic range and variability of action potential responses, particularly that underlying frontocortical signaling during more complex cognitive operations. To address this question, we recorded electrophysiological and imaged calcium activity in orbitofrontal cortex during olfactory discrimination learning. Orbitofrontal single-unit spiking signaled information about the sensory properties and meaning (reward prediction) of odor cues, essentially representing the specific associations acquired during learning. By contrast, imaging ensembles primarily contained reward prediction information. Even more surprisingly, using calcium to infer spikes further washed-out information beyond reward prediction. These findings have important implications for the interpretation, processing, and understanding of the use of calcium imaging as a stand in for single unit recording to understand how neural circuits mediate complex cognitive operations.

Disclosures: E.E. Hart: None. **M.P. Gardner:** None. **T. Kahnt:** None. **G. Schoenbaum:** None.

Poster

239. Hippocampal Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 239.04

Topic: H.08. Learning and Memory

Support: ZIA DA000587

Title: Schema cell formation in orbitofrontal cortex is facilitated by optogenetic inactivation of hippocampal output

Authors: *W. ZONG[1], J. ZHOU[2], M. GARDNER[3], K. M. COSTA[4], Z. ZHANG[4], G. SCHOENBAUM[4];
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Abstract: Previous studies have consistently supported the idea that the orbitofrontal cortex (OFC) contributes to cognitive mapping, and we have recently shown that neural activity in the OFC converges on a generalized cognitive map - a schema - when trained repeatedly on exemplars of a complex, 24-position, odor sequence task (Zhou et al, Nature, 2021). To further explore the representations of schemas in OFC, we repeated this study, using a much-simplified version of this task in which ten different odors were arranged in two “virtual” figure 8 mazes. The shape of the two mazes was identical however, each used unique odors. Neurons were recorded during performance on each maze, and we hypothesized that schema representations

would be characterized by similar firing patterns between the two mazes. Consistent with this, we found that while aspects of the individual mazes were represented by some neurons (odor, odor sequence, reward), many OFC neurons exhibited highly correlated firing patterns across mazes, consistent with extraction of the generalized cognitive map or schema common across the two mazes. Optogenetic inactivation of ventral subiculum had no impact on the prevalence of such schema cells in OFC in previously experienced maze pairs, however when inactivation was applied during learning of new maze pairs, the development of schema cells in OFC was significantly enhanced.

Disclosures: **W. Zong:** None. **J. Zhou:** None. **M. Gardner:** None. **K.M. Costa:** None. **Z. Zhang:** None. **G. Schoenbaum:** None.

Poster

239. Hippocampal Physiology I

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Program #/Poster #: 239.05

Topic: H.08. Learning and Memory

Support: NIH 1ZIADA000587
Center on Compulsive Behaviors Fellowship

Title: The selective D3-Receptor antagonist VK4-116 effectively treats behavioral inflexibility in rats following self-administration and withdrawal from cocaine

Authors: *M. C. PANAYI[1], S. SHETTY[1], Z.-X. XI[2], A. H. NEWMAN[1], G. SCHOENBAUM[3]; [1]NIH, NIDA IRP, Baltimore, MD; [2]NIH Nationl Inst. on Drug Abuse, Baltimore, MD; [3]NIDA IRP, NIH, Baltimore, MD

Abstract: Chronic psychostimulant use can cause long lasting changes to neural and cognitive function that persist even after long periods of abstinence. As cocaine and users transition from drug use to abstinence, a parallel transition from hyperactivity to hypoactivity has been found in orbitofrontal-striatal glucose metabolism, and striatal D2/D3 receptor activity. Targeting these changes pharmacologically, using dopamine D3 receptor antagonists, has shown significant promise in reducing drug-taking, and attenuating relapse in animal models of cocaine and opioid use disorder. However, much less attention has been focused on treating inflexible and potentially maladaptive non-drug behaviors following chronic psychostimulant use. Here we tested the selective D3-Receptor antagonist VK4-116 as a treatment for the long-term behavioral inflexibility in abstinent rats with a prior history of chronic cocaine use. Rats were first trained to self-administer cocaine (0.75 mg/kg/reinforcer) or a sucrose liquid (10%, .04 mL/reinforcer) for 2 weeks (FR1 schedule, max 60 reinforcers in 3 hrs), followed by 4 weeks of abstinence. Cognitive and behavioral flexibility were then assessed using a sensory preconditioning (SPC) learning paradigm. Rats were given an i.p. injection of VK4-116 (15 mg/kg) or vehicle 30 mins prior to each SPC training session, thus creating four drug-treatment groups: sucrose-vehicle,

sucrose-VK4-116, cocaine-vehicle, cocaine-VK4-116. The control groups (sucrose-vehicle, sucrose-VK4-116) demonstrated significant evidence of flexible SPC behavior, whereas cocaine use (cocaine-vehicle) disrupted SPC behavior. Remarkably, the D3 antagonist VK4-116 mitigated this cocaine deficit (cocaine-VK4-116), demonstrating flexible SPC to levels comparable to the control groups. These preclinical findings demonstrate that highly selective dopamine D3-receptor antagonists, particularly VK4-116, show significant promise as a pharmacological treatment for the long-term negative behavioral consequences of cocaine use disorder.

Disclosures: M.C. Panayi: None. **S. Shetty:** None. **Z. Xi:** None. **A.H. Newman:** None. **G. Schoenbaum:** None.

Poster

239. Hippocampal Physiology I

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Program #/Poster #: 239.06

Topic: H.08. Learning and Memory

Support: R01MH115304
R01NS105472

Title: Is the rat prefrontal cortex crucial for cognitive control during spatial cognition?

Authors: *E. PARK[1], G. GRUBBS[1], K. NICHOLAS[1], D. TABORGA[1], A. S. AHMED[1], K. C. O'REILLY[2], A. A. FENTON[1];
[1]New York Univ., New York, NY; [2]Child and Adolescent Psychiatry, New York State Psychiatric Inst., New York, NY

Abstract: Cognitive control tasks require using one class of information while ignoring another, competing class of information. The central role of the medial prefrontal cortex (mPFC) in cognitive control is well established because mPFC damage impairs tasks that may require cognitive control, as inferred, typically from the task design. We have previously established that cognitive control is required to perform an active place avoidance task on a rotating arena that requires rodents to avoid shock by using information about their location in the stationary room and ignoring information about their location on the rotating floor. Here we test whether rat mPFC lesion impairs cognitive control in the active place avoidance task to test the "central-computation" hypothesis that mPFC is essential for the computations required for cognitive control. Adult rats received either bilateral ibotenic acid or sham lesions of mPFC. Dorsal CA1 ensembles were recorded from lesion (n=3) and sham (n=2) rats during active place avoidance training. CA1 discharge alternates judiciously between representing stationary and rotating locations according to the proximity of shock (p's<0.005), demonstrating cognitive control in hippocampal discharge of both sham and lesion rats**.** Both lesion (n=10) and sham (n=8) rats learned to reduced errors of entering the shock zone across the initial 16 10-min trials (8

trials/day; Group: $F_{1,16} = 0.002$, $p = 0.9$; Day: $F_{1,16} = 26.34$, $p = 10^{-4}$; Trials: $F_{3.22, 51.50} = 36.76$, $p = 10^{-13}$). The time to first enter the shock zone on each trial increased in both groups as trials progressed (Group: $F_{1,16} = 0.1$, $p = 0.76$; Day: $F_{1,16} = 69.61$, $p = 10^{-7}$, Trials: $F_{5.33, 85.33} = 12.15$, $p = 10^{-9}$). Memory retention on day 3 was indistinguishable in the lesion and sham times to first enter the shock zone ($t_{16} = 1.01$, $p = 0.3$) and errors ($t_{16} = 0.95$, $p = 0.4$). The shock was relocated by 180° to additionally assess the impact of mPFC lesion on cognitive flexibility. The groups did not differ (errors: $F_{1,16} = 0.21$, $p = 0.65$). Cytochrome oxidase (CO) activity in the brains of a subset of the lesion (n = 8) and sham (n = 8) rats shows the lesion decreased coupling amongst multiple brain areas. Although the mPFC lesion was effective and altered the coordination of metabolic activity within the dorsal hippocampus, mPFC lesion did not impair cognitive control assessed by neural representations and active place avoidance. These findings support the alternative "local computation" hypothesis: the computations required for cognitive control can occur locally in brain networks independently of the mPFC as a central computational locus for cognitive control.

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Poster

239. Hippocampal Physiology I

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Program #/Poster #: 239.07

Topic: H.08. Learning and Memory

Support: IBS-R002-A1

Title: Variations in value and outcome processing along the longitudinal axis of the hippocampus

Authors: *M. YUN[1], J. HWANG[1], M. JUNG[2];
[1]Inst. For Basic Sci., Inst. For Basic Sci., Daejeon, Korea, Republic of; [2]Korea Advanced Inst. in Sci. and Technol., Daejeon, Korea, Republic of

Abstract: A large body of evidence indicates anatomical, transcriptional, physiological, and functional variations along the longitudinal axis of the hippocampus. It is unclear, however, how value-related signals vary along the longitudinal is of the hippocampus. In rodents, although strong value-related neural signals have been found in the dorsal CA1, it is unknown whether and how the ventral CA1 carries value-related signals. We addressed this issue by comparing value- and outcome-related neural activity between the dorsal and ventral CA1 regions (dCA1 and vCA1, respectively) in mice performing probabilistic classical conditioning tasks. Three odor cues were associated with 0, 25 and 75% of reward delivery in task 1, and with 75% of punishment delivery, no outcome, and 75% of reward delivery in task 2. We found that inactivation of the dCA1 (task 1, N = 9; task 2, N = 10 male mice) or vCA1 (task 1, N = 9; task

2, N = 8 male mice) by muscimol infusion impairs value-dependent anticipatory licking behavior. We then examined value- and outcome-dependent neural activity by *in vivo* Ca^{2+} imaging (dCA1, N = 6 male mice; task 1, n = 3304 neurons; task 2, n = 2743 neurons; vCA1, N = 6 male mice; task 1, n = 724 neurons; task 2, n = 799 neurons). Both the dCA1 and vCA1 showed value- and outcome-dependent activity, but their characteristics differed in several respects. First, as a population, dCA1 neurons monotonically increased activity as a function of value, while vCA1 neurons were preferentially responsive to the highest-value sensory cue following cue presentation and then to reward-predicting cues regardless of their values once licking was allowed. Second, once the trial outcome was revealed, value and outcome signals were stronger and persisted longer over multiple trials in the dCA1 than vCA1. Third, updated value signals were stronger in the dCA1 and vCA1. Fourth, vCA1 neurons showed more rapid responses to punishment and strongly biased responses to negative prediction error compared to dCA1 neurons. Collectively, these results indicate that both the dCA1 and vCA1 are involved in value and outcome processing, but in different ways. Our results suggest a dCA1 role in quantitative representation of stimulus value and a vCA1 role in preferentially processing behaviorally relevant, salient features of experienced events.

Disclosures: **M. Yun:** None. **J. Hwang:** None. **M. Jung:** None.

Poster

239. Hippocampal Physiology I

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Program #/Poster #: 239.08

Topic: H.09. Spatial Navigation

Support: KAKENHI(19H01131, 21H05296, 19K12768)
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Title: Role of VTA dopaminergic input to the hippocampus for novel learning during spatial navigation

Authors: *Y. TAMATSU[1], R. TAKAHASHI[1], H. AZECHI[2], K. IDE[2], S. TAKAHASHI[3]; [1]Doshisha Univ. - Kyotanabe Campus 1-3, Kyoto, Japan; [2]Lab. of Cognitive and Behavioral Neuroscience, Grad. Sch. of Brain Sci., Tezukayama Univ., Kyotanabe-Shi, Japan; [3]Doshisha Univ., Kyotanabe City, Japan

Abstract: For goal-directed behaviors, reward plays a significant role. Several lines of evidence revealed that the dopaminergic system conveys reward information to the hippocampus and is critical for maintaining the reward-related cognitive map in the hippocampus. The ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc) are two well-known sources of dopamine that project to the hippocampus. We anticipated that goal-directed navigational activities are influenced by neural connectivity between VTA dopaminergic neurons and the dorsal hippocampus. To clarify this, we devised a circular track based on previous studies. Mice

were trained to run on the circular track with four food bins located at the north, west, south, and east locations. Initially, we trained them to get a reward at only one of the food bins (fixed task). During this task performance, we calculated the learning rate. Three days after the trial began, a response rate of over 80% was achieved, and the rate remained stable over 3 days. Next, dopaminergic neurons in the bilateral VTA were lesioned with 6-OHDA. After the injury, the correct response rates did not significantly decrease while the number of laps was significantly lower than before the surgery. After the VTA was lesioned in mice, we performed a displacement task in which the position of a reward was moved in response with the change of the cue objects arrangement in the experimental room. The percentage of correct responses was initially lower than the chance level, but the percentage of correct responses rapidly increased. Our findings were comparable to those of previous study reporting that the suppression of D1/D5 dopamine receptors in the dorsal hippocampus (dCA1) prevent the learning of novel reward acquisition rules. However, the number of laps was decreased following the lesion. We speculate that the reason is mainly due to postsynaptic regions of VTA involved in motivation. We thus discuss specific causal relationship between the VTA dopaminergic neurons and the hippocampus using optogenetics to selectively manipulate VTA dopamine in the hippocampus.

Disclosures: **Y. Tamatsu:** None. **R. Takahashi:** None. **H. Azechi:** None. **K. Ide:** None. **S. Takahashi:** None.

Poster

239. Hippocampal Physiology I

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Program #/Poster #: 239.09

Topic: H.09. Spatial Navigation

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The Ministry of education and NRF BK21 program

Title: Value-dependent reactivation of place cells in the intermediate, but not in the dorsal hippocampus

Authors: *S.-W. JIN, I. LEE;
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Abstract: It is widely held that the hippocampus is important for episodic memory, which is composed of autobiographical events that occurred at specific times and places. One of the neural mechanisms of memory consolidation is the reactivation of hippocampal cells during sharp-wave ripples (SWR). It is postulated that events acquired during active behavior are

reactivated during sleep and transferred to the neocortex, where it becomes permanently stored. Because high-value events tend to be remembered more easily than low-value events, the reactivation may also be affected by the event's value information. we tested the hypothesis that value information influences the reactivation strength of place cells in the iHP. We used a place-preference task in the T-maze while simultaneously recording single units from the dorsal hippocampus (dHP) and iHP using 24-tetrodes in rats (n=6). In block 1, they tended to choose the sunflower seed-baited arm over the cheerios-baited arm through trial and error. In block 2, the starting position was moved to the opposite arm to make the hippocampal-dependent learning. In block 3, the starting position was the same as in block 1, but the arms associated with sunflower seeds and cheerios were reversed. Our preliminary results show that the amounts of ripples in the dHP and iHP decreased transiently after the spatial values were swapped, but overall ripple rates associated with high- and low-value rewards were similar both in dHP and iHP. Meanwhile, the high-value-coding place cells (HVC-PCs) in the iHP was reactivated more frequently during SWR than other cell types during the task. Furthermore, the reactivation probability of HVC-PCs was higher than other cell types during the post-sleep session compared to the pre-sleep session, and the higher probability of firing of HVC-PCs during post-sleep compared to pre-sleep was positively correlated with the learning speed on the next day. These findings were not observed in dHP. Our findings suggest that reactivation of HVC-PCs in the iHP during sleep may underlie the strong consolidation of high-value places.

Disclosures: S. Jin: None. **I. Lee:** None.

Poster

239. Hippocampal Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 239.10

Topic: H.09. Spatial Navigation

Support: NIH: R01MH106552
NIH: R01MH112523

Title: Attentive State Activity in Hippocampus During 2-Dimensional Contextual Fear Conditioning

Authors: *A. UYSAL, J. CHENG, D. JI;
Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Hippocampus is a crucial structure for contextual fear conditioning. After a rat receives a mild foot shock in a specific environment, they show fear responses such as avoidance and freezing when placed back. Previously, we have demonstrated that before an avoidance turn, awake replays in the hippocampus encode the path from a rat's current location to where they had received a shock in a linear track. However, it remains unknown what spatial contents are encoded in the hippocampal activity during freezing in the classical fear conditioning. This study

analyzed brain activities in the hippocampus during freezing in a 2-dimensional (2D) environment. Rats were placed in a shock box for 15 minutes and received three mild-foot shocks at the end of the session. After a rest period of 30 minutes outside, they were returned to the box for another 15 minutes. We recorded place cell activity and local field potential from the CA1 region of the hippocampus. We analyzed the multiunit activity and found that hippocampal cells' synchronized activity (frames) had increased after the shock. On the other hand, the rate of sharp-wave ripples, usually seen when the animal is awake-resting, or consuming a reward, remained the same. In addition, when we analyzed the content of the frame activities, the decoded positions were closer to the animal than the decoded positions from ripples. For local field potential, we focused on theta rhythm, which can be categorized further into two types depending on the animal's behavior. The power of type 1 theta rhythm (8-12 Hz), observed when the animal is active in the shock box, remained similar after the shock. On the contrary, the power of type 2 theta rhythm (4-8 Hz), which occurs when the animal is immobile but attentive, increased significantly after the foot shock when the animal is freezing. These results suggest that after a mild foot shock, even though rats show a fear response of freezing in a 2D environment, hippocampal neurons are processing the recent aversive event via increased frame activities, coding nearby locations, and through increased attentive type 2 theta rhythm.

Disclosures: **A. Uysal:** None. **J. Cheng:** None. **D. Ji:** None.

Poster

239. Hippocampal Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 239.11

Topic: H.08. Learning and Memory

Support: R01 AA027474

Title: Prosapip1 in dopamine D1-receptor-expressing neurons plays a role in learning and memory

Authors: *Z. HOISINGTON, Y. EHINGER, J. MOFFAT, D. RON;
Dept. of Neurol., Univ. of California San Francisco, San Francisco, CA

Abstract: ProSAP-interacting protein 1 (Prosapip1) is a brain-specific protein that interacts with the postsynaptic density proteins, Shank3 and SPAR, and is highly enriched in hippocampal neurons [1]. Prosapip1 promotes F-actin formation and plays a role in synaptic and structural plasticity [2]. Dopamine D1-receptor (D1R) expressing neurons in the hippocampus are necessary for learning and memory-associated behaviors [3-5]. We therefore examined the contribution of Prosapip1 in D1R-expressing neurons to learning and memory in mice. To do so, we crossed a Prosapip1 (fl/fl) mouse line with a D1-Cre line to obtain mice with a conditional Prosapip1 knockout specifically in D1 neurons (Prosapip1 cKO/D1). We found that male and female Prosapip1 cKO/D1 mice show a memory deficit in a novel object recognition task.

Similarly, in a 3-day rotarod test, Prosapip1 cKO/D1 mice of both sexes exhibited a significant motor learning/memory deficit compared to wildtype (WT) mice. We also conducted a 3-chamber social interaction test (3CSI), in which mice chose to either spend time with a WT juvenile sex-matched interaction partner or with an empty chamber. Both male and female WT and Prosapip1 cKO/D1 mice demonstrated a significant preference for interaction with a novel mouse over an empty chamber, indicating normal sociability. In contrast, while male and female WT mice prefer interacting with a novel partner over a familiar one, Prosapip1 cKO/D1 mice fail to significantly discriminate between the novel and familiar mouse, indicative of a potential memory and/or sociability deficit(s). Importantly, Prosapip1 cKO/D1 mice did not exhibit any motor coordination or locomotor impairments. Together, these data suggest that Prosapip1 in D1R-expressing neurons plays a role in learning and memory in male and female mice.
1.Wendholt, D., et al. J Biol Chem, 2006.
2.Laguesse, S., et al. Neuron, 2017.
3.Oliveira da Cruz, J.F., et al. Cell Rep, 2020.4.Sarinana, J., et al. Proc Natl Acad Sci U S A, 2014. 5.Tran, A.H., et al. J Neurosci, 2008.
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Poster

240. Neural Representations for Navigation

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Program #/Poster #: 240.01

Topic: H.08. Learning and Memory

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NIH (NIH Director's New Innovator Award (DP2-AG071918))
Arnold and Mabel Beckman Foundation (Beckman Young Investigator Award)

Title: Anatomical and Functional Evidence for an Entorhinal-like Region in Birds

Authors: *M. C. APPLEGATE, K. S. GUTNICHENKO, D. ARONOV;
Columbia Univ., New York, NY

Abstract: The entorhinal cortex (EC) is a major input to the mammalian hippocampus. Both the hippocampus and EC have been extensively characterized for their spatial firing properties: the hippocampus contains place cells that predominantly fire within a single field, while the EC contains multi-field firing patterns such as grid cells. In birds, the dorsal lateral hippocampal formation (DL) has been proposed to serve as an avian homolog of the EC based largely on developmental evidence. However, little is known about the exact pattern of connections between DL and the hippocampus in birds, and no functional recordings have been performed in

DL. We aimed to characterize anatomical and functional characteristics of DL in food-caching birds (black-capped chickadees and tufted titmice) and compare them to the mammalian EC. We first examined inputs to the avian hippocampus and DL using retrograde tracer CTB. We showed that DL provides a strong input to the hippocampus: both by volume and by the number of cells, DL was the largest avian neural structure with a projection to the hippocampus. Additionally, other avian cortical inputs sent more afferent projections to DL than to the hippocampus directly. We also found that DL projects topographically along its longitudinal axis to the hippocampus, with anterior and posterior parts of DL providing input to anterior and posterior parts of the hippocampus, respectively. All of these patterns of connectivity are reminiscent of the organization of the mammalian entorhinal-hippocampal circuit.
Next, we developed a preparation for 1-photon calcium imaging in DL of awake birds to functionally characterize its spatial response properties. We targeted anterior DL, which provides input to the region of the bird hippocampus that contains place cells. We performed a random foraging experiment and compared neural activity between the anterior hippocampus and anterior DL. We found that about half of the cells within anterior DL were spatially selective ($p<0.01$). Spatial firing fields in DL consistently had more fields than hippocampal neurons—while half of spatial hippocampal cells had a single spatial firing field, less than a quarter of spatial neurons in DL had one field. Multi-field activity is consistent with activity patterns observed in the EC of mammalian species. Additionally, we observed that avian hippocampal neurons (like those in mammals) were on average predictive of the bird's future location, while the DL neurons (like EC neurons) had firing that best correlated to the current location. Collectively, these anatomical and functional similarities between DL and EC strengthen the hypothesis that DL is an entorhinal homolog.

Disclosures: M.C. Applegate: None. **K.S. Gutnichenko:** None. **D. Aronov:** None.

Poster

240. Neural Representations for Navigation

Location: SDCC Halls B-H

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Topic: H.08. Learning and Memory

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NSF Graduate Research Fellowship Program
Simons Society of Fellows

Title: Representations of one-shot and consistent information in the hippocampus of memory-expert birds

Authors: *E. L. MACKEVICIUS, C. FANG, S. N. CHETTIH, S. HALE, D. ARONOV; Columbia Univ., New York, NY

Abstract: The hippocampus is relevant for tasks that require one-shot memory, but also represents information about consistent features of the environment. To compare hippocampal representations of one-shot information and consistent information, we use food-caching birds of the chickadee family, studied for their remarkable hippocampus-dependent memory abilities, and recently made accessible for systems neuroscience experiments.
We recorded the hippocampus of food-caching birds in a task that involves both one-shot information (location of food caches), and consistent information (location of a feeder). Hippocampal place cell fields were overabundant at feeder locations, as has been described in rodent studies. To measure how the hippocampus represents food caches, we allowed birds to cache food in 16 different locations, arranged in a circle. Place fields were not overabundant at cache locations. Instead, we observed transient patterns of activity during caching, and these patterns were reactivated during retrieval of the same caches. This reactivation was highly specific: the match between cache and retrieval patterns was stronger than between two non-caching visits to the same site, or between two caching visits to neighboring sites. These results suggest that the hippocampus generates different representations of one-shot and consistent information.
A promising theoretical framework for understanding the hippocampal representations underlying memory-guided behaviors are state-space predictive models, such as the Successor Representation (SR). To determine whether the SR can capture hippocampal representations in our caching task, we constructed a simple state-space model where each spatial location consists of two states: food-related visits (caching, retrieval from cache-site or feeder), and non-food-related visits. To generate the SR, we simulated trajectories through this state space, with caches modeled as food-related states visited only twice (once to cache, then later once to retrieve the cache), and feeders modeled as food-related states visited more frequently. SR is a good match to neural data, exhibiting enhanced reactivation specific to a particular cache site, and overabundances of place fields at feeder locations, but not at cache locations. These results suggest that, while hippocampal activity differs between locations of consistent vs. one-shot presence of food, these differences can be accounted for within the unifying theoretical framework of state-space predictive models.

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Poster

240. Neural Representations for Navigation

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Beckman Young Investigator Award
NIH DP2 New Innovator Award

Title: Neural signatures of memory retrieval in the hippocampus of freely caching chickadees

Authors: *S. N. CHETTIH, E. L. MACKEVICIUS, D. ARONOV;
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Abstract: How does the brain record individual experiences, and recall them to adaptively guide future behavior? This 'episodic-like' memory regime involves the hippocampus, but it has been difficult to identify neural signatures for the recall of specific memories. Studying food caching behaviors may provide a mechanistic understanding of this process. Scatter hoarding species, such as chickadees, cache individual food items, and use hippocampal-dependent memory to later retrieve them. We thus developed tools for behavioral and neural measurement in chickadees to begin identifying possible neural correlates of cache memories. We used a behavioral arena in which chickadees spontaneously cache and retrieve seeds, without explicit training or reinforcement. We adapted this arena for use with multi-view videography to reconstruct 3D posture, and automatically parsed behavior into discrete actions and interactions with arena features such as caching and retrieval of seeds. Finally, we developed ultra-light implants (<1.5g) for chronic silicon probe recordings compatible with freely moving behavior in small birds, enabling simultaneous recording of ~100 hippocampal neurons. Hippocampal activity during caching exhibited strong modulation preceding cache site interaction. This activity was distinct for each cache site and encoded the hidden contents of the site, consistent with a representation of memory for individual caches. Intriguingly, this site-specific activity was distinct from canonical place coding observed in rodents and birds, in both the smoothness of spatial representations and temporal dynamics of activity during cache site interactions. Our results reveal a novel population code which may underlie hippocampal-dependent cache memory.

Disclosures: S.N. Chettih: None. **E.L. Mackevicius:** None. **D. Aronov:** None.

Poster

240. Neural Representations for Navigation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 240.04

Topic: H.08. Learning and Memory

Support: Helen Hay Whitney Foundation Fellowship
New York Stem Cell Foundation–Robertson Neuroscience Investigator Award
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NIH Grant AG071918

Title: Neural representations of physical and visual space in food-caching birds

Authors: *H. L. PAYNE, D. ARONOV;
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Abstract: Both physical space (where you are) and visual space (where you are looking) are represented in neural activity patterns in the mammalian hippocampus. However, it is unclear how these activity patterns each arise, how they interact with each other, and how they influence goal-directed behavior. Like humans, many birds rely heavily on vision to navigate and form spatial memories. In particular, food-caching birds depend on visual landmarks and an intact hippocampus to find previously hidden food items. We recorded from the hippocampus of food-caching birds during 2D foraging tasks. First, we found rodent-like patterns of activity, including place cells and sharp wave ripples. These features were robustly organized along analogous axes to those in mammals: for example, place cell prevalence and precision varied along the long axis of the hippocampus. This suggests that the hippocampal circuit mechanisms that give rise to representations of physical space are similar between birds and mammals, despite 300 million years of independent evolution. In a non-food-caching bird species, place coding was present but less robust, suggesting that spatial representations can vary quantitatively with species-specific ethological needs. Second, to investigate how visual inputs give rise to these representations of physical space, we developed a system to estimate the location at which gaze is directed during free motion, and designed a modified foraging task to behaviorally dissociate place and gaze locations. As in the primate hippocampus, we found signals related to vision, including modulation of firing by gaze saccades and firing correlated to gaze location. Ongoing work addresses how vision interacts with hippocampal representations of space, by comparing the content and timing of representations of physical and visual space in visual input pathways to the hippocampus.

Disclosures: H.L. Payne: None. **D. Aronov:** None.

Poster

240. Neural Representations for Navigation

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Program #/Poster #: 240.05

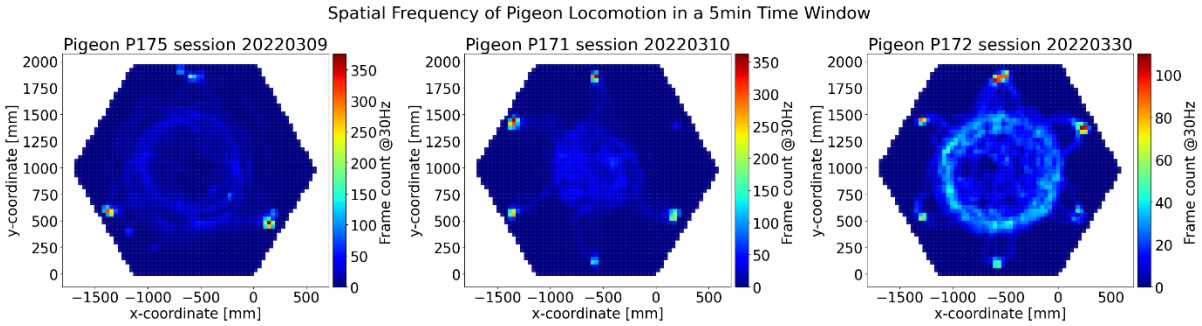
Topic: H.09. Spatial Navigation

Support: SFB 1372 project number 395940726, subproject Neu06
DFG Research Training Group "Situated Cognition" GRK 2185/1

Title: 3d tracking of freely moving pigeons reveals behavioral differences during space and color discrimination tasks

Authors: *G. HIDALGO GADEA, N. ROOK, V. LUDWIG, M. LI, M. INDA, J. M. TUFF, T. OTTO, R. PUSCH, O. GUNTURKUN;
Biopsychology, Ruhr Univ. Bochum, Bochum, Germany

Abstract: Measuring behavior has become increasingly popular in neuroscience, and decisive advances in machine learning have enabled robust quantification of animal movement in ever less restrictive setups. Pigeons are a great example of a standard model in cognitive neuroscience whose natural behavior has been systematically restricted during cognitive tasks and thus remains under-investigated. We, therefore, propose a versatile approach to investigate cognition-guided behavior in minimally restricted conditions. Such an approach would increase ecological validity in neuroscience, and the quantification of behavior would provide a statistical control measure for animal movement within tasks. To this end, we built a multi-camera 3D tracking arena for cognitive testing in freely moving pigeons using markerless pose estimation and robust camera triangulation at high resolutions, for long recording periods of up to 80 minutes. With over 100 hours of video data, we show that 3D pose estimation with 6 cameras can reliably reconstruct the naturally upright pose of pigeons in a $4m^2$ field of view at a distance of up to 2.8m. For instance, the body size of individual pigeons can be accurately estimated through the additional z-axis (length: 303.12mm ± 14.06mm; height: 239.74mm ± 5.65mm), and head movements during locomotion and pecking reveal characteristic kinematic components in vertical and horizontal axes. Comparing different cognitive tasks for space and color discrimination on multiple touch screens, we show that pigeons exhibit different spatial navigation strategies between feeders, using distinct path traces and different preferred sections within the arena. Moreover, animals show individual differences in the overall symmetry of their use of space, as well as in average locomotion speed and total distance traveled. Further quantification of pigeons' behavior on the tasks will be an important control to disentangle context- and task-associated neural activity in core brain structures.



Disclosures: **G. Hidalgo Gadea:** None. **N. Rook:** None. **V. Ludwig:** None. **M. Li:** None. **M. Inda:** None. **J.M. Tuff:** None. **T. Otto:** None. **R. Pusch:** None. **O. Gunturkun:** None.

Poster

240. Neural Representations for Navigation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 240.06

Topic: H.09. Spatial Navigation

Title: The influence of spatial experience on adult neurogenesis in the hyper- and mesopallium of the pigeon (Columba livia f.d.)

Authors: *J. MEHLHORN[1], S. KURUTAS[1,2], S. CASPERS[1,3], K. AMUNTS[3,2], C. HEROLD[2]; [1]Med. Fac., Inst. for Anat. I, Univ. of Düsseldorf, Duesseldorf, Germany; [2]C. and O. Vogt Inst. of Brain Res., Duesseldorf, Germany; [3]Inst. of Neurosci. and Med. (INM-1), Juelich, Germany

Abstract: Adult neurogenesis (AN) is a process that includes the generation, maturation and proliferation of new neurons in the brain over the lifespan. But even though there has been some progress in this field of research, the functional significance of this trait is still ambiguous. In birds, AN was detected in a variety of species. It is known that the occurrence of new neurons is not limited to a few brain regions, but is ubiquitously present throughout most of the avian telencephalon. Thus, adult neurogenesis in birds is well suited for experimental manipulations and the investigation of AN. In this study, 29 homing pigeons (*Columba livia* f.d.) originating from the same breeding stock were raised under identical conditions. Homing pigeons are well-known for their navigational skills and their brains are functionally adapted to homing with e.g. larger hippocampi. After fledging, 10 of them (Group I) remained permanently in the loft while the other 19 were allowed to fly around the loft and they participated successfully in pigeon races. After reaching sexual maturity, all pigeons were treated with 5-bromo-deoxyuridine (BrdU) to label dividing cells. After this, pigeons of Group I had to absolve an orientation learning task in a standard operant chamber. 10 of the other pigeons (Group II) got an individual training with several releases from unknown places (distance 35 km, east, north, west and south from the loft). The remaining 9 animals served as a control group (Group K) and did not receive any training. At the end of trainings, all pigeons were sacrificed, the brains were dissected and, immunohistochemically processed with BrdU, doublecortin (DCX), NeuN, S100ß, and GFAP to examine quantitatively different cell types and different stages of cell proliferation in the hyper- and mesopallium. The number of newly generated immature neurons, mature neurons and glial cells differs between the three groups. Pigeons of Group II and K showed more immature cells than pigeons of the Group I. Highest numbers of new mature neurons were found in pigeons that had to undergo a training (Groups I and II) with the highest number in the hyperpallium densocellulare of pigeons of Group I. Besides, hyperpallial structures showed more AN than the mesopallium. Our findings indicate that spatial learning processes have a positive effect on AN. This effect depends on the type of training. Moreover, individual life history has an influence on AN. The different distribution of maturing cells in the different forebrain structures support the idea that there is a functional specialization, respectively, that there is a link between brain-structure and function, species-specific requirements and AN.

Disclosures: **J. Mehlhorn:** None. **S. Kurutas:** None. **S. Caspers:** None. **K. Amunts:** None. **C. Herold:** None.

Poster

240. Neural Representations for Navigation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 240.07

Topic: H.08. Learning and Memory

Title: Scientific Abstract

Authors: *R. REICHERT[1], N. ROOK[2], O. GUNTURKUN[3];
[1]Ruhr Univ. Bochum, Bochum, Germany; [2]Biopsychology, Ruhr-University Bochum, Bochum, Germany; [3]Ruhr-Universitat Bochum, Bochum, Germany

Abstract: Reward prediction error (RPE) in the ventral tegmental area (VTA) facilitates extinction learning in mammals and birds. The avian and mammal dopaminergic systems in the context of extinction are developed to a comparable degree. Now, within the scope of the Sonderforschungsbereich 1280, we are examining whether the optogenetic manipulation of prediction error in the VTA terminating in the avian PFC, the caudolateral nidopallium (NCL), produces memory extinction and subsequent behavioural changes in pigeons. Both decreased and increased learning rates during extinction are hypothesized to be caused by RPEs. Though already established, manipulating extinction optogenetically has not yet been analysed in the temporal dimension on trial by trial dynamics. Using pigeons instead of mammals allows for superior analysis in an extinction paradigm, since the animals are able to undergo 1000+ trials completing one cycle of acquisition, extinction and renewal within one day. Though functionally similar, the avian dopaminergic system differs structurally to mammals. Hence, we used tyrosine hydroxylase (TH) specific antibodies in the avian midbrain to establish precise injection targets. In addition, we counterstained dopamine-beta-hydroxylase differentiating dopaminergic neurons from noradrenergic cells. We also injected biotinylated dextran amines as an anterograde tracer to stain the axonal projection targets of the VTA in the NCL. To manipulate dopaminergic cell bodies in the VTA as well as dopaminergic axonal projections in the NCL we created a TH-specific viral vector to express the membrane proteins necessary for optogenetic excitation and inhibition. Optogenetic manipulation of VTA terminals in the NCL enables subsequent dissociation of the context dependent role of RPEs in the VTA similar to RPEs in the striatum. The behavioural experiment builds upon an established extinction paradigm created in our lab. This way, we will behaviourally manipulate the animals during extinction and renewal to quantify the trial by trial dynamics and contextual dependencies that subsequently aides our understanding of cognition and behaviour in the extinction context.

Disclosures: R. Reichert: None. **N. Rook:** None. **O. Gunturkun:** None.

Poster

240. Neural Representations for Navigation

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Program #/Poster #: 240.08

Topic: H.09. Spatial Navigation

Support: Howard Hughes Medical Institute

Title: Accurate angular integration with only a handful of neurons

Authors: *M. NOORMAN, B. K. HULSE, V. JAYARAMAN, S. ROMANI, A. M. HERMUNDSTAD;
Janelia Res. Campus, Ashburn, VA

Abstract: To flexibly navigate, many animals rely on internal spatial representations that persist when the animal is standing still in darkness, and update accurately by integrating the animal's movements in the absence of localizing sensory cues. Theories of mammalian head direction cells have proposed that these dynamics can be realized in a special class of networks that maintain a localized bump of activity via structured recurrent connectivity, and that shift this bump of activity via angular velocity input. Although there are many different variants of these so-called ring attractor networks, they all rely on large numbers of neurons to generate representations that persist in the absence of input and accurately integrate angular velocity input. Surprisingly, in the fly, *Drosophila melanogaster*, a head direction representation is maintained by a much smaller number of neurons whose dynamics and connectivity resemble those of a ring attractor network. These findings challenge our understanding of ring attractors and their putative implementation in neural circuits. Here, we analyzed failures of angular velocity integration that emerge in small attractor networks with only a few computational units. Motivated by the peak performance of the fly head direction system in darkness, we mathematically derived conditions under which small networks, even with as few as 4 neurons, achieve the performance of much larger networks. The resulting description reveals that by appropriately tuning the network connectivity, the network can maintain persistent representations over the continuum of head directions, and it can accurately integrate angular velocity inputs. We then analytically determined how performance degrades as the connectivity deviates from this optimally-tuned setting, and we find a trade-off between network size and the tuning precision needed to achieve persistence and accurate integration. This work shows how even small networks can accurately track an animal's movements to guide navigation, and it informs our understanding of the functional capabilities of discrete systems more broadly.

Disclosures: M. Noorman: None. **B.K. Hulse:** None. **V. Jayaraman:** None. **S. Romani:** None. **A.M. Hermundstad:** None.

Poster

240. Neural Representations for Navigation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 240.09

Topic: H.09. Spatial Navigation

Support: Howard Hughes Medical Institute

Title: A multimodal rotational velocity signal required for angular path integration in Drosophila

Authors: *B. HULSE[1], A. STANOEV[2], D. TURNER-EVANS[4], J. D. SEELIG[5], V. JAYARAMAN[3];

[1]Howard Hughes Med. Institute, Janelia Res., Howard Hughes Med. Institute, Janelia Res., Ashburn, VA; [3]Howard Hughes Med. Institute, Janelia Res. Campus, [2]Howard Hughes Med. Institute, Janelia Res. Campus, Ashburn, VA; [4]Univ. of California, Santa Cruz, Univ. of California, Santa Cruz, Santa Cruz, CA; [5]Max Plankck Inst. for Neurobio. on Behavior, Caeser, Bonn, Germany

Abstract: Navigating animals continuously integrate self-motion signals to update internal representations of their directional heading and spatial location in their environment. How neural circuits combine sensory and motor information to construct these velocity estimates and how these signal update internal representations supporting navigation computations is not well understood, despite considerable theoretical interest. Recent work in *Drosophila* has identified a neural circuit that performs angular path integration to compute the fly's head direction, but the nature and neural identity of the velocity signal remains unknown. Here we identify a bilateral pair of neurons—known as GLNO(s)—that encode the fly's rotational velocity with high accuracy using both motor and visual signals and whose activity is necessary for angular path integration. Reciprocal inhibition generates flip-flop dynamics that can resolve sensorimotor conflicts that arise when visual and motor signal convey opposing velocity estimates. Under most circumstances, when visual and motor signals are largely congruent, GLNO(s) rely on motor information at the expense of optic flow. Together, these results suggest that flies update their head direction representation by constructing a rotational velocity signal from sensorimotor cues arranged in a hierarchy according to cue reliability and motor context.

Disclosures: B. Hulse: None. **A. Stanoev:** None. **D. Turner-Evans:** None. **J.D. Seelig:** None. **V. Jayaraman:** None.

Poster

240. Neural Representations for Navigation

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Program #/Poster #: 240.10

Topic: H.09. Spatial Navigation

Support: Howard Hughes Medical Institute

Title: Sun compass cues provide fly head direction cells with global references for robust navigation in naturalistic environments

Authors: *S. S. CHITNIS[1,2], H. HABERKERN[1], P. M. HUBBARD[1], T. GOULET[1], A. M. HERMUNDSTAD[1], V. JAYARAMAN[1];
[1]HHMI Janelia Res. Campus, Ashburn, VA; [2]Indian Inst. of Sci. Educ. and Res., Pune, India

Abstract: Several animals, including many species of insects, rely on internal representations of head direction (HD) to navigate flexibly in their environments. Flies depend on HD cells in the central complex — a highly conserved region in the insect brain — to select and maintain their bearings relative to directional cues around them. This compass-like HD representation is

updated by self-motion and tethers to visual and mechanosensory cues in the environment. Some of these cues, like the position of the sun, can act as global compass cues for the animal. In natural settings, however, the sun can get obscured by clouds or terrestrial landmarks, such as trees or blades of grass. Here we used a novel, immersive virtual reality (VR) environment to study the behavior of tethered flies walking in an open field under different naturalistic visual settings. We found that flies exhibited stable heading preferences in the presence of directional global cues that mimicked the natural sky such as bright sun-like disks and gradients of light intensity. Silencing the fly's HD cells disrupted this flexible, individualized behavior, and caused all flies to revert to phototaxis. We then combined two-photon calcium imaging in head-fixed Drosophila with behavior in VR, and asked if and how central complex neurons maintain a reliable estimate of HD in the presence of these visual cues. We found that the HD representation remained stable in the presence of intensity gradients with high contrast. However, in low contrast gradients, the HD representation became unstable and flies were unable to maintain their heading preference, instead walking in curved paths. When we placed flies in dense visual environments with patches of grass, flies continued to maintain a reliable HD estimate by tethering to available global cues even though they could be temporarily obscured. Our results demonstrate that the fly's HD system enables it to perform goal directed behaviors like maintaining stable heading preferences even in complex natural environments.

Disclosures: S.S. Chitnis: None. **H. Haberkern:** None. **P.M. Hubbard:** None. **T. Goulet:** None. **A.M. Hermundstad:** None. **V. Jayaraman:** None.

Poster

240. Neural Representations for Navigation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 240.11

Topic: H.09. Spatial Navigation

Support: Howard Hughes Medical Institute

Title: Flexible control of behavioral variability mediated by an internal representation of head direction

Authors: *C. DAN, R. KAPPAGANTULA, B. HULSE, V. JAYARAMAN, A. HERMUNDSTAD;
Janelia Res. Campus, HHMI, Ashburn, VA

Abstract: Internal representations enable flexible behavior in many animals. Although these representations are usually tethered to sensory cues, they allow animals to achieve behavioral goals even in the absence of the cues[1]. In many natural settings, animals develop these internal representations while identifying locations and actions of value within the environment.
Here we delve into the dynamic process by which internal representations, goals, and behavioral policies develop and work together during rapid, visually guided operant learning in flies. We

adapted a well-established paradigm to explore the behavioral policy and the underlying circuit architecture that enables flies to modify their actions in response to heat punishment associated with one of two repeating visual patterns arranged symmetrically around them[2]. In double-blind experiments, we found that silencing the fly's head direction (HD) cells significantly impairs performance. We analyzed the behavior of individual flies to understand the structure of their actions and isolated a set of control parameters that govern action selection. We showed that flies' behavior is consistent with a heading-dependent policy, but only if the symmetry of the setting evokes a predictable instability in the HD representation.
The fly's HD representation is observable as a `bump' of calcium activity in a population of neurons within a brain region called the central complex (CX)[3]. We performed two-photon calcium imaging of these HD neurons in tethered flying flies and found that the symmetry of the visual setting indeed induces a structured instability in the HD representation. We constructed a model for how CX circuits might use this representation to both learn a goal heading in the fly's environment and select actions that are driven by its current heading relative to its goal. Importantly, the structure of the fly's behavioral policy relative to this goal heading appears to be hardwired, allowing rapid learning and immediate adjustments to new goal headings. Finally, we used the model to understand the interplay of the processes governing the generation of the HD representation, the inference of a goal heading, and the selection of actions.
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2. Wolf, R. & Heisenberg, M. Basic organization of operant behavior as revealed in Drosophila flight orientation. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* 169, 699-705 (1991).
3. Seelig, J. D. & Jayaraman, V. Neural dynamics for landmark orientation and angular path integration. *Nature* 521, 186-191 (2015).

Disclosures: C. Dan: None. **R. Kappagantula:** None. **B. Hulse:** None. **V. Jayaraman:** None. **A. Hermundstad:** None.

Poster

240. Neural Representations for Navigation

Location: SDCC Halls B-H

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Program #/Poster #: 240.12

Topic: H.09. Spatial Navigation

Support: Howard Hughes Medical Institute
NINDS R01NS104934

Title: Sign-inverting vectors underlie a coordinate transformation in the Drosophila central brain

Authors: *I. G. ISHIDA[1], S. SETHI[1], T. L. MOHREN[1], L. F. ABBOTT[2], G. MAIMON[1]; [1]Lab. of Integrative Brain Function, The Rockefeller Univ., New York, NY; [2]Mortimer B. Zuckerman Mind Brain Behavior Institute, Dept. of Neurosci., Columbia Univ., New York, NY

Abstract: We studied a mechanism by which the *Drosophila* brain represents directions in an allocentric reference frame. We focused on the PFNa neurons, a system in the *Drosophila* central complex that signals wind direction. Using a combination of 2-photon calcium imaging, electrophysiology, and mathematical modeling, we found that the activity of PFNa neurons can be thought of as encoding a set of vectors whose sum yields the direction of wind relative to external cues like the sun, as opposed to relative to the animal's head. We also discovered that PFNa neurons switch signaling modes to convey the positive and negative signs of a vector. Specifically, when wind hits the fly's head from the front, PFNa neurons signal in the canonical way, by raising the membrane potential and spike rate of the relevant cells. When wind hits the fly's body from the back, however, PFNa neurons lower their membrane potential and experience oscillatory calcium spikes, which are expected to release neurotransmitter. This switch between a depolarized, spike-rate signaling mode and a hyperpolarized, oscillation-based signaling mode is a new mechanism for representing positive and negative values for vector computations in the fly brain.

Disclosures: I.G. Ishida: None. **S. Sethi:** None. **T.L. Mohren:** None. **L.F. Abbott:** None. **G. Maimon:** None.

Poster

240. Neural Representations for Navigation

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Program #/Poster #: 240.13

Topic: H.09. Spatial Navigation

Support: NINDS R01NS104934
HHMI

Title: A neural circuit for comparing heading direction with an internal goal to drive steering in Drosophila

Authors: *P. MUSSELLS PIRES[1], L. F. ABBOTT[2], G. MAIMON[1,3];
[1]Lab. of Integrative Brain Function, The Rockefeller Univ., New York, NY; [2]Mortimer B. Zuckerman Mind Brain Behavior Institute, Dept. of Neurosci., Columbia Univ., New York, NY; [3]Howard Hughes Med. Inst., New York, NY

Abstract: Animals are capable of astonishing feats of spatial navigation: fruit bats fly kilometers to re-visit the same tree night after night and desert ants return to their nest in a near straight line following foraging excursions that can bring them thousands of body lengths away from their home. Regardless of the specific behaviour, a navigating animal is confronted with a problem: is the animal heading in the right direction, and if not, what is the appropriate locomotor behaviour to correct its course? Decades of research have revealed several classes of neurons whose activity covaries with different spatial variables (e.g. an animal's heading direction). In *Drosophila*, a population of neurons—called EPG neurons—are anatomically arranged in a ring

and carry a sustained bump of calcium activity whose position along the ring tracks the fly's heading direction. EPG neurons synapse onto a population of neurons—called PFL3 neurons—that have been hypothesized to control goal-directed steering. However, experimental evidence for how heading information is combined with goal information to guide locomotor behaviour is lacking. Using electrophysiology, two-photon calcium imaging and mathematical modelling, we provide evidence that PFL3 neurons combine heading and goal information to produce a signal that tells the fly which way to turn. We found that PFL3 neurons receive inputs from a population of neurons that carry a bump of calcium activity in an array-like structure of the fly's brain called the fan-shaped body. By manipulating the position of this bump of activity along the fan-shaped body, we found that the position of the bump can control the fly's goal heading direction. Our work provides mechanistic insight into how neural correlates of spatial variables guide navigational behaviour.

Disclosures: P. Mussells Pires: None. **L.F. Abbott:** None. **G. Maimon:** None.

Poster

240. Neural Representations for Navigation

Location: SDCC Halls B-H

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Program #/Poster #: 240.14

Topic: H.09. Spatial Navigation

Support: ERC

Title: Fragmented replay of very large environments in the hippocampus of bats

Authors: *T. ELIAV, S. R. MAIMON, A. SAREL, S. PALGI, D. BLUM, L. LAS, N. ULANOVSKY;
Brain Sci., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: In sleeping or immobile rodents, ensembles of hippocampal place-cells exhibit sequential reactivations of previously-experienced trajectories (termed 'replay'). Such neuronal sequences are considered to be important for memory consolidation. Here we looked for replay sequences in the hippocampus of bats flying in a 200-meter long tunnel. Specifically, we asked whether there are ultra-long replay sequences in bat hippocampus, which cover the entire large naturalistic environment. To investigate this, we decoded neuronal activity when bats were stationary - during rest-times between flight epochs in the 200-meter long tunnel, and during subsequent sleep. The decoding analysis revealed many replay sequences. Surprisingly, these sequences depicted trajectories that covered relatively small pieces of the environment, between a few meters and ~20 meters - i.e. less than 10% of the environment size - in striking contrast to replay sequences in rodents in small setups, which typically cover the entire environment. These sequences were time-compressed, with a compression-ratio similar to rodents. These findings provide the first demonstration of hippocampal sequential replay in a non-rodent species. Our findings may have important implications for understanding hippocampal replay in mammals.

Specifically, the fragmented replay of information in this large environment might reflect: (i) Communication between hippocampus and neocortex - where the short replay fragments resemble the small information-packets used for transmitting large messages in artificial communication systems. (ii) Training of the neocortical network by the hippocampal network, using snippets of long experiences - as seen in training protocols of deep neural networks.

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Poster

240. Neural Representations for Navigation

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Program #/Poster #: 240.15

Topic: H.09. Spatial Navigation

Support: NINDS R01NS104934
HHMI

Title: Intracellular dynamics in the fly head-direction network during a virtual navigation task

Authors: *S. SETHI[1], G. MAIMON[1,2];
[1]Lab. of Integrative Brain Function, The Rockefeller Univ., New York, NY; [2]Howard Hughes Med. Inst., Chevy Chase, MD

Abstract: Recent studies have uncovered a head-direction system in the fly central complex, which is an important center for navigation-related computations in the arthropod brain. We decided to systematically characterize the intracellular activity dynamics of all the major, columnar cell types in the primary head-direction network of the central complex during a simple navigational task. We placed head-fixed *Drosophila* in a standard virtual reality environment, where a visual cue rotated in closed loop with their left/right (yaw) turns on an air-cushioned ball. In this scenario, flies maintain straight trajectories for tens of minutes, reminiscent of long-range dispersal trajectories seen in more natural settings. As flies performed this behavior, we performed single-cell, patch-clamp recordings from the columnar cell types that interconnect the ellipsoid body and the protocerebral bridge. We observed a diverse array of physiological responses. Consistent with past models of this network, for example, there were head direction cells with narrow tuning and broad tuning, with some showing conjunctive responses to heading and turn-velocity. When the flies stood still for a long time, we could measure persistent activity and subthreshold membrane potential oscillations in specific cell-types, whose nature may illuminate new aspects of how this network stores angular information and updates it over time. Alongside the existing connectomic and single-cell transcriptomic data sets, this sort of comprehensive patch-clamp data set should help to constrain a more detailed description of how a sense of direction is built in the nervous system, and how that sense helps to guide behavior.

Disclosures: **S. Sethi:** None. **G. Maimon:** None.

Poster

240. Neural Representations for Navigation

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Program #/Poster #: 240.16

Topic: H.09. Spatial Navigation

Support: HFSP
EMBO
ISF

Title: Hippocampal representations during natural social behaviours in a bat colony

Authors: *S. RAY, I. YONA, L. LAS, N. ULANOVSKY;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Highly-social animals live in complex communities, and interact with each other at times, locations and manner of their choosing. However, neurophysiological investigations of social responses are rarely conducted in rich multi-animal settings that allow such natural behaviours. To understand how the brain represents social information - when the animals' behaviour is not experimentally constrained to a particular task - we established a laboratory-based "bat cave" for a mixed-sex colony of 5-10 Egyptian fruit bats. The bats lived there 24/7 for several months, free to engage in any behaviours. We tracked the identities and social behaviours of all the bats using a set of high-resolution cameras. Simultaneously, we tracked their 3D locations during flights using a radiofrequency-based localization system, while conducting wireless single unit neuronal recordings in 1-2 male and female bats for several hours every day. This allowed us to understand what information is represented by dorsal CA1 neurons during naturalistic, rich and unconstrained behaviours. We found that the bats exhibited three distinct and interleaved behavioural phases: (i) Flight phase - where a large fraction of "classical" place cells exhibited social modulation and identity coding. (ii) Social Interaction phase - where a subset of hippocampal neurons encoded specific social interactions (e.g. allogrooming or aggression). (iii) Sedentary phase - where we utilized generalized additive models (GAM, a nonlinear extension of GLM) and explainable machine learning methodologies (like Shapley values) - and found that hippocampal neurons simultaneously encoded the positions of both self and others. This information was represented either allocentrically or egocentrically. Some of these neurons exhibited sparse coding, and represented only a few behavioral dimensions, while other neurons encoded many dimensions. Overall, we found that hippocampal dorsal CA1 neurons combine complex social and spatial information to form a multidimensional representation of the natural world.

Disclosures: S. Ray: None. **I. Yona:** None. **L. Las:** None. **N. Ulanovsky:** None.

Poster

240. Neural Representations for Navigation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 240.17

Topic: H.09. Spatial Navigation

Support: ERC

Title: Natural switches in behavior rapidly modulate hippocampal coding

Authors: A. SAREL[1], ***S. PALGI**[1], D. BLUM[1], J. ALJADEFF[2], L. LAS[1], N. ULANOVSKY[1]; [1]Dept. of Brain Sci., Weizmann Inst. of Sci., Rehovot, Israel; [2]Section of Neurobiology, Div. of Biol. Sci., UCSD, San Diego, CA

Abstract: In the real world, both humans and animals constantly switch between different behaviors. However, neuroscience research typically studies the brain while the animal is performing *one* behavioral task at a time - and little is known about how brain circuits rapidly switch between different behaviors. Navigation is a complex and dynamic behavior that enables testing these kinds of behavioral switches: It requires the animal to know its own location within the environment, while also paying attention to incoming sudden events such as obstacles, predators or conspecifics. Here we set out to test how brief attentional switches to 'things out there' affect the representation of space in the hippocampus. To this end, pairs of bats flew in a 135-meter flight tunnel, alternating between flying alone and flying towards each other ('cross-over' events). During cross-overs, bats had to be attentive to the other bat in order to avoid collision. Indeed, bats increased their echolocation call rate ~20 meters before the moment of cross-over, indicating that the bats were attentive during these events. We recorded the neural activity in hippocampal area CA1 and tracked the positions of the bats using wireless-electrophysiology and custom tracking devices. We found that during cross-overs, many CA1 neurons encoded the distance to the other bat: The neurons switched from place coding to a conjunctive distance-by-place coding - and then switched back to place coding after the bats passed each other. These neuronal switches were very rapid, as fast as 100 ms. This switching was correlated on a trial-by-trial basis with the attention signal, as indexed by the bat's echolocation calls - suggesting that spatial attention and active-sensing are controlling these major switches in neural coding. Interestingly, we found that in place-cells, the different place-fields of the same neuron often exhibited very different tuning to inter-bat distance - creating a non-separable coding of position by distance. Theoretical analysis demonstrated that such a non-separable code leads to better decoding of distance. Together, our results suggest that attentional switches during navigation - which in bats can be measured directly by their echolocation signals - elicit very rapid dynamics of hippocampal spatial coding. More broadly, this study demonstrates that during natural behavior, neural circuits can rapidly and flexibly switch between several computations, to represent the relevant behavioral variables - thus potentially supporting behavioral flexibility.

Disclosures: A. Sarel: None. **S. Palgi:** None. **D. Blum:** None. **J. Aljadeff:** None. **L. Las:** None. **N. Ulanovsky:** None.

Poster

240. Neural Representations for Navigation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 240.18

Topic: H.09. Spatial Navigation

Support: ERC

Title: Object × position coding in the entorhinal cortex of flying bats

Authors: *G. GINOSAR, L. LAS, N. ULANOVSKY;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Successful navigation requires knowledge of the locations of specific objects, such as landmarks and goals. However, it is unclear where in the brain this information is integrated into the cognitive map of space. So far, only an object-invariant signal was discovered, conveying general information regarding all objects at all locations (carried by so-called 'object-vector cells' in the superficial layers of the medial entorhinal cortex (MEC)). It is thus unknown whether and where does this general object-invariant signal converges with a signal for a specific location (as carried by place cells in the hippocampus) – and whether these two variables are encoded conjunctively. We hypothesized that if indeed the brain represents object × position in a conjunctive manner, such encoding will be found at the "end" of the MEC-hippocampal-MEC loop – namely, the deep layers of MEC – where all-object information from the superficial layers of MEC converges with location-specific information from hippocampal place-cells, and is then sent to the neocortex. Here we recorded from MEC of flying bats as they foraged for food in a large flight-room where 6–11 identical rest-objects were placed at various heights and locations. We found that a substantial fraction of cells in the deep layers of MEC (but not superficial layers) fired at the vicinity of specific rest-objects at specific locations. These cells fired near the rest-object either when the bat flew from or flew towards the object, but not when it flew through the same location without object-engagement – thus encoding object × position. Our results suggest a broader prevalence than currently thought for conjunctive coding of navigational variables – including the encoding of specific objects at specific locations, which are crucially important for navigation.

Disclosures: G. Ginosar: None. **L. Las:** None. **N. Ulanovsky:** None.

Poster

240. Neural Representations for Navigation

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Program #/Poster #: 240.19

Topic: H.09. Spatial Navigation

Title: Temporary hypoxia in ovo produces persistent movement deficits in the adult leopard gecko, Eublepharis macularius

Authors: N. S. BOON[1], E. E. KINERSON[1], K. M. OZIMAC[1], K. C. ADORNO[1], D. G. WALLACE[3], S. L. PARKER[2], *R. M. YODER[1];
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Abstract: Flooding and other natural events can cause temporary periods of anoxia or hypoxia in reptile nests, but no evidence currently exists to indicate whether this temporary insult affects reptilian brain development or behavior. Here, we evaluated the organization and kinematic properties of visual and non-visual exploration in leopard geckos following temporary hypoxia *in ovo*. The control group of gecko eggs (n = 13) were incubated in normoxic (~21% oxygen) conditions, whereas the experimental group (n = 3) included 10 days of hypoxic (8% oxygen) conditions. All eggs were then permitted to hatch and geckos were housed individually throughout behavioral procedures. At 6+ months of age, gecko exploration was evaluated in dark and light conditions. The behavioral test included one 60-min trial where the animal explored a round table within a square featureless enclosure. An infrared-capable overhead camera recorded all movements at 30 fps. Recordings were analyzed offline with Ethovision (Noldus) and custom movement analysis software (Microsoft Excel). Trials were separated into five 10-min epochs, and the total distance, peak speed, movement scaling (correlation between progression path length and peak speed), distance ratio, heading error, total stop time, mean stop time, number of stops, number of progressions, and progression distance were compared between groups and across epochs with a mixed Group X Epoch ANOVA. None of the measures changed significantly across epochs for either group. However, in darkness, the hypoxia group showed reduced peak speeds, $F(1,15) = 6.18$, $p = .025$, and greater movement scaling scores, $F(1,15) = 5.78$, $p = .03$, relative to controls. The significant group differences indicate that the brain damage caused by *in ovo* hypoxia caused adult geckos to more slowly than controls, and the hypoxia group's strong correlation between progression distance and peak speed suggests they are able to accurately estimate distance. These preliminary results suggest that brief early hypoxia permanently alters movement characteristics, and these deficits could affect reptilian species' survival.

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Poster

240. Neural Representations for Navigation

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 240.20

Topic: H.09. Spatial Navigation

Title: A brainstem integrator for self-location memory and positional homeostasis

Authors: *E. YANG[1], M. ZWART[3], M. RUBINOV[4], B. JAMES[5], Z. WEI[7], S. NARAYAN[8], N. VLADIMIROV[9], B. MENSH[6], J. E. FITZGERALD[2], M. B. AHRENS[10];
[1]HHMI Janelia Res. Campus, Ashburn, VA; [2]HHMI Janelia Res. Campus, Reston, VA; [3]Cambridge Electronic Design Ltd., Ashburn, VA; [4]Biomed. Engin., Vanderbilt Univ., Nashville, TN; [5]HHMI Janelia, Ashburn, VA; [6]HHMI Janelia, San Rafael, CA; [7]Janelia Res. Campus, Ashburn, VA; [8]HHMI- Janelia Res. Campus, Ashburn, VA; [9]Max-Delbrueck Ctr., Berlin, Germany; [10]Janelia Res. Campus / HHMI, Ashburn, VA

Abstract: To track and control self-location, animals integrate their movements through space. While self-location is represented in the hippocampal formation, it is unknown how such representations arise from integrated self-motion, whether they exist in more ancient brain regions, and by what pathways they control locomotion. Fish can be carried by water currents to potentially dangerous areas; here we report that larval zebrafish track their displacements to later return to previous locations. Whole-brain functional imaging revealed the circuit enabling this 'positional homeostasis'. A newly identified brainstem positional integrator stores a memory of past displacements and induces an error signal in the inferior olive, which controls future corrective swimming. Optogenetically manipulating functionally-identified integrator cells evokes displacement-memory behavior; ablating them, or downstream olivary cells, abolishes positional homeostasis. These results reveal a multiregional hindbrain circuit in vertebrates for integration of self-motion, memory of self-location, and control of locomotor behavior.

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Poster

241. Speech, Language, and the Brain

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 241.01

Topic: H.11. Language

Support: 'Chen Guang' project supported by Shanghai Municipal Education Commission and Shanghai Education Development Foundation (WBH4307002)

Title: Event-related potentials and brain oscillations index retrieval and integration effort in natural sentence comprehension: Evidence from a Chinese relative clause study

Authors: *K. XU[1], C. MA[2];
[1]Inst. of Modern Languages and Linguistics, [2]Dept. of Chinese languages and literature, Fudan Univ., Shanghai, China

Abstract: Despite increasing evidence for depicting the neural mechanism underlying the processing of complex sentences using electroencephalography (EEG), the degree to which brain oscillations reflect the primary sentence processing mechanism is still a matter of debate. In the

present study, we conducted an EEG study to explore the comprehension of Chinese relative clauses whose processing difficulty could be manipulated by only changing word order. We aimed to elucidate how the neural activity, indexed by event-related potentials (ERPs) and brain oscillations (i.e., event-related synchronization/desynchronization, ERS/ERD), attuned to sentences with different processing difficulties. Twenty-three right-handed, healthy native speakers of Mandarin Chinese (13 females; mean age = 21.2 years, SD = 1.6) were recruited to read natural relative clauses in the Rapid Serial Visual Presentation (RSVP) paradigm. First, we applied the cluster-based permutation test on ERP data and found waveform differences between subject relative clauses (SRCs) and object relative clauses (ORCs) mainly on the head noun. A more significant N400 effect was elicited in the centro-parietal region by ORCs, which required a thematic-role shift on the head noun and was more demanding in memory retrieval. Meanwhile, a larger P600 effect was elicited in the parietal region when comparing sentences with a non-canonical word order (i.e., SRC) to those with a canonical word order (i.e., ORC), indicating more integration effort in SRCs. Second, to characterize the relationship between ERPs and ERS/ERDs, we extracted the theta- and alpha-band oscillatory power in the specified time period showing significant ERP differences, and implemented the correlation analysis on an individual level. A negative correlation was found between alpha-band power and ERP amplitude in SRCs, whereas the theta-band power and ERP amplitude negatively correlates in ORCs. We propose that an N400/P600 biphasic effect indicates the interaction of memory retrieval and integration stage in the comprehension of natural sentences with various processing difficulties. Meanwhile, the theta-band brain oscillation is attuned to the demand for memory retrieval. In contrast, the alpha-band power is associated with integration effort during the comprehension of more difficult sentences with a non-canonical word order. Therefore, this study not only provides clear evidence in support of the relation between ERP amplitude and event-related power changes during sentence processing, but also sheds light on the interpretation of theta and alpha oscillations during the comprehension of natural sentences.

Disclosures: **K. Xu:** None. **C. Ma:** None.

Poster

241. Speech, Language, and the Brain

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 241.02

Topic: H.11. Language

Support: UCONN SPARK Fund

Title: A dynamic model of neural oscillation can synchronize with natural speech

Authors: I. E. ROMAN[1], E. RABINOVITCH[2], E. ZION GOLUMBIC[2], *E. W. LARGE[3]; [1]Music and Performing Arts Professions, New York Univ., New York, NY; [2]The Gonda Ctr. for Brain Res., Bar Ilan Univ., Ramat Gan, Israel; [3]Psychological Sci. and Physics, Univ. of Connecticut, Storrs, CT

Abstract: Speech is a pseudo-periodic signal with an envelope frequency that dynamically fluctuates around 5Hz. One influential hypothesis proposed in recent years is that intrinsic neural oscillations entrain to the speech rhythm to track and predict speech timing. However, given that natural speech is not strictly periodic, but contains irregular pauses and continuous changes in speech-rate, the question of whether this type of stimulus can be effectively tracked or predicted by neural oscillations has been highly debated. Here we present a simple and parsimonious computational model of neural oscillation that is able to dynamically and continuously synchronize with the speech envelope. Our work is an adaptation of a previously proposed model that captures rhythmic complexities in music (Roman, Roman and Large 2020), extended to deal with stimuli that are not strictly periodic. The model has a natural frequency of oscillation, which it dynamically adapts to match stimulus frequency using Hebbian learning. Additionally, an elastic force pulls the system back towards its natural frequency in the absence of a stimulus. Using automatic differentiation in tensorflow and gradient descent to optimize parameters, the model was trained to maximize the correlation between its activity and the speech onsets in a corpus of spoken utterances. The model was validated on an independent set of stimuli not included in the training data. First, using phase coupling only, performance reached a mean predictive power of $r = 0.33$ ($0.24 < r < 0.42$). Next, when frequency was also allowed to adapt dynamically, the model achieved a mean predictive power of $r = 0.40$ ($0.29 < r < 0.50$). For comparison we ran an ablation study in which the oscillator model was decoupled from the stimulus (i.e., neither phase coupling nor frequency adaptation). In the ablation study we observed chance-level predictive power of $r = -0.001$. These results demonstrate the theoretical plausibility that neural oscillations synchronize to continuous speech, exploiting the principles of neural resonance and Hebbian learning. This model paves the way for future research to empirically test the mechanistic hypothesis that speech processing is mediated by entrainment of neural oscillations.

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Poster

241. Speech, Language, and the Brain

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Program #/Poster #: 241.03

Topic: H.11. Language

Support: NIH Grant R01NS065395
NIH Grant U01NS113339

Title: Intelligibility of audiovisual speech drives multivoxel response patterns in human superior temporal cortex for words and sentences

Authors: *Y. ZHANG[1], J. F. MAGNOTTI[1], J. RENNIG[2], M. S. BEAUCHAMP[1];
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[2]Div. of Neuropsychology, Univ. of Tübingen, Tübingen, Germany

Abstract: Regions of the human posterior superior temporal gyrus and sulcus (pSTG/S) respond to the visual mouth movements that constitute visual speech and the auditory vocalizations that constitute auditory speech. Neural responses in pSTG/S may underlie the perceptual benefit of visual speech for the comprehension of noisy auditory speech. We examined this possibility through the lens of multivoxel pattern responses in pSTG/S. BOLD fMRI data was collected with from 37 participants. Stimuli consisted of sentences or single words presented in five formats: clear auditory speech paired with a video of a talking face (AcV); noisy auditory speech with a face video (AnV); clear auditory-only (Ac); noisy auditory-only (An); and visual-only (V). Following the presentation of each item, participants rated intelligibility with a button press. Noisy speech was often rated as intelligible, but only if it was paired with a face video (mean of 76% with face video, 45% without). For these conditions, the fMRI data was *post hoc* sorted into intelligible and unintelligible trials. The pairwise correlations were averaged across hemispheres. The patterns evoked in pSTG/S by physically-similar noisy audiovisual speech differed, depending on intelligibility. The response pattern to intelligible AnV speech was more similar to that evoked AcV speech (mean $r = 0.38$) while the response pattern to unintelligible AnV speech was less similar to that of AcV speech (mean $r = -0.09$). The cross-correlations were Fisher z-transformed and entered into a linear mixed-effects model. There were main effects of intelligibility ($p = 10^{-15}$) and stimulus type, with a stronger intelligibility effect for words than sentences ($p = 10^{-6}$), without a significant interaction. To visualize the pairwise correlations, multidimensional scaling (MDS) was applied to the average correlation matrix for sentences and words. The MDS for sentences and words were qualitatively similar. Plotting the pairwise correlations for correlation ranks across words and sentences against each other showed a significant positive correlation of correlation ranks, $r = 0.68$; $p = .0008$.Seeing the face of the talker significantly improves the perception of noisy speech. Across two independent experiments using single word or sentences, we found that noisy but intelligible audiovisual speech evoked brain activation patterns in pSTG/S similar to those of clear audiovisual speech. The successful integration of visual and auditory speech produces a characteristic neural signature in pSTG/S, highlighting the importance of this region in generating the perceptual benefit of visual speech.

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Poster

241. Speech, Language, and the Brain

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Program #/Poster #: 241.04

Topic: H.11. Language

Support: NIH-NIDCD Grant 5R01DC014279

Title: End-to-end automatic speech recognition reveals the hierarchical encoding of language in the auditory pathway

Authors: *M. KESHISHIAN[1], S. THOMAS[2], B. KINGSBURY[2], S. AKKOL[3], S. BICKEL[3], A. D. MEHTA[3], N. MESGARANI[1];
[1]Electrical Engin., Columbia Univ., New York, NY; [2]IBM Res. AI, Yorktown Heights, NY; [3]Feinstein Inst. for Med. Res., Manhasset, NY

Abstract: Constructing computational models of spoken language processing in the human auditory system is hindered by the scarcity of neural data with the spatiotemporal resolution necessary for training models with sufficient complexity that can accurately explain the neural responses to novel stimuli. A common approach to handle such limitation is to train an artificial deep neural network on speech perception tasks using large amounts of data and learn insights about the brain processes by comparing the model and actual neural representations. There is increasing interest in deep language models trained to predict the next word in a sequence, such as GPT2. A crucial difference between these models and the brain is that the input to the auditory system is a highly variable sound which such models ignore by using the transcript. Because of this unrealistic assumption, the similarity between the transformation of sound to meaning in biological and computational neural networks remains underspecified.
Here, we chose an RNN-Transducer as our computational model of speech perception which consists of 6 LSTM layers that process sound into meaning, and 1 feedback LSTM layer that uses the model's previous predictions to influence the next decisions. The outputs of these two branches are then combined to form the final output of the model. The model was trained on datasets of transcribed telephone conversations with state-of-the-art accuracy.
Our analysis consists of two parts. We first map the RNN-T layer activations to the neural activity at electrode locations. The intracranial (ECoG and sEEG) neural data for this analysis is recorded from 15 patients undergoing epilepsy surgery who listened to 30 minutes of speech. Our analysis was focused on the auditory cortex, including the Heschl's gyrus, planum temporale, and the superior temporal gyrus. By comparing the predictability of the neural activation at each site from different layers of the network, we grouped neural recording sites by their best predictive layer of the network and observed better predictability of deeper layers of the model for downstream areas in the auditory pathway.
To shed light on the mechanisms behind this improved prediction accuracy, we determined the degree of linguistic feature encoding in RNN-T layers from subphonetic to semantic levels. This analysis revealed a hierarchy of language encoding in the model such that earlier layers are best at predicting phoneme-level information and later ones at word-level information. Together, these two levels of analysis show a progressive encoding of linguistic information across different layers of the network as well as different regions of the auditory cortex.

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Poster

241. Speech, Language, and the Brain

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Program #/Poster #: 241.05

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Support: WF Einzelprojekt P31230

Title: Speech intelligibility changes the temporal evolution of neural speech tracking

Authors: *Y.-P. CHEN[1], F. SCHMIDT[1], A. KEITEL[2], S. RÖSCH[3], A. HAUSWALD[1], N. WEISZ[1];
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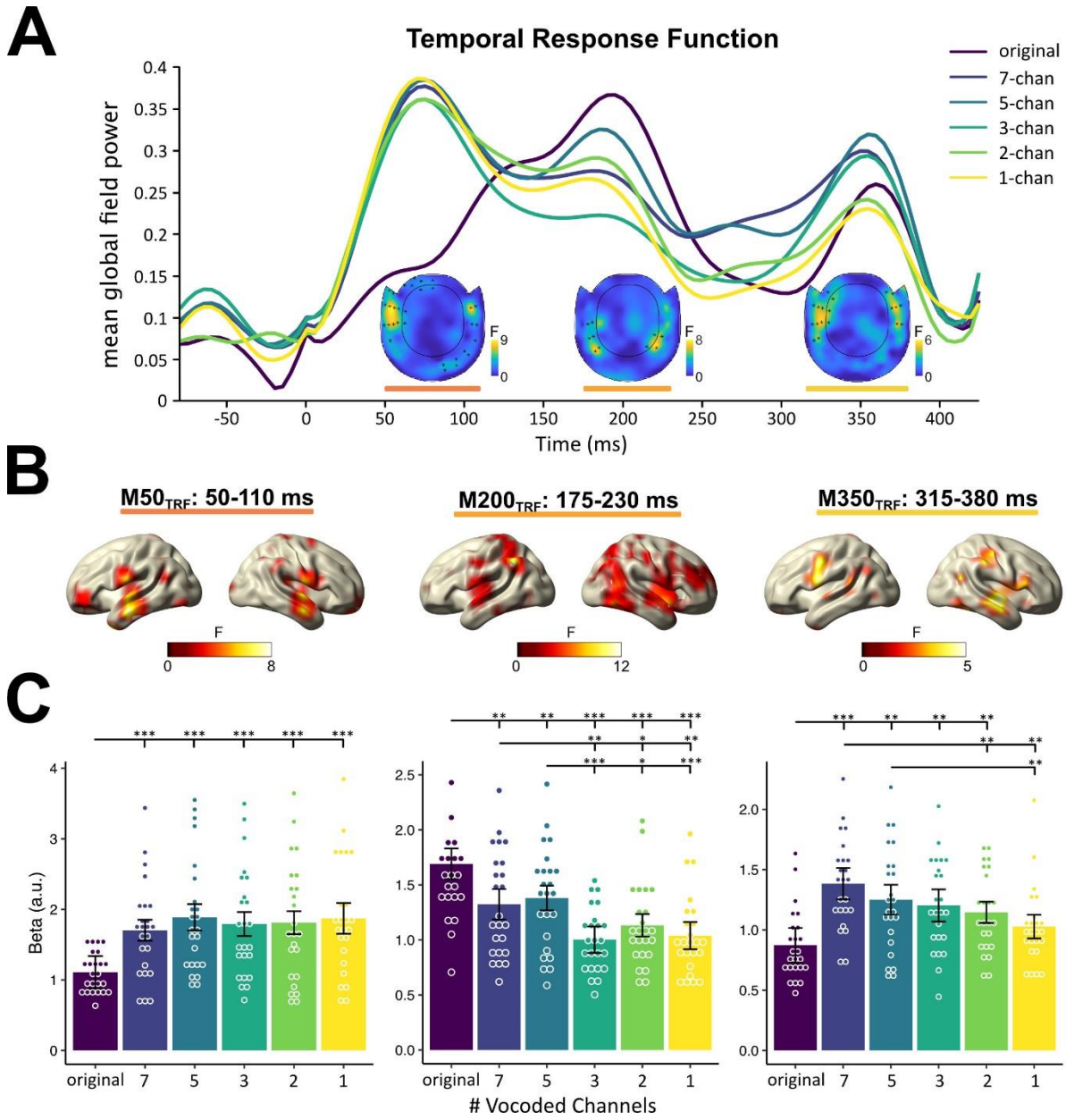
Abstract: Listening to speech with poor signal quality is challenging. Neural tracking of degraded speech has been used to advance the understanding of how brain processes and speech intelligibility are interrelated, however the temporal dynamics of neural speech tracking are not clear. In the present MEG study, we exploited temporal response functions (TRFs) and generated spectral-degraded speech to depict the temporal evolution of intelligibility modulation on neural speech tracking. In addition, we inter-related other facets of neural speech tracking (e.g., speech envelope reconstruction, speech-brain coherence, and components of broadband coherence spectra) to endorse our findings in TRFs. Our TRF analysis yielded marked temporally differential effects of vocoding: reduced intelligibility went along with large increases of early peak responses (~50-110 ms, $M50_{TRF}$), but strongly reduced responses ~175-230 ms ($M200_{TRF}$). For the late responses ~315-380 ms ($M350_{TRF}$), the maximum response occurred for degraded speech that was still comprehensible then declined with reduced intelligibility. Furthermore, we related the TRF components to our other neural tracking measures and found that $M50_{TRF}$ and $M200_{TRF}$ play a differential role in the shifting center frequency of the coherence spectra. Overall, our study highlights the importance of time-resolved computation and parametrization of coherence spectra on neural speech tracking and provides a better understanding of degraded speech processing.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Temporal Response Function
original
7-chan
5-chan
3-chan
2-chan
1-chan
mean global field power
Time (ms)
B
M50TRF: 50-110 ms
M200TRF: 175-230 ms
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C
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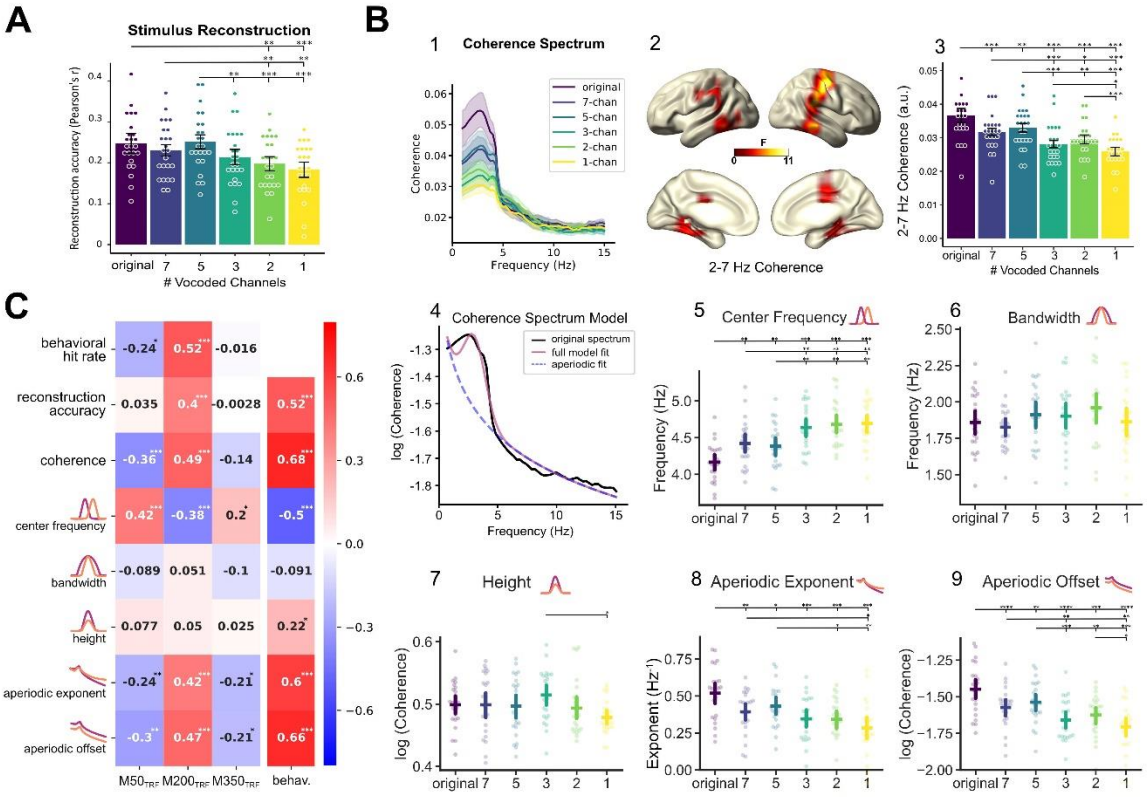
Vocoded Channels



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Poster

241. Speech, Language, and the Brain

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Program #/Poster #: 241.06

Topic: H.11. Language

Support: NYUAD Research Institute Grant G1001

Title: Bilateral temporal involvement in predictive morphological segmentation and processing during spoken word comprehension: MEG evidence from Arabic

Authors: *S. MATAR[1], A. MARANTZ[1,2];
[1]New York Univ., New York, NY; [2]NYU Abu Dhabi Res. Inst., Abu Dhabi, United Arab Emirates

Abstract: In speech comprehension the brain must segment a continuous input stream into units such as words or single sounds. But it is unclear whether and how the brain also places within word boundaries between the smallest meaningful units in language, called morphemes (e.g., ‘bake’ and ‘-ing’ in ‘baking’). We test three nested models: 1. Naïve model: has acoustic, word- and sound-level predictors, but no morpheme information; 2. Passive model: sensitive also to morpheme onset; 3. Predictive model: sensitive also to predictive morpheme information and segmentation. 27 participants listened to single words in Arabic while we recorded brain activity using magnetoencephalography (MEG). Words had a verb stem and a direct object pronoun (e.g., qayyama-ni=‘(He) evaluated-me’; hyphen=morpheme boundary). Stems were long (all from the Arabic template _a__a_a) or short (a shorter template with the same onset: _a__a). In Arabic, root consonants are inserted into templates to produce verbs (e.g., root {j,r,b} in the long template produces jarraba=‘(he) tested’; {j,r} in the short one produces jarra=‘(he) dragged’). We had two conditions. Ambiguous stems were either long or short: long stems had corresponding short stems with the same onset (jarra vs jarraba), producing temporary ambiguity during listening. Unambiguous stems were all long (qayyama), with no derivable shorter stems (qayya is not a verb/stem). All stems across conditions became uniquely identifiable at the same point (offset of _a__a). Using source localization, we estimated cortical activity in bilateral temporal and inferior frontal cortex. We used a Temporal Response Function framework to estimate responses to models’ predictors, measuring models’ power to explain cortical activity. The passive model explained significantly more activity than the naïve model in bilateral temporal and inferior frontal ROIs ($p=0.0001$). The predictive model explained more activity than the passive model in bilateral temporal cortex ($p<0.0001$). We also compare evoked responses across conditions by averaging long-stem words time-locked to stem uniqueness points (t=0). We found an early effect, 200-50ms before uniqueness point, in bilateral superior temporal cortex, with more negativity for ambiguous vs. unambiguous stems ($p=0.002$). This could index ‘eager’ predictive processing of a potential morpheme boundary in ambiguous stems. A late effect, 50-125ms after uniqueness point (left temporal:$p=0.006$; right temporal:$p=0.02$), shows the opposite pattern, possibly reflecting boundary revision in ambiguous stems. Our results support predictive and proactive morpheme-level segmentation in speech processing.

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Poster

241. Speech, Language, and the Brain

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Program #/Poster #: 241.07

Topic: H.11. Language

Support: MEXT KAKENHI Grant 22H02945
MEXT KAKENHI Grant 22H03913
JSPS KAKENHI Grant 21K12610

Title: Phase advance of theta-alpha oscillation in anterior temporal lobe during semantic processing

Authors: *N. SATO[1], R. MATSUMOTO[2], A. SHIMOTAKE[3,4], M. OTANI[3], T. KIKUCHI[5], T. KUNIEDA[6], R. TAKAHASHI[3], A. IKEDA[4];
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Abstract: Semantic processing is maintained by a neural network of multiple cortices, of which the anterior temporal lobe (ATL) has been shown to play a dominant role in multi-modal semantic integration. Synchronization of electrocorticogram (ECoG) oscillations has produced important clues for the understanding of the cortical interactions, while synchronized oscillations with lags gradually distributed over the cortical surface, called “cortical traveling waves”, has been also reported. According to the communication-through-coherence theory, such lagged oscillations can regulate information transfer between connected regions in association with their conduction delay. Moreover, our connectome-based neural network simulation predicted that theta-alpha oscillation effectively contributes to asymmetric coupling of cortical regions and these lag patterns are organized by relative activations of regions comparing in multiple spatial scales. Such lag-structure is also expected to be available for the evaluation of cortical interaction during semantic processing. In the present study, we evaluated spatio-temporal patterns of lags in theta-alpha-band ECoG oscillations during a picture naming task. Results are as follows: (1) Consistent with previous reports, topographical patterns of lags depended on the individual. However, our group analysis revealed that the ATL showed earlier-phase oscillations, and the inferior frontal gyrus (IFG) and posterior middle temporal gyrus (pMTG) showed later-phase oscillation, during overall task-period. (2) Topographic patterns of lags were not strongly modulated by task-procedure along the time, while ATL oscillation-phases were earlier, and pMTG oscillation-phases were later, during after-articulation than before-articulation. (3) Trial-by-trial lags were not significantly correlated to articulation-delays, except for later lags in the temporal pole. (4) Additionally, stabilities of lag-structures against variety of cognitive tasks (e.g., motor-response, face recognition) were evaluated using an open ECoG database (Miller, 2019; Nat Hum Behav) and found that lag-structure during a picture naming task was similar to those during a motor response task, but dissimilar to those during a face recognition task. These results suggested that lag-structures become similar in response to similar task-demands. In summary, theta-alpha-band phase advances in the ATL against other regions including the IFG and pMTG characterize asymmetric cortical interaction during semantic processing, which is thought to reflect both preparation and implementation of semantic processing dominated by the ATL.

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Poster

241. Speech, Language, and the Brain

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 241.08

Topic: H.11. Language

Support: Gravitation Grant 024.001.006 of the Language in Interaction Consortium from Netherlands Organisation for Scientific Research

Title: Alpha and beta oscillations shape naturalistic language comprehension based on low-level operations

Authors: *I. ZIOGA[1], H. WEISSBART[1], A. G. LEWIS[1], S. HAEGENS[1,2], A. E. MARTIN[1,3]; [1]Radboud Univ., Donders Ctr. for Cognitive Neuroimaging, Nijmegen, Netherlands; [2]Dept. of Psychiatry, Columbia Univ., New York, NY; [3]Max Planck Inst. for Psycholinguistics, Nijmegen, Netherlands

Abstract: Out of the many neural mechanisms that exist, brain oscillations are prevalent in all species and are involved in numerous perceptual operations. In particular, alpha oscillations are related to facilitated processing through the inhibition of task-irrelevant networks, while beta oscillations have been linked to the reactivation of content representations. Can the proposed functional role of alpha and beta oscillations be generalized from low-level operations to higher-level cognitive processes, and especially naturalistic speech processing? Twenty-two Dutch native speakers listened to stories in both spoken Dutch and French while magnetoencephalography (MEG) was recorded. We used dependency parsing to identify three dependency states at each word, as the number of 1) newly opened dependencies, 2) dependencies that remained open, and 3) resolved dependencies. We then constructed linear forward models (Temporal Response Functions, TRFs) to predict alpha and beta power from the dependency features. Reconstruction accuracy was measured as the correlation between the actual brain signal and the reconstructed signal, following a leave-one-out cross-validation approach. Results showed that dependency features successfully predict alpha and beta power in language-related regions beyond low-level linguistic features. Left temporal, basic language regions are involved in language comprehension in the alpha band, while parietal, higher-order language regions, and motor regions are involved in the beta band. Critically, alpha and beta band dynamics seem to subserve comprehension by contributing to low-level operations, potentially associated with cognitive load and concept reactivation, during meaning composition. Overall, this study sheds light on the role of alpha and beta oscillations during naturalistic language processing, providing evidence for the generalizability of these dynamics from perceptual to complex linguistic processes.

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Poster

241. Speech, Language, and the Brain

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Program #/Poster #: 241.09

Topic: H.11. Language

Support: Whitehall Foundation
Alfred P. Sloan Foundation
Burroughs Wellcome Fund

Title: Semantic decoding of continuous language from non-invasive brain recordings

Authors: *J. TANG[1], A. LEBEL[2], S. JAIN[1], A. G. HUTH[1];
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Abstract: A brain-computer interface that decodes continuous language from non-invasive recordings would have many scientific and practical applications. Currently, however, decoders that reconstruct continuous language use invasive recordings from surgically implanted electrodes, while decoders that use non-invasive recordings can only identify stimuli from among a small set of letters, words, or phrases.
We introduce a non-invasive decoder that reconstructs continuous natural language from cortical representations of semantic meaning recorded using functional magnetic resonance imaging (fMRI). To overcome the low temporal resolution of fMRI, we used a Bayesian approach that combines a neural network language model and a voxel-wise encoding model. The language model generates linguistically coherent word sequences, and the encoding model predicts how the brain would respond to each sequence. Our decoder then identifies the most likely stimulus by comparing the predicted brain responses to the recorded brain responses.
Given novel brain recordings, our decoder generates intelligible word sequences that recover the meaning of perceived speech, imagined speech, and even silent videos, demonstrating that a single language decoder can be applied to a range of semantic tasks. To study how language is represented across the brain, we tested the decoder on different cortical networks, and found that natural language can be separately decoded from multiple cortical networks in each hemisphere. To test whether decoder predictions are modulated by attention, we instructed subjects to attend to a different speaker for each repeat of a multi-speaker stimulus, and found that the decoder selectively reconstructs the attended stimulus. Finally, as brain-computer interfaces should respect mental privacy, we tested whether successful decoding requires subject cooperation. We found that decoders trained on cross-subject data performed substantially worse than decoders trained on within-subject data, suggesting that subject cooperation is necessary to train the decoder. Further, we found that subjects could consciously resist decoding of perceived language by performing a different cognitive task, suggesting that subject cooperation is also necessary to apply the decoder.
Our results demonstrate that continuous language can be decoded from non-invasive brain recordings, enabling future multipurpose brain-computer interfaces.

Disclosures: **J. Tang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Board of Regents, The University of Texas System. **A. LeBel:** None. **S. Jain:** None. **A.G. Huth:** E. Ownership

Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Board of Regents, The University of Texas System.

Poster

241. Speech, Language, and the Brain

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Program #/Poster #: 241.10

Topic: H.11. Language

Support: JSPS KAKENHI JP20K07951

Title: Concordance of lateralization index for hemispheric asymmetry to find a reliable language task

Authors: *K. MATSUO[1], N. YASUI-FURUKORI[2], K. SHIMODA[2], Y. KAJI[3], K. AKIYAMA[4];
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Abstract: A problem in measuring hemispheric asymmetry using fMRI lies in the uncertain reliability of the computed index in regard to the "true" asymmetry degree. We developed a method to evaluate lateralization index (LI) using reproducibility, or concordance, of index value rankings. The underlying idea was that, although various language tasks activate various cortical regions related to the task procedures, an individual who showed a strong leftward asymmetry in a task would also show a leftward asymmetry in another task, at least to an extent. Kendall's coefficient of concordance (W) was used as the statistical measure. We previously used this procedure to evaluate multiple LI computation algorithms [1]. Among six algorithms, AveLI [2] yielded the highest W across four language tasks, which indicated that the individuals' rankings of LI values were similar across the four tasks. Now, we used this procedure to evaluate language tasks, considering that a reliable language task would reproduce similar LI values across various LI algorithms. We used the same datasets with those for evaluating LI types above [1], but the direction of the analysis using Kendall's W was the opposite. We added another new LI algorithm and these seven LI types were computed for 38 people who performed the four language tasks. Four regions-of-interest (ROIs) [3] and the sum of these ROIs were used to compute the LIs. We conducted a one-way ANOVA for W (after angular transformation) and detected a tendency of difference among tasks ($p=0.10$). The post-hoc paired test did not detect significant differences. The means of W (standard deviation) were as follows: verb generation 0.877 (0.058), narration listening with word detection 0.863 (0.039), lexical decision with visual presentation 0.813 (0.023), and lexical decision with aural presentation 0.812 (0.064). Detailed observation indicated that the concordance of LI rankings depended on ROIs. For around Brodmann's area 44, W of verb generation was 0.946 and that of narration listening was 0.866, whereas for the peri-Sylvian language area, the former was 0.789 and the latter was 0.882. Thus,

to examine asymmetry of Brodmann's area 44, verb generation task yielded similar LI rankings regardless of LI computation types and thus being recommended, whereas to examine the peri-Sylvian area, narration listening would be recommended, because they would provide stable index values regardless of LI algorithms. References. [1] Matsuo et al., 2021, J. Neurolinguistics 57, 100943. [2] Matsuo et al., 2012, J. Neurosci Methods 205, 119-129. [3] Glasser et al., 2016, Nature 536, 171-178.

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Poster

241. Speech, Language, and the Brain

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Program #/Poster #: 241.11

Topic: H.11. Language

Support: Vontobel Foundation (Zurich)
SNSF 100014_182135/1

Title: Investigating the role of the human limbic system during whispered affective speech processing using intracranial recordings

Authors: *M. BOBIN[1,2], T. FEDELE[3], J. SARNTHEIN[4], S. FRÜHHOLZ[1,2,5];
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Abstract: The human brain excels at perceptual adaptation. For instance, when faced with reduced acoustic information, functional auditory networks actively seek to reconstruct degraded acoustic signals into a meaningful perception. Perceiving whispered speech precisely illustrates this process. Despite signal degradation, listeners are still able to identify various paralinguistic features of speech including those indicating a speaker's affective state. In addition to the auditory cortex, the medial temporal limbic system (MTL) is part of the functional network that classifies acoustic components of affective voices and matches them to stored emotional pattern templates. However, the specific role of individual subcomponents of the MTL during this process is still unclear. The present research aims to understand the functional part of the amygdala (AMY) and hippocampus (HPC) during the perception of degraded affective speech. We hypothesize that compared to normal affective voices, the processing of whispered voices relies more on retrieval of information from long-term memory (e.g. previous experience, affective templates), mediated by the HPC as a functional support to the broader MTL. We recorded single- and multi-unit (MUA) and local field potentials (LFPs) in the limbic system of 14 participants (seven females, mean age 38 ± 13, range [19, 57]) while they performed an

emotion identification task on records of normal and whispered affective voices. Spiking analyses show increased neuronal firing rate in the AMY at 900ms post-stimuli and posterior HPC at 1200ms post-stimuli, lateralized to the left hemisphere, for whispers vs. normal voices. Right AMY displays an earlier, 500ms post-stimuli increase in spiking activity for emotional vs. neutral voices, but also shows a temporal delay for peak activity related to whispers in comparison to normal voices. LFP analyses reveal that whispered vs. normal voices generate event-related potentials shifted in amplitude for the anterior HPC and delayed in time for the AMY, bilaterally. Our results provide evidence for the role of the AMY and the HPC in the perception of emotions from whispered voices, a context in which processing acoustic information is more challenging. Neural dynamics recorded at the local (i.e. MUA) and global (i.e. LFPs) scale with high temporal and anatomical resolution suggest local preference of hippocampal activity for whispers perception, while the amygdala is reactive to both emotional and whispered voices, with slower temporal dynamics for the latter.

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Poster

241. Speech, Language, and the Brain

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Program #/Poster #: 241.12

Topic: H.11. Language

Title: Probabilistic mapping of human language and naming networks derived from direct cortical stimulation

Authors: *O. WOOLNOUGH, N. TANDON;
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Abstract: The prevailing gold-standard for causal, pre-surgical mapping of language cortex is the artificial depolarization of cortical regions by passing electrical currents into the brain during a language task - cortical stimulation mapping (CSM). Integration of CSM data from large populations of patients into a common spatial reference frame may allow for the generation of spatial probability maps of essential language sites - allowing for an *a priori* determination of the risk of resection for a particular region in a given patient. Data were collected from 224 language mapping sessions (123 intraoperative, 101 extraoperative) performed with 181 adult patients, over a fifteen-year period. Sites were considered positive if they resulted in speech arrest, articulatory difficulty, problems with comprehension, or anomia. We co-registered 8,712 tested stimulation sites to create population-level, probabilistic, surface-based maps of stimulation-induced language disruption. Language testing was performed using auditory naming (2,043 positive sites), auditory repetition (1,234 positive sites) and visual naming (811 positive sites). Additionally, we mapped sites leading to disruption of motor control (535 sites) and vision (400 sites). We isolated five main functional clusters resulting in causal disruption of language or naming function: 1) Superior temporal gyrus, 2) Posterior middle temporal gyrus, 3) Inferior

frontal gyrus, 4) Posterior middle frontal gyrus (pMFG), and 5) Mid-fusiform cortex (mFus). Auditory naming was substantially disrupted during stimulation across all five functional clusters (35-51% disruption probability), and all clusters resulted in preferential disruption of auditory naming over auditory repetition (OR = 1.3 - 12.2). Stimulation of mFus equiprobably disrupted both auditory and visual naming function (OR = 1.1 (0.90 - 1.5)), however all other regions showed significantly higher odds of auditory naming disruption (OR = 1.9 - 4.1). We observed substantial inter-individual variability in localization, with the probability of disruption peaking at ~50% at any given anatomical location. In conclusion, we have generated a surface-based probabilistic map of stimulation-induced language and naming deficits. This work highlights the importance of two crucial language areas that are often overlooked in pre-surgical mapping - pMFG and mFus. pMFG appears to be involved in higher-order semantics and working memory manipulation, both essential for naming from a definition. mFus acts as a multi-modal lexico-semantic hub, crucial for both auditory and visually cued naming.

Disclosures: O. Woolnough: None. **N. Tandon:** None.

Poster

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Title: Heard or understood? Evaluating neural tracking of higher-level language features to assess speech understanding

Authors: *M. GILLIS, J. VANTHORNHOUT, T. FRANCART;
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Abstract: Over the last years, more attention has been devoted to understanding and characterizing the neural responses associated with speech understanding. The differentiation can be made between hearing the stimulus and understanding the presented speech, whereby the listener can connect to the meaning of the storyline. To investigate the neural response to speech, one can investigate neural tracking, i.e., the phenomenon whereby the brain time-locks to specific aspects of the speech. Although envelope tracking is thought to capture certain aspects of speech understanding, this is not always true (Verschueren et al., 2022). A possible solution can be found by focussing on identifying higher-level language features, derived from the speech's content, which can capture the neural correlates of speech understanding (e.g., Brodbeck et al., 2018; Broderick et al., 2018; Weissbart et al., 2020).
This study evaluated whether neural tracking of these higher-level language features, i.e.,

linguistic tracking, gains more insight into whether the listener understood the presented speech. We investigated the EEG responses of 19 normal-hearing young participants (6 men) who listened to a Dutch story, a Frisian story whereby Frisian was not familiar to the participants, and a word list whereby individual words were understood but the context did not make sense. We hypothesized that the Dutch story would show linguistic tracking as the storyline can be understood, while this would not be the case for the Frisian story and the word list. Preliminary results indicate the Dutch story showed more linguistic neural tracking than the Frisian story, which shows higher linguistic tracking than the word list. The results obtained by analyzing linguistic tracking converged with the subjectively rated speech understanding, i.e., the answer to the question 'how much of the content of the speech did you comprehend?'. The Dutch story was fully intelligible, followed by the Frisian story, rated around 50%, while the speech understanding for the word list was rated around 10%.
Our preliminary results indicate that linguistic tracking can capture the effect of speech understanding. These results open doors toward understanding language disorders and improving their diagnosis and treatment.

Disclosures: **M. Gillis:** None. **J. Vanthornhout:** None. **T. Francart:** None.

Poster

241. Speech, Language, and the Brain

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Program #/Poster #: 241.14

Topic: H.11. Language

Support: R21 DC017563

Title: Examining learning mechanisms involved in auditory motor adaptation

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Abstract: Speech motor learning involves learning new or modifying the current speech movements. Generally, the speech motor system accomplishes learning by reducing errors over successive productions. Theoretical models of speech production suggest that sensory errors are generated when predicted sensory outcomes do not match the incoming sensory (e.g., Guenther, 2016). A large body of literature suggests that motor learning depends on implicit and explicit learning mechanisms (e.g., Krakauer et al., 2019). Implicit learning is a gradual and sub-conscious process that relies on sensory prediction error and results in acquiring the underlying abstract structure of a motor skill. Explicit learning is a conscious and intentional process resulting in acquiring systematic, rule-based information about a motor skill. One approach for examining speech motor learning processes in laboratory settings is to use auditory-motor adaptation paradigms. Previous studies have suggested that auditory-motor adaptation may primarily rely on implicit processes. However, the contributions of explicit learning in auditory-

motor adaptation are not clear. The current study aimed to examine whether explicit learning induced by explicit strategies could complement implicit learning in auditory-motor adaptation. In the present study, we recruited 19 healthy adults. Subjects completed two adaptation tasks in which we altered their formant frequencies. The first adaptation task was a typical auditory-motor adaptation in which no information about the perturbation was given to the subject. In the second adaptation task, we provided subjects with an explicit strategy to compensate for the effect of auditory perturbations. We measured adaptive responses for each subject and compared the extent of the responses between the two tasks to examine how much of the response was from implicit mechanisms and how much was from explicit mechanisms. Our preliminary results showed that subjects were able to use the explicit strategy to overcome the errors induced by the perturbations; however, when subjects switched back to speaking without using strategy, they did not maintain what they learned. In other words, subjects quickly used the strategy to complement implicit learning processes but did not maintain what they acquired through explicit learning processes.

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Poster

241. Speech, Language, and the Brain

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Title: Relationship between “tip-of-the-tongue” word retrieval errors and EEG features

Authors: E. A. RILEY[1], C. SANDBERG[2], D. MC. FARLAND[3], ***D. GUPTA**[3,4];
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Abstract: *Background:* Temporary word retrieval failures that occur when a person cannot recall a specific word, but can recall information about the word (e.g., number of syllables) are known as “tip-of-the-tongue” (TOT) errors. TOT errors are common, especially in older adults. They are thought to involve inaccurate phonological retrieval after lexical selection has occurred, which may be similar to the underlying cause of word retrieval errors that some persons with aphasia (PWA) experience. Previous work has demonstrated that word retrieval in PWA can be predicted using EEG features. The aim of the current study was to investigate the predictive relationship between word retrieval errors and EEG features in healthy adults using a Famous People naming

task.
Method: Eighteen healthy adults (mean age=57.22yrs; 3m/15f) participated in a behavioral screening session and 4 EEG sessions. Participants who advanced beyond screening produced error responses for at least 10% of the items and were able to recognize at least 250 pictures from the set. In EEG sessions, stimuli (pictures recognized in the screening session) were presented in random order in the first 2 sessions and repeated in the next 2 sessions. Stimuli were displayed for a maximum of 5 sec/picture, during which the participant verbally named the person/icon in the picture, recorded by a microphone. 32-channel non-invasive EEG was acquired (Brain Vision, 1000Hz sampling) simultaneously. BCI2000 software was used to present stimuli and synchronize EEG with the spoken response. All trial responses were scored by two researchers independently, on an 8-point rating scale that encoded responses as correct, nil, circumlocutions, verbal fillers, partially correct responses and other related and unrelated errors. Data was collected with informed consent (Penn Uni. IRB).
Analysis & Results: EEG was common average referenced, band pass (0.2-50Hz) filtered, and epoched (-2s to 2.5s, with respect to the stimuli presentation). Data was denoised with Independent Component Analysis and Xdawn. Mean response time was 2s. Based on literature, we focus on the frontal and parietal regions, 1-2s post stimulation (mean response time was 2.3 ± 0.05s). Compared to the correct trials, the incorrect trials had significantly large positivity in the right frontal region, and a significantly large negativity in the left. Parietal regions showed reduced negativity in both left and right, although not significant. Classification (logistic regression, l1 penalty, 10-fold cross-validation) of correct vs incorrect trials, based on the above ERP features, showed a statistically significant ($p<0.01$) mean classification accuracy of 61%.

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Poster

241. Speech, Language, and the Brain

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Topic: H.11. Language

Support: NIH NIDCD R01 DC014589

Title: A shared cortical language network for heteromodal speech production

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Abstract: Lexical access describes the process involved in the mapping between conceptual representations and phonology and is an integral component of speech production. While various brain regions have been hypothesized to support separable language processes, the complete cortical language network and its functional mapping to lexical access remain unclear. We used

electrocorticography to identify cortical brain networks involved in lexical cognition in the context of heteromodal sensory input modalities with convergent design. Data were obtained from epilepsy patients who underwent invasive electrophysiology. Recordings were acquired during three cued naming tasks using pictures (n=120; 16,284 electrodes), auditory descriptions (n=82; 11,215 electrodes), and orthographic descriptions (n=68; 9,282 electrodes). High gamma power (65-115 Hz) was used to measure cortical engagement, and electrode recording zones were defined on the cortical surface. Surface-based mixed-effects multilevel analysis was used to estimate group-level high gamma activity and compared across tasks to identify regions with significant activation. Analyses showed left-lateralized heteromodal activity in the inferior frontal gyrus (IFG), the middle fusiform gyrus (mFus), and the intraparietal sulcus (IPS) following initial sensory processing. Activation of mFus peaked 150 ms before stimulus offset for auditory (15.2%, $p < 10^{-6}$) and orthographic (21.4%, $p < 10^{-6}$) naming and 350 ms after picture onset (39.0%, $p < 10^{-6}$) for visual naming. Activation of IPS peaked just prior to stimulus offset for auditory (38.1%, $p = 7.7*10^{-5}$) and orthographic (27.5%, $p = 0.008$) naming and 450 ms after picture onset (43.0%, $p < 10^{-6}$) for visual naming. Activation of IFG also peaked just prior to stimulus offset for auditory (30.1%, $p < 10^{-6}$) and orthographic (40.1%, $p = 10^{-6}$) naming and 500 ms after picture onset (36.4%, $p < 10^{-6}$) for visual naming. There was also heteromodal activity in the left posterior middle temporal gyrus (pMTG) for auditory and orthographic descriptions but not for pictures with peak activation occurring prior to the convergence of all tasks 250 ms before stimulus offset (auditory: 34.1%, $p < 10^{-6}$; orthographic: 25.5%, $p = 0.0035$). These results reveal that a shared, heteromodal brain network consisting of IFG, mFus, and IPS supports lexical access. Furthermore, our findings also implicate the role of pMTG in phonological access. Altogether, this work further characterizes the functional roles of key brain regions within language networks and provides important insights that are critical to the development of improved treatment methods for speech-related disorders.

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Poster

241. Speech, Language, and the Brain

Location: SDCC Halls B-H

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Program #/Poster #: 241.17

Topic: H.11. Language

Title: The temporal profile of linguistic units in natural speech and its neural correlates

Authors: *C. IAIA[1,2], A. TAVANO[1], M. GRIMALDI[2];
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Abstract: Cortical oscillations are thought to temporally align to low-frequency modulations of the speech envelope, possibly reflecting temporal regularity of low-level speech units such as syllables. However, it is unclear whether such regularity assumption can be projected to higher-

order linguistic information, such as syntactic chunking. To assess the temporal profile of meaningful linguistic information, we have mapped the variability in speech unit duration across four levels (phonemes, syllables, words, and sentences), as well as across four types of syntactic constituents (Noun Phrases, Verb Phrases, Adverbial Phrases, and Clauses). In a behavioral and EEG study, twenty-three subjects (5 male, mean age = 23.3, std ± 3.5) listened to the first chapter of two audiobooks in the Italian language, read by a professional voice actor. Both chapters were segmented into 10 trials each of roughly 50 seconds, for a total of 18 minutes. Units of interest in the acoustic signal of the stimuli were manually annotated at the phoneme, syllable, word, syntactic phrase, and sentence time scales using PRAAT software. Syntactic analysis was performed automatically using Stanza module in Python. Then, the extracted constituents were manually segmented. We then estimated the duration of each unit and compared variance across levels using the coefficient of variation and individual nonlinear fits.As for speech units, significant differences in variance were found between the phonemic/syllabic levels and the word/sentence levels with larger variance for the latter. Specifically, phonemic and syllabic highly correlated with each other, but no other significant correlation was found for the remaining speech levels. The larger variance for word/sentence levels was due to a bimodal distribution of duration estimates, relative to the unimodal profile of phonemic/syllabic levels. As for syntactic constituents, the largest variance was found for the duration of NPs, which have a bimodal distribution, while the remaining syntactic units were unimodally distributed. Pairwise phase consistency in neural data reflected variability in stimulus duration, congruently displaying one or two peaks of activity. Hence, we conclude that the human brain tracks the temporal profile of speech units, displaying a polymodal capacity.

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Poster

241. Speech, Language, and the Brain

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Title: Visual cortex responses to speech measured with iEEG: modulation by sensory modality and stimulus content

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Abstract: Humans communicate face-to-face using auditory information from the talker's voice and visual information from the talker's face. We used iEEG to record responses from the visual cortex of epileptic patients as they were presented with single words that differed in their sensory

modality (V, visual-only; AV, audiovisual; A, auditory-only). Four words were presented: voice-leading (VL) words ("known", "meant") that the auditory speech preceded the visual speech; mouth-leading (ML) words ("drive", "last") that the visual speech preceded the auditory speech. Brain responses were recorded at 2000 Hz, with broadband high-frequency activity (BHA) of 70 Hz - 150 Hz to measure the temporal dynamics of speech responses. All preprocessing was conducted using RAVE. Together 35 electrodes in occipital lobe from 4 patients were examined.All electrodes responded to both V and AV speech, as they contained a visual stimulus consisting of a moving face; there were minimal responses or slight deactivations in response to A speech. We observed two significant effects of contrasts: sensory modality (V vs. AV) ($p <$ .001) and stimulus content (VL vs. VL) ($p <$ .001). The response to AV was less than V speech. The responses to V and AV speech diverged shortly after speech auditory onset (~300 ms after clip onset). Averaging across a time window of 600 ms - 1000 ms, the mean response was 1.3 (z-score from fixation baseline) for V and 1.0 for AV speech.In addition, the response was greater for ML than VL words. This effect began shortly after the face began moving (~100 ms after clip onset). Averaging across a time window of 200 ms - 600 ms, the mean response was 1.5 for ML and 1.4 for VL speech.To test this double-dissociation between the presenting timing and stimulus content vs. sensory modality, we constructed an LME with fixed factors of time window, sensory modality, and stimulus content. The model showed a significant interaction between sensory modality and time window [X2(1) = 9.6, p = .002], driven by a greater (V - AV) difference for the late (+0.29, $p <$ .001 Bonferroni-corrected for 4 comparisons) than early time window (+0.1, p = .08). The model also showed a significant interaction between stimulus content and time window [X2(1) = 10.7, p = .001]. The response to ML words was greater than the response to VL words in the early window (+0.14, p = .001), but difference was reversed and not significant in the late window (-0.07; p = .09). There was no three-way interaction.Our results showed that visual cortex response is modulated by both the speech modality and the relative timing of auditory and visual speech.

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Poster

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Title: Neurocomputational transformations of sequences into mental structures

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Abstract: Understanding how the brain represents and binds complex information distributed over time is a challenging problem, requiring computationally and neurobiologically informed approaches to solve. Human language is a salient example, whereby syntactic knowledge facilitates "movement" and transformation of sequential information into hierarchical mental structures. By some accounts a fundamental A/B "Merge" function is responsible for creating these mental structures, and may be implemented in other cognitive domains. We previously proposed a computational model, VS-BIND, which applies vector-symbolic operations to population-level codes to bind A/B pairs of items segregated in time, forming a composite representation. VS-BIND also specifies mechanistic roles for prefrontal areas 44/45 and motor/premotor cortex during interactions with temporal cortex (Calmus et al., Phil. Trans. Roy. Soc. B, 2019). Here, we test this model using human intracranial recordings obtained during an Artificial Grammar Learning (AGL) task, in neurosurgery patients being monitored for epilepsy treatment. During the task, 12 patients listened to auditory speech sequences containing dependencies between adjacent and non-adjacent nonsense words, before being tested on their ability to distinguish novel "grammatical" and "ungrammatical" sequences. We analyzed intracranial data using traditional methods, demonstrating engagement of the fronto-temporal network. Additionally, we applied a battery of established and novel multivariate analyses to reveal the representational geometry of regional speech encodings and the causal representational flow between frontal and temporal regions. The results show that certain prefrontal areas integrate relational information, including the ordinal position of items in a sequence, in concordance with the mechanistic and site-specific predictions of the VS-BIND model. Furthermore, we observed net causal representational flow consistent with feed-forward and feedback predictive signaling, suggesting that expectation-driven predictions are fed back to primary auditory cortex from prefrontal areas including 44/45. We are testing tenets of VS-BIND using neural patterns directly derived from the neurophysiological signals recorded in the human patients, and comparing it to other models. The results indicate a critical role for fronto-temporal areas in transforming the sensory world into mental structures, and they provide initial evidence for fundamental A/B binding mechanisms associated with the transformation of sequential information into mental structures.

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Poster

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Title: Gender and race differences in language related functional connectivity brain networks in healthy young adults.

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Abstract: Gender and age-related differences in functional connectivity of brain networks have been reported in task- based fMRI. But, it is unclear whether race/ethnicity may interact with gender and age in determining functional connectivity. Understanding these effects is important as we develop computational algorithms to predict and improve individualized patient clinical outcomes. Using the Human Connectome Project (HCP) Young Adult dataset we conducted a surface-based analyses of static functional connectivity for language comprehension networks of young healthy adults. Language tasks included vocabulary comprehension tasks with math as a control. N=140 (avg age 27.5 + 3.2, range 22-37 years). Subjects were matched for level of education: 15.6 years and handedness (Edinburgh Handedness Inventory). 50% females and 50% males. Racial distribution was 67% white, 18% Asian, 4% Black, and 15% Hispanic. Subjects demonstrated no gender or age differences by race, though Hispanics were slightly younger (26.0 ±3.2, p=0.046). Image acquisition: 3T Siemens Connectome Skyra MRI; FOV:208x 180; Slice thickness: 2.0mm; TR: 720ms. We used structural T1 and T2 weighted sequences for brain segmentation and preprocessed the images using AFNI for image distortion and motion-correction. We overlaid Desikan-Killiany parcellation on the pre-processed functional images to extract the corresponding time-series for each region and calculated activated network correlations using Spearman rank correlations with Bonferroni correction for multiple comparisons. Only common network connections were included in our analyses. We defined "common networks" as those activated in at least 85% of subjects. We found sex differences in 24% of activated network connections (range $p<0.001$ to 0.047) and a race effect in 34% of the common connections (range $p<0.001$ to 0.049). The race effect was prevalent despite matched educational levels. It was not predictive of task accuracy. Behavioral performances were comparable across racial and gender groups. Our findings confirm reported gender differences in static functional network connectivity but suggests that race/ethnicity may also serve as a contributing factor. A caveat is that the distribution of the racial groups is uneven and these findings may only be representative of this cohort of subjects. Larger, more diversified cohorts will be needed for future research. Although race is not considered a biological construct, these findings suggest that gender, age, and possibly race may require some weighting in computational models that predict individualized functional network plasticity and/or rehabilitation follow CNS injury.

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Poster

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Title: Low and high frequency spectral signatures of language processing in human ECoG

Authors: *N. M. CHAPOCHNIKOV[1,2], L. YU[4], W. K. DOYLE[2,3], O. DEVINSKY[1,2], A. FLINKER[1,2,3,4];
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[4]Tandon Sch. of Engin., NYU, New York, NY

Abstract: Language comprehension and production are unique human abilities that require the interplay of many brain regions. Neurosurgical intracranial recordings in humans provide an ideal platform to study speech, with a combined spatial and temporal resolution and virtually no motor artifacts during articulation. Although broad-band high gamma activity (HGB, >70Hz) has been well studied over the past two decades, neural activity at lower frequencies leading to and during speech production is poorly understood.To address this gap, here, we characterize the power spectral densities (PSD) of >3000 electrodes in a large cohort of 23 ECoG subjects undergoing 5 word-retrieval tasks (50-100 words each): picture naming (naming an object), word reading, auditory word repetition, auditory naming (production based on definition), sentence completion (production to complete the end of a sentence). Per electrode, we calculated 20 (5 tasks x 4 segments) PSDs: baseline (500ms pre-stimulus), stimulus presentation (first 500ms), pre-articulation (500ms prior to speech), and articulation (first 500ms of articulation). We characterized the periodic activity by parametrically fitting PSDs during baseline in the 4-40Hz range and found the frequency peak with maximal power. We found a systematic posterior-anterior gradient of peak frequency on the brain. Temporal cortex was alpha (7-12Hz)-dominant; parietal and occipital cortex: alpha and beta (12-30Hz) dominant; frontal cortex: beta dominant; in particular, motor cortex (precentral gyrus) contained a significant proportion of gamma (30-60Hz) dominant electrodes.Next, to understand the functional organization of the brain oscillations during language tasks, we clustered PSDs in an unsupervised manner using a K-means approach. We consistently found functional clusters: occipital clusters responding to visual stimuli (increased HGB and decreased alpha); clusters in the superior temporal gyrus responding to speech sounds (increased HGB and theta, decreased alpha and beta); clusters in motor regions, activated prior to and during articulation (increased HGB, decreased beta). Lastly, several distributed clusters over prefrontal (IFG, MFG) and supramarginal cortices activated during pre-articulation and selectively for auditory naming and sentence completion, which require increased semantic load. Taken together, our findings further our understanding of neural oscillatory activity, its relation to functional organization of the brain during language processing, and provide a link between low and high frequency activity, which is unavailable in non-invasive recordings (fMRI, M/EEG).

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Poster

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Title: Brain directed connectivity analysis shows evidence of auditory corollary discharge

Authors: *A. KHALILIAN-GOURTANI, R. WANG, X. CHEN, L. YU, W. DOYLE, O. DEVINSKY, Y. WANG, A. FLINKER;
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Abstract: Introduction: A direct consequence of any motor action is the activation of the relevant sensory system. It is critical for the brain to dissociate self-generated action from external sensation. A hallmark neural circuit addresses this issue by a blueprint of motor signals informing the sensory cortex of the impending action; known as corollary discharge (CD). While there is ample evidence of CD signals across the animal kingdom, the source and dynamics of CD in the human auditory system is not known. **Methods:** We leveraged the excellent spatiotemporal resolution of electrocorticography and acquired recordings from 8 neurosurgical patients while they performed an auditory repetition task (subjects were instructed to listen and then repeat single words). We used the high-gamma broadband (70-150 Hz) signal which is a common marker for underlying neural activity. In order to study the information flow between brain regions, we developed a directed connectivity analysis framework based on autoregressive Granger causality measures and applied unsupervised clustering techniques. Our approach elucidates dominant information flow (source and target) as well as prototypical temporal connectivity patterns (tested against permutation at $p<0.05$ for statistical significance) with their corresponding directed connections. **Results:** To understand the dynamics of information flow we applied our directed connectivity analysis framework to the neural recordings. Our results show three distinct phases during the auditory repetition task likely related to comprehension, pre-articulatory preparation, and speech production. Locked to word articulation we find a distinct component peaking at -100 ms relative to articulation onset with directed influence from speech motor cortex onto auditory cortex (STG). Unlike high-gamma analysis alone, which revealed pre-articulatory neural activity in multiple regions and subsequent STG suppression, only the connectivity approach was able to extract the directed information flow which originated in ventral precentral gyrus targeting STG. The CD component replicated within patients and the degree of directed influence on auditory electrodes significantly predicted

speech induced suppression in STG (Pearson Correlation, R=0.43, p=1.4e-4). **Conclusions:** In humans, an auditory CD is hypothesized to increase sensitivity to our own speech and its impairment can lead to auditory hallucinations. Our results provide the first evidence for the source and timing of a corollary discharge signal in the human auditory system and has great implication for speech motor control as well as the study of psychotic symptoms in humans.

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Poster

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Topic: H.11. Language

Support: NSF BCS-1756313
NIH 1S10OD023561
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Title: Evidence for a Spoken Word Lexicon in the Auditory Ventral Stream

Authors: *S. DAMERA[1], L. CHANG[1], P. P. NIKOLOV[1], J. A. MATTEI[1], S. BANERJEE[1], L. S. GLEZER[2], P. H. COX[3], X. JIANG[1], J. P. RAUSCHECKER[1], M. RIESENHUBER[1]; [1]Georgetown Univ. Med. Ctr., Washington, DC; [2]San Diego State Univ., San Diego, CA; [3]George Washington Univ., Washington, DC

Abstract: The existence of a neural representation for whole words (i.e., a lexicon) is a common feature of many models of speech processing. Prior studies have provided evidence for a visual lexicon containing representations of whole written words in an area of the ventral visual stream known as the “Visual Word Form Area” (VWFA). Similar experimental support for an auditory lexicon containing representations of spoken words has yet to be shown. Using fMRI rapid adaptation techniques, we provide evidence for an auditory lexicon in the “Auditory Word Form Area” (AWFA) in the human left anterior superior temporal gyrus that contains representations highly selective for individual spoken words. Furthermore, we show that familiarization with novel auditory words sharpens their selectivity in the AWFA. These findings reveal strong parallels in how the brain represents written and spoken words, showing convergent processing strategies across speech modalities in the visual and auditory ventral streams.

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Poster

241. Speech, Language, and the Brain

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Topic: H.11. Language

Title: Action reading requires motor simulation to activate the motor system

Authors: *W. DUPONT, C. PAPAXANTHIS, F. LEBON, C. MADDEN;
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Abstract: Reading about actions has been shown to engage motor simulations, yielding modulations in activity in the primary motor cortex (Barsalou 2008; Fischer and Zwaan 2008). However, it has been argued that such motor system activation is not automatic, and that individuals may or may not trigger a motor simulation of the action described in the sentence depending on factors such as task demands and individual differences in reading and cognitive strategies (Tomasino et al. 2008). According to this view, the primary motor cortex would only be activated for individuals/situations in which the motor simulation is explicitly or implicitly triggered. To investigate this idea, we tested aphantasic individuals, who have an inability to evoke motor simulation, i.e., to mentally create motor images (Zeman et al. 2015; Keogh and Pearson 2018). We used transcranial magnetic stimulation (TMS) to assess corticospinal excitability during action reading and motor imagery in phantasic (normal imagining) and aphantasic individuals. If an implicit or explicit motor simulation is required to activate the motor system during action reading, we would expect to observe in increase in cortical excitability compared to rest for phantasic but not aphantasic individuals.Thirty-four participants (17 phantasic and 17 aphantasic) were asked to read manual action sentences (e.g., “I have a hair on my arm, I pull it out”), or to explicitly imagine a maximal pinch movement. We delivered TMS pulses over the finger/hand area of the left primary motor cortex to assess corticospinal excitability at rest, during action reading (at 200, 300, 400 and 500ms after action verb display) and during motor imagery (2 seconds after a visual cue onset). We observed group-dependent modulations of corticospinal excitability during action reading, characterized by a greater corticospinal excitability when phantasic individuals read action sentences compared to aphantasic individuals. Corticospinal excitability increased relative to rest for phantasic individuals, but not for aphantasic individuals. We observed similar results for motor imagery, with greater corticospinal excitability for phantasic compared to aphantasic individuals. The absence of corticospinal excitability modulations during action reading for individuals with an inability to evoke mental simulation (implicitly or explicitly), support the idea that action reading activates the motor system only when a motor simulation is triggered.

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Poster

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Arizona Biomedical Research Commission Grant ADHS16-162413
NIH Grant F31MH122107

Title: Verbal fluency in ASD: the impact of aging across the adult lifespan

Authors: *D. OGBEAMA[1], S. CORTES CORIA[1], M. WALSH[2], B. BRADEN[1];
[2]Speech and Hearing Sci., [1]Arizona State Univ., Tempe, AZ

Abstract: Introduction: It is known that as one ages, cognitive function begins to decline. However, cognitive aging research on adults with Autism Spectrum Disorder (ASD) is scant. Verbal fluency is language-based executive function that is impaired in many with ASD and is negatively affected by normal age-related processes. In an ongoing longitudinal study, we investigated cross-sectional and age-related changes in verbal fluency behavior and functional brain activity via task-based MRI adults with ASD versus neurotypical (NT) adults. **Methods:** Participants were adults with (n=114) and without (n=92) ASD who were ages 18 to 71 years old. The Controlled Oral Word Association Test (COWAT) measured verbal fluency (i.e. word production) based on either a letter or a category. Word production during the first 30 seconds and last thirty seconds of the task were compared to determine latency of challenges. All participants also completed an fMRI version of the letter fluency task in the scanner. Participants who were >40 years old completed behavioral and fMRI tasks longitudinally at two year intervals (n=70; follow up duration=2.78(±0.84) years). Main effects of diagnosis, and diagnosis group by age, and diagnosis group by time interactions were explored using SPSS 27 and SAS software for behavior data, and the Sandwich Estimator in Statistical Parametric Mapping 12 (Matlab) for fMRI data. **Results:** The results showed adults with ASD produced fewer letter words during the first 30 seconds of the test (p=0.036), and fewer category words in the second 30 seconds of the test (p=0.009), compared to the NT control participants. There were no diagnosis group by age or diagnosis group by time interactions. **Discussion:** The results suggest that adults with ASD across a wide age range have persistent difficulties with verbal fluency production, but these abilities may not change differently between ASD and NT groups as aging ensues. Research determining the neural underpinnings of persistent verbal fluency challenges in adults with ASD is in progress. Taken together, findings will contribute to strategies for best supporting aging autistic adults across the lifespan.

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Poster

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Topic: H.11. Language

Support: NIH Grant R01DC019653
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AAUW

Title: Single neuronal decoding of human speech: a deep learning approach

Authors: J. CAI[1], ***M. JAMALI**[1], Y. KFIR[1], Y. BELINKOV[3], A. C. PAULK[2], S. S. CASH[2], Z. WILLIAMS[1];
[1]Neurosurg., [2]Neurol., Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA; [3]Technion, Haifa, Israel

Abstract: From complex combinations of words and sentence constructions, humans are able to comprehend extraordinarily rich and nuanced meanings through speech. Human speech processing is known to involve a wide set of interconnected brain regions within the frontotemporal cortex. The precise cellular process by which we derive meanings through speech or represent complex combination of words given through phrases and sentences, however, remains largely unknown. Here, we utilized a rare opportunity to record single-neuronal activities from frontotemporal cortices of participants undergoing planned intraoperative neurophysiology to discover robust responses to specific phonetic, lexico-semantic, grammatical and thematic word features. By further comparing neuronal activities to that of deep learning models (e.g., GPT-2), we also find that these cell ensembles displayed higher predictivity at the deeper layers of the pre-trained model embeddings, suggesting compositional phrase and sentence level representations integrated over the course of narratives. Finally, we find that, rather than passively processing the linguistic signal, these neurons actively predicted the upcoming words before they were heard. Together, our findings reveal a coordinated assembly of single neurons that encode a variety of linguistic features, and a dynamical cellular process that could potentially serve to support speech comprehension.

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Poster

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Support: National Institute of Neurological Disorders and Stroke (NS098981)

Title: Mapping the concreteness network with intracranial recordings and cortical stimulation

Authors: *E. MURPHY[1], O. WOOLNOUGH[2], C. W. MORSE[2], X. SCHERSCHLIGT[2], N. TANDON[2];
[1]Elliot Murphy, [2]Univ. of Texas Hlth. Sci. Ctr., Houston, TX

Abstract: Language comprehension relies on rapidly navigating the boundary between concrete words (perceptual: 'table') and abstract words (non-perceptual: 'justice'). Concrete words are typically faster to make lexical decisions about. Any neurobiological model of human semantics will ultimately need to make reference to the concrete-abstract formatting of conceptual representations. While behavioral effects between concrete/abstract words are consistent, the neurobiological effects differ substantially across studies. There remains no consensus concerning the contribution of nodes in the semantics network to concreteness. We present an intracranial investigation in a cohort of epilepsy patients (n = 15) of the spatiotemporal dynamics of concreteness using an orthographic single-word concreteness judgment paradigm. We additionally conducted cortical stimulation mapping (n = 5) to attribute causality to nodes in the semantic network. Patients were presented with a single word on a screen and asked to judge whether it referred to something you could touch, taste, smell, see or hear. A fixation cross was presented in the center of the screen for 700 ms, after which orthographic single-word exposures lasting 1000 ms were presented. 1500 ms of blank screen followed during which patients had to press either the left arrow (Concrete) or right arrow (Abstract). We recruited a large list of concrete, abstract and 'midscale' words (occupying the middle of the concreteness scale; e.g. 'magic') from a comprehensive review of databases for concreteness ratings. Data were acquired from either subdural grid electrodes or stereotactically placed depth electrodes. Behavioral performance was high (>80% for all) and only correct trials were analyzed. Analyzing broadband high gamma activity (BGA; 70-150 Hz), we discovered a frontotemporal network for concreteness, implicating mid-fusiform gyrus (mFus), parahippocampal cortex (PHC), inferior frontal gyrus (IFG), orbitofrontal cortex (OFC) and frontal operculum (FO). The earliest effects were found in OFC, mFus and PHC. Analyzing directionality via amplitude-envelope correlations in BGA, information flowed from OFC to FO, PHC, temporoparietal junction (TPJ) and both aIFG and pIFG (at ~350 ms for pIFG). Only in FO at approximately 800-1000 ms did patient subjective responses for midscale words impact BGA, showing greater activity for concrete-rated midscale items. Lastly, we used direct cortical stimulation to attribute causal involvement of certain nodes in this network (ventral temporal, inferior frontal) to concreteness, successfully disrupting judgments for concrete words.

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Poster

241. Speech, Language, and the Brain

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Support: NIH
INCLUDE Project

Title: The neural circuitry of human language: Multimodal imaging, Heschl's gyrus, interhemispheric connectivity and the genetic basis of language in Down syndrome (DS) and identical twins discordant for DS

Authors: *J. KORENBERG[1], O. ABDULLAH[3], J. S. ANDERSON[2];
[1]Univ. of Utah, Salt Lake City, UT; [2]Neuroradiology, Univ. of Utah, Eugene, OR; [3]NYU, Abu Dhabi, United Arab Emirates

Abstract: Language and verbal communication remain the most human of primate evolutionary domains and are disturbed in most human genetic conditions, prominently so in Down syndrome. Although human neural circuitry and regional brain functions are in part understood, the relationship of these to deficits in verbal and auditory processing, language production and cognitive functions are elusive as is the genetic contribution to language brain and cognition in DS. Filling this gap becomes a possibility by using emerging high resolution technologies to simultaneously query genetics, volumetric (MRI), functional (fMRI) to study the spectrum of language related features in individuals with genetic conditions such as Down syndrome. Here we report the integrated results from studies of multimodal imaging, cognition, language and verbal features of a cohort of 30 individuals with DS (validated by blood-based high resolution genome-wide arrays and karyotypes) and age and gender matched controls (ages 15-32 yo). Volumetric analysis revealed a significant correlation of verbal IQ with increased volume of the right hemisphere (RH) TTG (transtemporal gyrus) equivalent of left hemispheric (LH) language region, Heschl's gyrus. Tractographic analysis (using DSI Studio) revealed hyper interhemispheric connectivity of Heschl's gyrus to RH structures in DS versus controls. fMRI seeded at Heschl's gyrus revealed significantly decreased functional connectivity to the RH language region correlated with verbal IQ (above) in DS versus controls. In summary, distinct imaging modalities distinguish three independent properties of human language circuitry and a brain basis for language and verbal dysfunction in DS: aberrant volumes, increased physical interhemispheric and local connectivity and altered functional lateralization. The fragility of the neural systems for human language, its LH lateralization and disturbance in DS suggest genetic contributions to disturbed hemispheric specialization as novel mechanisms for a spectrum of human language deficits that may be related to their recent evolution and raise the possibility of modulation with unilateral stimulation.

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Poster

241. Speech, Language, and the Brain

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Title: Decoding speech production from intracranial depth electrodes

Authors: *T. THOMAS, A. SINGH, L. BULLOCK, N. TANDON;
Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Brain-computer interface (BCI) research has demonstrated the potential to restore communication abilities by decoding speech-related components from intracranial recordings. BCIs using electrocorticography are normally limited to decoding from only one region, e.g. speech motor cortex, due to the risks associated with placing multiple subdural grids on the cortical surface. Alternatively, stereo-electroencephalography (sEEG) is a less-invasive method to achieve widespread coverage across multiple regions. sEEG depth electrodes have been extensively used to study the distributed nature of the speech production network, but have yet to be widely explored for speech decoding and BCI research. We investigated the decoding potential of widespread sEEG coverage across 8 subjects. Subjects were presented with individual sentences from the TIMIT corpus in a trial-based manner and instructed to read each sentence aloud. Each sentence was divided into different types of speech components (phonemes, place of articulation, and manner of articulation), and the onset and offset times for each component label were extracted from microphone recordings. The neural recordings were processed to compute the broadband high-gamma power (BHG: 70-150 Hz) in each electrode and annotated with the speech component onset and offset times. Linear discriminant analysis was used to train and test a classification model for each type of speech component using 5-fold cross-validation. The average classification accuracy across all subjects was significantly above chance for phonemes (5.4% across 38 phonemes), place of articulation (18.1% across 9 labels), and manner of articulation (26.5% across 5 labels). Place and manner of articulation resulted in the highest accuracies relative to chance accuracies. We estimated each electrode's decoding power from the weights assigned by the classifiers. Across all subjects, we found that the electrodes with high decoding power were distributed across multiple cortical sites for both phonemic and articulatory classification models. These sites included sensorimotor cortex, inferior frontal gyrus, mid-fusiform cortex, and auditory cortex. While some of these electrodes were close to the cortical surface, we also observed many contributing electrodes deeper in the gyri and sulci regions distributed across the dominant and non-dominant language hemispheres. This comparison of the classification of different speech components sheds light on the advantages of safely accessing and decoding speech representations across a wide range of cortical regions using intracranial depth electrodes.

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Poster

241. Speech, Language, and the Brain

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Program #/Poster #: 241.30

Topic: H.11. Language

Support: NIH/NIDCD 1F30DC017083-01
NIH/NIDCD 5U01NS098981-03
NIH/NIDCD 1R01DC014589-01A1

Title: Cortical State Space Sequences for Human Language Production

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Abstract: Language is a defining human behavior that emerges from a broadly distributed set of cortical substrates. The transient, interactive, and global orchestration of this network is central to neurobiological models of language, yet empirical evaluation of these dynamics has been limited. We used direct intracranial recordings in a large cohort to create a complete spatiotemporal atlas of word generation. Next, we resolved network dynamics comprising sequences of state space transitions between patterns of directed interactional strength. Finally, we related the functional activity of this network to its causal perturbation with direct cortical stimulation. First, cortical activity was measured with high-gamma power using intracranial electrodes (n=26067, 135 patients) during picture naming of common objects. Global mean dynamics were integrated across the cohort with a surface-based mixed-effects multilevel analysis in narrow time windows to render a high-resolution movie of picture naming in the language-dominant hemisphere. Second, distributed interactions were studied using group autoregressive hidden Markov models. This principled probabilistic framework integrates stochastic linear dynamical systems with the switching Markov property. Such an approach retains the interpretability of multivariate autoregressive analysis while efficiently capturing nonlinearities inherent to neural data. We revealed 5 states engaged during picture naming. Each of these states was dually characterized by timing within a trial-specific transition sequence and by functional network structure comprising directed information exchange. Third, we investigated the response properties of this distributed cortical network relative to canonical linguistic features and causal perturbation with cortical stimulation. Semantic familiarity, lexical frequency, and lexical selectivity were associated with reaction time and with activity throughout the distributed networks identified during the second and third states in prior analysis: middle fusiform gyrus, pars triangularis and opercularis, supplementary motor area, and superior frontal sulcus. Stimulation in these regions caused anomia consistent with disruption of conceptualization and formulation processes.These data reveal the network architecture of speech production, constraining the neurobiological instantiation of extant theories for language cognition. In addition to providing new insights for language production theory, our approach investigates the broad utility of linking cognitive processes to network states rather than to activity in isolated substrates.

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Poster

242. Sample Preparation and Novel Probes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 242.01

Topic: I.03. Anatomical Methods

Support: FWF P 35614-B
WWTF CS18-019

Title: Human tissue and tumor imaging by light sheet microscopy

Authors: *H.-U. DODT[1,2], J. A. OAKES[1,2], C. FUCHSSTEINER[2], S. KIRNBAUER[2], M. FOROUGHIPOUR[1], M. FOROUGHIPOUR[1], K. BECKER[1,2], S. SAGHAFI[1];
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Abstract: Light sheet microscopy has been applied very successfully to the investigation of chemically cleared mouse brains. However its application to human tissue has been hampered by difficulties to obtain specific cellular staining. The use of endogenous fluorescent marker is not possible in humans and staining with antibodies is cumbersome due to diffusion problems. Furthermore standard clearing protocols like 3DISCO take weeks to months to clear human tissue. Notoriously difficult to clear are tumors due to their high cell density. We therefore developed in the last years a new clearing protocol, pathoDISCO, which allows clearing of tumor tissue in the centimetre range within days by using a chemical reaction for dehydration[1]. This method used boosted autofluorescence for cellular visualization but did not yet provide specific subcellular staining. We now searched for specific fluorescent stainings for cytoplasm and nuclei which also survive our harsh tumor clearing. Applying suitable image processing we were able to generate Hematoxilin/Eosin (HE) stained like images of various human tissues. Also stainings for other tissue components will be presented. With our ultramicroscope volume recordings of pathology cassette sized tumor pieces of 4 mm thickness could be obtained with subcellular resolution. Our single optical sections provide a resolution and appearance equivalent to histological slides but now for whole tissue volumes. We applied our technology to various tissues like human brain, human brain metastases and mamma. We are confident that this approach will open up completely new ways for histological diagnostics in pathology.
[1]Sabdyusheva-Litschauer I et al. (2020) 3D histopathology of human tumours by fast clearing and ultramicroscopy, Sci Rep.10:17619

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Poster

242. Sample Preparation and Novel Probes

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Program #/Poster #: 242.02

Topic: I.03. Anatomical Methods

Support: NIH-1RF1MH123402
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Title: Epic-clear: enzyme powered ihc-compatible clearing for whole organs and whole animals

Authors: M.-C. CHENG[1], ***M. E. MCGLOTHLIN**[1], Y. LI[1], L. A. WALKER[2,3], D. CAI[1,2,4]; [1]Cell and Developmental Biology, Michigan Med., [2]Biophysics, [3]Dept. of Computat. Med. and Bioinformatics, [4]Michigan Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: Hydrogel embedding and tissue clearing have transformed molecular in situ profiling into a tool for multiplexed and multimodal analysis. Additionally, hydrogel expansion enables super-resolution microscopy with regular imaging modalities in thick tissues. Applying this technique to whole organs has become possible, but its widespread implementation is limited by light scattering biomolecules in the tissue. Currently, two types of tissue clearing methods have been broadly used to make hydrogel embedded tissue transparent for 3D volumetric imaging. The first type, adapted in CLARITY, MAP, and miriEx, etc., utilizes a strong detergent, such as SDS, to strip off lipids and carry them out of the tissue in the form of micelles. The second type, adapted in ExM, proExM, and EASI-FISH, etc., utilizes Proteinase K to digest proteins into small polypeptide chains so that biomaterials, including protein fragments and lipids, are removed from the expanded tissue. While these two methods are effective, SDS clearing eliminates native fluorescent protein (FP) labeling, and Proteinase K digestion destructs protein antigens prohibiting multiplex proteomic profiling. Here, we present enzyme powered IHC-compatible clearing (EPIC-clear), a novel clearing method utilizing solely enzymes that preserves native FP fluorescence and endogenous proteins for multiplex molecular profiling. Using only passive incubation at mild temperatures, EPIC-clear eliminates the drawbacks of SDS/electrophoresis yet clears mammal brain samples at the same speed. For instance, thick sections or whole mouse brains can be completely cleared in a few hours to several days, respectively. Tissues previously considered very challenging, such as kidney, liver, skins and whole adult Drosophila, are also turned completely transparent. EPIC-clear presents a fast and easy to use tissue clearing method for many embedding hydrogel formulas, which can be broadly adapted in the neuroscience research community.

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Poster

242. Sample Preparation and Novel Probes

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Program #/Poster #: 242.03

Topic: I.03. Anatomical Methods

Support: NSERC Discovery

Title: Neuronal quantification and brain size comparison in mice fixed with solutions used in human gross anatomy laboratories

Authors: E.-M. FRIGON[1], A. GÉRIN-LAJOIE[1], M. DADAR[2], D. BOIRE[1], ***J. MARANZANO**[1,3];
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Abstract: We previously demonstrated that mice brains fixed with different solutions used in human gross anatomy laboratories, namely an alcohol solution (AS) and a salt saturated solution (SSS) preserve cell antigenicity and are compatible with histology studies (Frigon EM. et al. BioRxiv. 2022). We further observed that the brains fixed with AS had greater weight than those fixed with SSS or a 4% formaldehyde solution (FAS), standardly used in animal fixation (Frigon EM. et al. SfN Conference. 2021). Since FAS is known to shrink the brain, whereas AS was developed to prevent brain deformation, we aimed to compare the size of brains' slices and the number and size of the neurons in mice fixed with the three different solutions.We perfused 9 C57BL/6J mice (6 months old; 3 females-6 males) with FAS (N=3), SSS (N=3) or AS (N=3). The brains were extracted, cryoprotected, frozen and cut into 40 μm sections for immunohistochemical staining of neuronal nuclei (NeuN). We then acquired photomicrographs (20X) of the closest section of 0.22mm posterior to bregma (The Mouse Brain Atlas, Franklin & Paxinos) to assess the slice surface, using a brightfield microscope (Olympus BX51W1) controlled by Neurolucida (MBF Bioscience). In these sections, we also acquired three photomicrographs of regions of interests (ROIs) (100X, 191x152mm; 2752x2192px) in the 3rd layer of the primary motor cortex. We then assessed the following variables using Display (MINC Tool Kit, McConnell Brain Imaging Center): 1-total cell surface, 2-cellular size, and 3-number of cells. We found that the slice surface was significantly greater in brains fixed with AS (mean=29,764,777px) than in brains fixed with SSS (mean=23,901,864px; p=0.047) and FAS (mean=23,448,856px; p=0.037). The total cell surface in the ROIs was also significantly greater in brains fixed with AS (mean=2,140,330px) than the FAS-fixed brains (mean=1,708,817px; p=0.042). The individual cells in AS-fixed brains were significantly larger (mean=42,727px) than in the brains fixed with SSS (mean=36,521px, p<0.0001) and FAS (mean=36,392px, p<0.0001), which explains the higher total cell surface in AS brains. Finally, ROIs of AS-fixed brains showed significantly fewer cells (mean=78) than ROIs of FAS-fixed specimens (mean=102.22, p=0.019).In conclusion, AS fixation produces larger brains, with larger but fewer cells in the same geometric ROIs, showing less brain shrinkage than FAS and SSS specimens. These results are important to consider in further use of brains fixed with these solutions to quantify cells in neurodegenerative pathologies

Disclosures: E. Frigon: None. **A. Gérin-Lajoie:** None. **M. Dadar:** None. **D. Boire:** None. **J. Maranzano:** None.

Poster

242. Sample Preparation and Novel Probes

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Program #/Poster #: 242.04

Topic: I.03. Anatomical Methods

Support: NSERC - BESCD3-559015-2021
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Title: Antigenicity of brains fixed with formaldehyde or solutions used in human gross anatomy laboratories

Authors: *E.-M. FRIGON[1], A. GÉRIN-LAJOIE[1], D. BOIRE[1], J. MARANZANO[1,2];
[1]Univ. Du Quebec A Trois-Rivieres, Univ. Du Quebec A Trois-Rivieres, Trois-Rivieres, QC, Canada; [2]McGill Univ., Montreal, QC, Canada

Abstract: Brain tissue for neuroscientific research is obtained from brain banks that provide small tissue samples, while gross anatomy laboratories could become a source to neuroscientists by providing full brains. However, these brains are preserved with solutions appropriate for dissections that are different than the classic 4% formaldehyde solution (FAS) used in brain banks. Our previous work in mice shows that two different solutions used in human gross anatomy, either a salt saturated solution (SSS) and an alcohol-formaldehyde solution (AFS) preserve antigenicity of the main brain cell populations (Frigon et al. BioRxiv. 2022). Our goal is now to compare the histology quality and antigenicity of human brains fixed with FAS vs. those fixed with a SSS and an AFS.
We used a convenience sample of 16 brains (10 males, 6 females; 44-87 years old at time of death) fixed in our anatomy laboratory using: FAS (N=5), SSS (N=9), or AFS (N=2). The brains were cut into small samples of approximately 1 cm^3 that were cryoprotected, frozen and cut into 40 μm sections.We assessed the following variables: 1-ease of manipulation (slice consistency, presence of tears, and presence of rolling slices), 2-microscopic quality (neuropil uniformity and cellular shape), 3-antigenicity by immunohistochemistry of neuronal nuclei (NeuN), glial fibrillary acidic protein (GFAP), ionized calcium binding adaptor molecule1 (Iba1) and myelin proteolipid protein (PLP), 4-cell distribution (homogeneous cells/in patches/isolated cells), 5-background (dark/intermediate/light) and 6-antibody penetration (complete/incomplete).
We found that the FAS-fixed brains were the easiest to manipulate. FAS and AFS-fixed brains showed a higher microscopic tissue quality (uniform neuropil and regular cell contours). The specimens fixed with AFS showed positive antigenicity of all targeted antigens and homogeneous cellular distribution, while SSS and FAS showed negative specimens for astrocytes (SSS=1, FAS=2) and myelin (SSS=2, FAS=2), in addition to a higher occurrence of patched or isolated cell distribution. We also found a higher incidence of light backgrounds in AFS, and dark backgrounds in FAS-fixed brains. Finally, SSS-fixed brains showed a higher occurrence of incomplete penetration for all antigens.
Despite a more difficult manipulation and poorer quality of the SSS-fixed brains, antigenicity was preserved in human brains fixed with solutions used in human gross anatomy. Furthermore,

AFS-fixed brains were superior to FAS-fixed brains for some specific variables. These results are promising for neuroscientists interested in using full brains from anatomy laboratories.

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Poster

242. Sample Preparation and Novel Probes

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Program #/Poster #: 242.05

Topic: I.03. Anatomical Methods

Support: NSF DGE 2137420

Title: Comparative mechanics of brain tissue and blood clot towards a next-generation brain phantom material

Authors: *G. M. JEANPIERRE[1], M. K. RAUSCH[2], S. R. SANTACRUZ[3];
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Abstract: Soft tissue phantoms are critical tools to understanding, modeling, and optimizing medical devices. For example, brain tissue phantoms are used to study brain imaging modalities and medical device-brain tissue interactions. Most of these phantoms are based on hydrogel systems which do not mimic soft tissues across all spatial and temporal scales. That is, hydrogels' mechanics differ in their large-deformation and viscoelastic response from brain tissue. To overcome the limitation of current phantoms, we pursue an innovative, alternative approach. Here, our goal is to study blood clot as a potential phantom for brain tissue. The specific objective of this proposal is to test and validate a mechanical test protocol with which we can compare brain tissue, composite hydrogel, and blood clot mechanics.
Fresh porcine brain was submerged in 1 x phosphate buffer saline (PBS) solution to prevent dehydration of the tissue. 5 mm slices were cut with a brain knife using a unidirectional slicing motion. 5 mm cubes were cut from the corona radiata and basal ganglia. Sample size was chosen due to spatial limitations of the two brain areas investigated and sample shape was chosen for simplicity of cutting. Non-human primate brain tissue was prepared using the process above. However, 1 x PBS was substituted for 1 x artificial cerebral spinal fluid. The composite hydrogel was prepared by first 3D printing a mold with 5 mm^3 internal dimensions. 6% polyvinyl alcohol and 0.85% phytagel were individually dissolved in deionized water at 90 °C for 1 hour. The two solutions were mixed in a 1:1 weight ratio at 70 °C for 30 minutes (mins), cooled, poured into the 3D molds, and frozen at -20 °C for 18 hours. The same 3D printed mold was used for the preparation of blood clot. 1.5 ml of bovine blood was prepared with CPDA-1 anticoagulant and 20 mM of $CaCl_2$ to reverse the anticoagulant. The blood was poured into the 3D mold and coagulated for 60 mins in a 37 °C incubator. The top and bottom of the sample cubes were fixed onto a specimen holder using superglue. A triaxial tester was used to apply 15% compression to

the samples for 2 mins followed by a 1-min relaxation, 15% tension to for 2 mins followed by 1 min relaxation, 15% tension and compression cycling for 5 cycles, and 30% shear cycling for 5 cycles. A 0.05 mm/s deformation rate and 5 Hz sampling frequency were used.
The aim of this work was to develop a mechanical testing protocol to compare the mechanics of brain tissue, a composite hydrogel, and blood clot toward the goal of establishing blood clot as a brain-mimicking phantom. Specifically, our goal is to identify a phantom that better mimics the dynamic, non-linear, viscoelastic mechanical properties of brain tissue.

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Poster

242. Sample Preparation and Novel Probes

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Topic: I.03. Anatomical Methods

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Title: Low-cost diolistic delivery in non-human primate and rodent brain slices to reveal species differences in astrocyte morphology

Authors: *K. S. HEFFERNAN[1], A. GALVAN[2];
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Abstract: Most recent morphological studies rely on transgenic animals and/or viral vectors to label individual cells. In non-human primate (NHP) research, viral transduction of cells is possible, but poses several practical and financial challenges, limiting the number of researchers that can thoroughly investigate cell morphology in NHP tissue. Golgi impregnation, a long-used technique to reveal cell morphology in both NHPs and rodents, is limited in resolution due to its reliance on brightfield microscopy. The use of a gene gun system to deliver fluorescent lipophilic dye-coated gold or tungsten particles into brain tissue ("diolistic delivery") has been used previously, however available gene gun systems are expensive with limited application, and therefore an uncommon piece of equipment in most labs (Seabold et al., 2010). On the other hand, most studies of cell morphology in NHPs that have used either viral delivery methods or diolistic dye delivery have focused on neurons, despite known differences in astrocytes across species. Previous studies have found increased morphological complexity and size of astrocytes in primate brains (Oberheim et al., 2009). However, quantitative comparisons across species relied on glial fibrillary acidic protein (GFAP) which may only label 15% of a given astrocyte (Bushong et al., 2002). To overcome these limitations of available techniques and to expand our

understanding of astrocyte morphology across species, we have constructed our own low-cost diolistic delivery system to deliver dye-coated gold or tungsten particles into NHP and rodent brain slices. We have determined optimal parameters (pressure of 100 psi, distance of 3 cm from the top of the slice, and 20 µm filter paper between the barrel and slice) of our diolistic system to achieve penetration of DiI-coated particles to depths of 50-100µM, allowing for complete reconstruction of individual cells within a slice. DiI is a lipophilic carbocyanine dye that emits in the red spectrum. Additionally, we have found that cardiac perfusion with 4% PFA is sufficient for diffusion of DiI through cell membranes, while perfusion solutions containing glutaraldehyde prevent diffusion and result in leakage from the cell membrane. Studies are in progress to apply this technique and compare astrocyte morphologies between NHP and rodents in evolutionarily conserved (basal ganglia) and divergent (prefrontal cortex) brain regions. In sum, our diolistic delivery system is a low-cost and low maintenance method that can be readily established in neuroanatomical labs to reliably label cells, including astrocytes, in both species.

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Poster

242. Sample Preparation and Novel Probes

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Title: Genetically-encoded probes for labeling neurotransmitter-defined synaptic vesicles

Authors: *A. J. FLORES[1], L. ORIOL[1], T. STEINKELLNER[3,1], J. HU[1,2], M. MACKEY[1,2], D. BOASSA[1,2], T. S. HNASKO[1,4];
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Abstract: Vesicular transporters represent highly specific targets for the development of molecular tools for mapping brain circuits by allowing precise specification and imaging of synaptic vesicles (SVs) and synapses based on their neurochemical identity. Conventional tools used to visualize neurotransmitter-defined SVs and synapses rely on immunolabeling. While such studies have been incredibly informative, they have key limitations, e.g., tissue processing

reduces antigenicity and the use of detergents needed for bulky antibody permeability damages tissue ultrastructure, making immunolabeling suboptimal for use with some modern techniques, such as serial block-face scanning 3D electron microscopy (EM). Here, we expand on our approach for labeling SVs using tagged vesicular transporters. This approach is fully compatible with a wide array of light and EM techniques. We developed a suite of genetically encoded probes for identification of glutamatergic, GABAergic, cholinergic, and monoaminergic SVs using both light and EM applications by fusing mini singlet oxygen generator (miniSOG) or HaloTag to intralumenal domains of vesicular transporters for glutamate (VGLUT1 & VGLUT2), GABA & glycine (VGAT), monoamines (VMAT2), and acetylcholine (VAChT). We packaged these tagged transporters into Cre-dependent adeno-associated virus (AAV) vectors and assessed their expression and trafficking, by both light and EM, both in primary neuronal culture, and *in vivo* using appropriate Cre-expressing mouse lines. In particular, we highlight the photooxidation labeling of SVs catalyzed by miniSOG and Janelia Fluor HaloTag ligands for visualization by EM. These novel tools represent a new resource for accessing subcellular structure and molecular machinery in SVs and presynaptic terminals defined by their neurotransmitter.

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Poster

242. Sample Preparation and Novel Probes

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Topic: I.03. Anatomical Methods

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Title: Correlative chemical imaging identifies amyloid peptide signatures of neuritic plaques and dystrophy in human sporadic Alzheimer’s disease

Authors: *S. KOUTARAPU[1], J. GE[1], D. JHA[1,3], K. BLENNOW[1], H. ZETTERBERG[1], T. LASHLEY[4], W. MICHNO[6], J. HANRIEDER[2,5];
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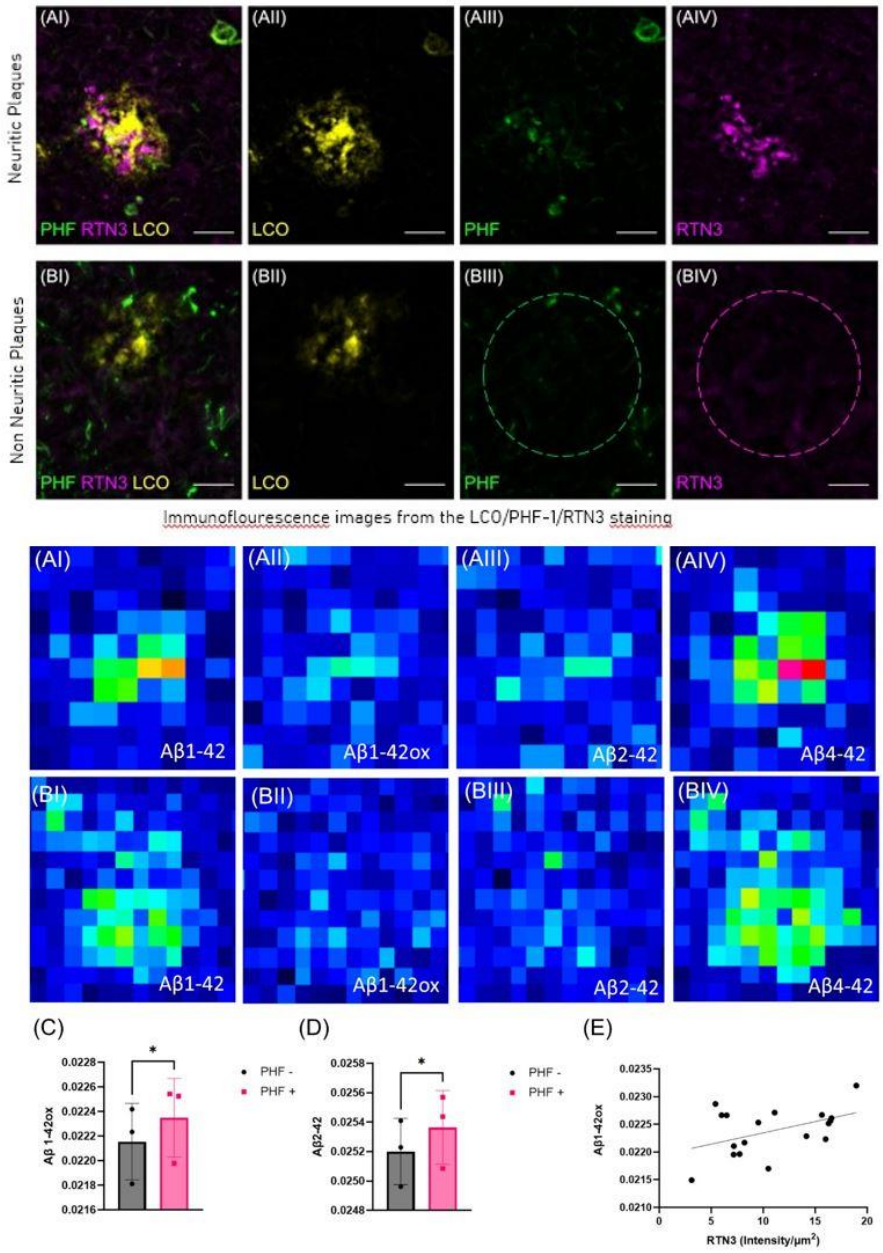
London, Inst. of Neurol., London, United Kingdom; [6]Neurosci. Studies, Macalester Col., Saint Paul, MN

Abstract: Alzheimer's disease (AD) is the most common form of dementia and neurodegenerative disease, with the majority cases belonging to sporadic variant (sAD). Current efforts to combat the disease focus beta-amyloid (Aβ) plaque pathology. Plaque pathology is very heterogeneous, where a subset of plaques, neuritic plaques, are considered most neurotoxic. This makes characterizing heterogeneity of the plaque population in Aβ pathology essential to understand Aβ pathogenicity. The aim of this exploratory research was to spatially elucidate Aβ signatures specific to various plaque polymorphs. We devised a correlative chemical imaging paradigm based on immunofluorescence microscopy and mass spectrometry imaging (MSI), to probe senile Aβ pathology in post mortem human sporadic AD brain sections stained towards PHF-1 and RTN3 in combination amyloid fluorophores: qFTAA and hFTAA. Cored and neuritic plaques showed increased levels of Aβx-40 peptides, associated with compact core formation. Neuritic plaques were characterized by Aβ1-42ox and Aβ2-42 as compared to cored, PHF negative plaques. Moreover, correlation with a marker of dystrophy (reticulon 3, RTN3) identified key Aβ species, incl. Aβ1-42ox and Aβ4-42 that both delineate neuritic plaques and display association with neuritic dystrophy, driving the notion towards Aβ pathogenicity and neurotoxicity. These data highlight the great potential of mass spectrometry imaging to investigate biochemical specific plaque morphologies, which cannot be delineated with conventional staining approaches. Together these correlative imaging data shed light on the complex biochemical architecture of senile plaque pathology in AD, specifically for neuritic plaques and associated dystrophic neurites. which can be a potential novel biomarker of Aβ pathogenicity.



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Poster

242. Sample Preparation and Novel Probes

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Support: NIH Grant R44MH119989

Title: Brain-wide visualization of neuronal activity developed with cellular resolution measurement of Npas4 and cFos

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Abstract: Recent advances in optical clearing and light sheet imaging have opened an exciting new avenue for brain-wide, cellular resolution immunostaining at the forefront of a dimensional shift from 2D to 3D histology. Providing access to the intricate anatomy of the whole intact brain, new tissue clearing methods can provide neuroscientists with unbiased and complete pictures of brain anatomy and function. With our optimized iDISCO-based clearing methods and our Mesoscale Imaging System for the ZEISS Lightsheet Z.1 microscope, we produce high-resolution terabyte scale whole-brain image sets. To stitch these images, we developed Stitchy, a standalone software tool that can process raw files from common light sheet microscope systems (e.g. ZEISS Lightsheet Z.1 or LaVision/Miltenyi Ultramicroscope II), allowing for both automated and manual alignment and then stitching images to commonly used output formats like ims, ome.tif, and ngff. By reading native files and writing directly to the desired output, Stitchy avoids proprietary intermediates and provides significant speed increases. To generate actionable quantitative data from these stitched images, our machine learning-enabled 3TK software identifies individual cells throughout the brain and aligns them to the Allen Reference Atlas to produce a regionalized read-out of cellular patterns across hundreds of brain areas. We have applied this technology to generate cellular resolution maps of neuronal activity by measuring the immediate-early gene (IEG) products Npas4 and cFos. Expression of cFos is driven by Ca^{2+}-signaling downstream of neuronal activity and is commonly used to mark ensembles of active neurons responding to environmental cues. However, cFos expression is also driven by cAMP elevations and signaling pathways engaged by neurotrophins or other paracrine factors. In contrast, Npas4 expression is neuron-specific and tightly tuned to Ca^{2+}-dependent signaling pathways. Using our Activity Signaling anti-Npas4 recombinant rabbit monoclonal antibody and a commercial anti-cFos antibody, we can measure brain-wide responses extrinsic factors. Our data indicate that the divergent signaling pathways that control the expression of cFos and Npas4 reveal distinct, but overlapping, populations of neurons in response to both behavioural and pharmacological stimuli. These data indicate that the simultaneous cellular-

resolution measurement of both cFos and Npas4 can provide rich data sets that allow one to distinguish pure neuronal activity responses from responses that involve GPCRs or trophic factors.

Disclosures: **D.G. Wheeler:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems, Inc. **J.H. Zeitoun:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc. **A. Reksoatmodjo:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc. **C. Lo:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc. **N. Guanzon:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc. **Y. Gallegos:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc. **C. Redd:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc. **E. May:** A. Employment/Salary (full or part-time):; chase@translucencebio.com. **M. Peters:** None. **R. Azevedo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems, Inc. **S.P. Gandhi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems, Inc..

Poster

242. Sample Preparation and Novel Probes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 242.10

Topic: I.03. Anatomical Methods

Support: NIH Grant U24NS119916

Title: Expanding our NABORhood: A call to diversify and further validate the Neuroscience AntiBody Open Resource

Authors: M. REGO[1], **K. H. DEMAIO**[1], A. RHODES[1], A. BALTUS[1], E. PERKINS[1], D. BOURGES[1], M. TAKVORIAN[1], K. D. MURRAY[2], K. M. TEMPLETON[2], J. S. TRIMMER[2], *M. FAN[1];
[1]Addgene, Addgene, Watertown, MA; [2]Univ. of California, Davis Sch. of Med., Univ. of California, Davis Sch. of Med., Davis, CA

Abstract: Neuroscientists require a steady source of validated antibodies for many of their research applications. The current antibody market, however, is plagued by unreliable stocks, variable quality and a lack of transparency surrounding antibody sequences and associated data. To address these issues, Addgene, in collaboration with Dr. James Trimmer and colleagues at UC Davis, launched the Neuroscience AntiBody Open Resource (NABOR), an open-access library of neuroscience-focused plasmid-based recombinant antibodies. Unlike traditional antibody resources, NABOR antibody sequences are open-access so end-users know the molecular identity of the materials they are using. As part of this collaboration, Addgene has

produced the recombinant versions of a number of Dr. Trimmer's NeuroMabs as ready-to-use antibody preparations. After validation in the Trimmer lab these antibodies are being made available to the neuroscience community through Addgene's website. Addgene has also begun producing antibodies against common tags and proteins that can be used by both neuroscientists and the wider scientific community. The NABOR collection is actively growing and we are looking for collaborators who are willing to share their recombinant antibody plasmids through Addgene. In addition to sharing physical materials, a major goal of the NABOR initiative is to make antibody-related data fully transparent and accessible. Through collaboration with the Trimmer lab, NABOR antibodies have been tested in a variety of common applications such as western blotting, immunocytochemistry and immunohistochemistry. These antibodies, however, may be suitable for a variety of applications for which they have not yet been tested. To this end, Addgene is creating a platform for end-users to share data regarding antibody performance in various applications. Neuroscience techniques, in particular, evolve rapidly and NABOR's success will depend on community support to test and report on suitability in these applications. Our goal is to enhance the visibility, access, and value of the antibodies used by the neuroscience community and to promote change from a model of affinity reagent sharing in which companies control the reagents to one in which scientists are empowered. Openly shared recombinant antibodies will improve reproducibility, increase discoveries, and evolve these valuable research tools.

Disclosures: **M. Rego:** None. **K.H. DeMaio:** None. **A. Rhodes:** None. **A. Baltus:** None. **E. Perkins:** None. **D. Bourges:** None. **M. Takvorian:** None. **K.D. Murray:** None. **K.M. Templeton:** None. **J.S. Trimmer:** None. **M. Fan:** None.

Poster

242. Sample Preparation and Novel Probes

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 242.11

Topic: I.03. Anatomical Methods

Support: NIH Grant NS109113

Title: Generation and open source dissemination of recombinant antibodies for neuroscience research

Authors: *J. S. TRIMMER, R. J. BROWDE, Q. G. LE, P. A. SINGH, K. M. TEMPLETON, D. A. VAN DER LIST, N. C. VIERRA, K. D. MURRAY;
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Abstract: Previously, we developed a widely-used collection of monoclonal antibodies (mAbs), termed NeuroMabs (neuromab.ucdavis.edu) that have been extensively validated in essential neuroscience research applications including immunohistochemistry on mammalian brain samples. We recently made the hybridomas producing these NeuroMabs available through two

open access academic resources, the Developmental Studies Hybridoma Bank at the University of Iowa (DSHB, https://dshb.biology.uiowa.edu/collections/neuromab) and the UC Davis MMRRC (https://www.mmrrc.org/catalog/overview_Major_Collection.php), allowing for low-cost production of mAbs at any scale. DSHB also disseminates ready-to-use mAbs. We are converting these mAbs into recombinant form allowing for enhanced archiving and dissemination as DNA sequence and as plasmid DNA. Plasmid-encoded recombinant mAbs or R-mAbs have additional advantages as molecularly defined reagents that yield higher and more consistent expression levels. We previously used an RT-PCR based approach to generate heavy and light chain variable ("VH" and "VL") domain amplicons from hybridoma-derived RNA, which were then used to generate R-mAb mammalian expression plasmids (Andrews et al., 2019, eLife 8: e43322). We are now employing a high-throughput Illumina-based sequencing platform to determine mAb VH and VL domain sequences directly from hybridomas. To date we have posted sequences from >6,000 hybridomas on the open access NeuroMabSeq website (neuromabseq.ucdavis.edu). We are using selected VH and VL domain sequences to generate R-mAb expression plasmids. After validation of the produced R-mAbs, the expression plasmids are being disseminated through the open access non-profit resource Addgene (https://www.addgene.org/James_Trimmer/), with over 315 R-mAb expression plasmids deposited to date. In addition, these plasmids are being used to produce ready to use recombinant mAbs being distributed by Addgene under the NIH-funded NABOR Initiative (https://www.addgene.org/antibodies/). We are also using these VH and VL domain sequences to engineer mammalian expression plasmids for miniaturized single chain variable fragments (scFvs), which at 1/5 the size of conventional antibodies have enhanced tissue penetration and enable higher resolution imaging. These scFv expression plasmids are also being disseminated through Addgene (over 30 deposited to date). Together, these efforts provide open-source renewable recombinant research reagents to support neuroscience research efforts.

Disclosures: J.S. Trimmer: None. **R.J. Browde:** None. **Q.G. Le:** None. **P.A. Singh:** None. **K.M. Templeton:** None. **D.A. van der List:** None. **N.C. Vierra:** None. **K.D. Murray:** None.

Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.01

Topic: I.04. Physiological Methods

Title: Toxicity risk assessment method for compounds using human iPS cell-derived neurons

Authors: *Y. ISHIBASHI, N. NAGAFUKU, I. SUZUKI;
Dept. of Electronics, Tohoku Inst. of Technol., Sendai, Miyagi, Japan

Abstract: In vitro human iPSC-derived neuronal systems are an alternative platform for neurotoxicity testing to animal models and primary cultures. Microelectrode array (MEA),

measurement system of the electrophysiological activity, are suitable to evaluate the neurotoxicity of compounds. We have previously reported the electrophysiological responses to known neurotoxicity compounds using MEA in human iPSC-derived neurons. However, the identification of analytical parameters to detecting toxicity of unknown compounds remains an important issue. Here we established analytical parameters and multivariate analysis method capable of detecting toxicity of compounds using MEA measurement of human iPS cell-derived neurons. Human iPS cell-derived neurons (SynFire Co-Culture Kit, Neucyte Inc.) and human iPS cell-derived astrocytes were co-cultured on MEA (Maestro, Axion Inc.). More than 10 pesticides and industrial chemicals were cumulative administered in 5 weeks cultured samples. We identify the analytical parameters enabling the separation of responses between neurotoxicity and negative control, and the separation the mechanism of action by using principal component analysis. By comparing the optimized analytical parameters of each testing compound with the standard deviation (SD) of negative control, we can predict general neurotoxicity in relatively quantitatively scale, for example, low risk for lower than SD range, medium risk for 2xSD, and high risk for over 2xSD. Known neurotoxicity compounds increased toxicity risk with increased concentration. Negative compounds did not increase toxicity risk. Almost pesticides and industrial chemicals increased toxicity risk with increased concentration. In addition, the principal component analysis method was able to estimate the mechanism of action of pesticides by selecting appropriate parameters. In this study, we evaluated the toxicity risk of pesticides and industrial chemicals. We demonstrated that the toxicity risk can be detected by MEA measurement of human iPSC-derived neurons. This predictability can help select appropriate concentration levels to avoid toxicity/adverse effects.

Disclosures: **Y. Ishibashi:** None. **N. Nagafuku:** None. **I. Suzuki:** None.

Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.02

Topic: I.04. Physiological Methods

Title: A SYSTEM FOR INVESTIGATING BIOELECTRONIC THERAPIES USING A MULTICONTACT NERVE CUFF ELECTRODES AND AN IMPLANTED STIMULATOR/RECORDER.

Authors: *M. SCHUETTLER[1], T. DENISON[2], I. GILLBE[3], R. PFEIFER[4], J. RICKERT[5]; [1]Cortec GmbH, Cortec GmbH, Freiburg Im Breisgau, Germany; [2]MRC Brain Network Dynamics Unit, Univ. of Oxford, Oxford, United Kingdom; [3]Engin., Bioinduction Ltd, Bristol, United Kingdom; [4]Cortec Gmbh, Freiburg, Germany; [5]CorTec GmbH, Freiburg, Germany

Abstract: 1. Introduction The field of bioelectronic medicine has moved quickly in the past years from fundamental research on rodents to first clinical studies, e.g., for treatment of rheumatic arthritis or hypertension. New applications are on the horizon but the transfer from

fundamental research to clinical studies requires hardware suitable for large animal or human use, which is not commonly available today. Here, we describe a platform suitable for investigating new biomedical treatment paradigms in large animals and humans.
2. Methods and Materials The platform combines the Picostim ultracompact stimulator/recorder implant system by Bioinduction (Bristol, UK), the DyNeuMo research application developed in partnership between Bioinduction and Oxford, and the AirRay electrode technology developed by CorTec (Freiburg, Germany).
3. Results The Picostim implant is powered by a rechargeable battery and features 8 channels of electrical stimulation (up to 15mA), neural recording, and a 3-axis accelerometer. The implant is very compact at just 7cc and is programmed remotely using an external wireless controller. The implant allows smart adaptive therapy employing circadian, motion and neural signal feedback, which are combined and processed in real time on the implant.
4. Discussion The nerve interface consists of a novel self-sizing spiral nerve cuff electrode with 8 contacts. Two of the contacts are ring shaped, arranged at the ends of the cuff. The remaining 6 contacts are dot-shaped and describe a segmented ring in the middle of the cuff. The cuff is 18mm in length and has an inner diameter of 2.5mm. It is made from laser-micromachined soft silicone (PMDS) and platinum iridium contacts. The cuffs are attached to multi-lumen polyurethane-based cables/connectors.
5. Conclusions Combined, the platform can be used to develop new and more precise treatments using recording and fascicle selective stimulation on the vagus nerve as well as other nerves. Currently, preclinical versions of the system are undergoing tests and validations, and clinical versions are planned. Here, we present the technical details of the system and its application.

Disclosures: **M. Schuettler:** A. Employment/Salary (full or part-time):; CorTec GmbH. **T. Denison:** F. Consulting Fees (e.g., advisory boards); CorTec GmbH. **I. Gillbe:** A. Employment/Salary (full or part-time):; Bioinduction Ltd. **R. Pfeifer:** A. Employment/Salary (full or part-time):; CorTec GmbH. **J. Rickert:** A. Employment/Salary (full or part-time):; CorTec GmbH.

Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.03

Topic: I.04. Physiological Methods

Support: MRC Grant ST11725

Title: Application of high-density transcranial magnetic stimulation (hd-tms) in amyotrophic lateral sclerosis (als): lessons learnt from a pilot study in healthy volunteers

Authors: *A. CAROBIN, J. BASHFORD, V. SANTORO, I. PREMOLI, L. ROCCHI, M. RICHARDSON, C. SHAW;
King's Col. London, London, United Kingdom

Abstract: ALS is a neurodegenerative disorder that causes progressive paralysis and death on average within three years of symptom onset. There is currently no effective treatment, and the discovery of new potential therapies is held back by the lack of biomarkers for timely diagnosis and reliable progression monitoring of the disease. Transcranial magnetic stimulation (TMS) combined with single-channel EMG has identified early signatures of cortico-spinal hyperexcitability in individuals with ALS and demonstrated that this subclinical biomarker of motor neurons dysfunction is an early pathogenic mechanism preceding irreversible muscular atrophy. However, how well different TMS parameters of abnormal cortico-spinal excitability track disease progression is less well understood and has led to some contrasting results.
This study piloted the novel combination of TMS with the 64-channel high-density surface EMG (HDSEMG), instead of the commonly used single-channel EMG for the first time. Established TMS protocols probing cortical inhibition through the duration of the cortical silent period (CSP), the magnitude of short interval intracortical inhibition (SICI) and intracortical facilitation (ICF), were measured during simultaneous HDSEMG registration from the first dorsal interosseous (FDI) muscle of the dominant hand in 15 healthy volunteers (9 males, 6 females, mean age 69.3).
This study indicated high data quality and methodological validity of our novel electrophysiological approach as well as allowed us to build a HD-TMS data library on healthy volunteers before commencing our main research study with ALS patients. The improved spatial resolution resulting from the high-density approach we developed, allowed for the incorporation of CSP, SICI and ICF into a more detailed 3D anatomical map of the FDI firing.The outcome of this study is now being applied to a 12-month longitudinal clinical study with ALS patients. The application of these findings to the ALS population has the potential to improve our understanding of the topographical distribution and temporal evolution of disinhibition or excess facilitation that has been postulated to underlie cortical hyperexcitability in ALS. Precisely determining how excitability abnormalities evolve and spread over time may help the progression of a detailed anatomical map of disease trajectory in ALS.

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Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.04

Topic: I.04. Physiological Methods

Support: DFG JA 1999/5-1
DFG GE 3008/3-1
ERC StG MEMCIRCUIT 758032
Else-Kroener-Fresenius Foundation

Title: Human intraoperative microelectrode recordings with broad cortical access, single neuron resolution and parallel behavioural monitoring

Authors: V. EISENKOLB[1], **A. UTZSCHMID**[1], L. HELD[1], S. M. KRIEG[2], B. MEYER[2], J. GEMPT[2], *S. N. JACOB[1];
[1]Translational Neurotechnology Lab., [2]Dept. of Neurosurg., Technische Univ. München, Munich, Germany

Abstract: The human frontoparietal association cortex is crucial for executive control. Little is known about how individual neurons and neuronal networks in these brain regions give rise to higher-level cognitive functions such as abstract categorization and working memory. Non-invasive methods such as EEG or fMRI provide good brain coverage, but preclude the investigation of cognitive functions at the cellular level. More specialized methods such as depth electrode recordings in epilepsy patients provide single unit resolution, but do not typically cover the frontoparietal cortex. Here, we report techniques to record from multi-channel microelectrode arrays (MEAs) implanted into the left prefrontal and posterior parietal cortex of neurosurgical patients operated awake for resection of left-hemispheric brain tumors. The arrays can be placed flexibly within the craniotomy, providing broad access to the lateral cortical convexity. We obtained high quality local field potentials (LFPs) in every recording (n = 6). In contrast, the extent of spiking activity depended on the array configuration: lower-density MEAs with 25 channels outperformed standard density MEAs with 96 channels (n = 3 recordings each), yielding an average of one well-isolated single unit per electrode. While recording, we administered a task to the participants that probed their number sense, a cognitive function involving the representation and manipulation of abstract numerical categories. Intraoperative performance was only mildly reduced compared to pre-operative training, but not distorted, and showed all key behavioral signatures of numerical cognition (n = 4 participants). Intraoperatively acquired data permitted us to explore neuronal correlates of the human number sense with single-neuron resolution and on a single-subject basis. Our findings suggest that (1) the human parietal cortex harbors single units tuned to number, (2) at the single-neuron level, non-symbolic set sizes are coded with graded and continuous responses, displaying no sign of neuronal subitizing, (3) the number code partially generalizes across notations with number-congruent responses for non-symbolic and symbolic stimuli, and that (4) symbolic numbers are coded with distinct temporal dynamics and more categorical responses than non-symbolic quantities. The combination of multi-channel recordings directly from the human association cortex with controlled behavioral tasks opens up new possibilities to study species-independent principles of cognitive functioning and to address neuronal mechanisms that govern human-specific cognition on a microcircuit level.

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Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

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Program #/Poster #: 243.05

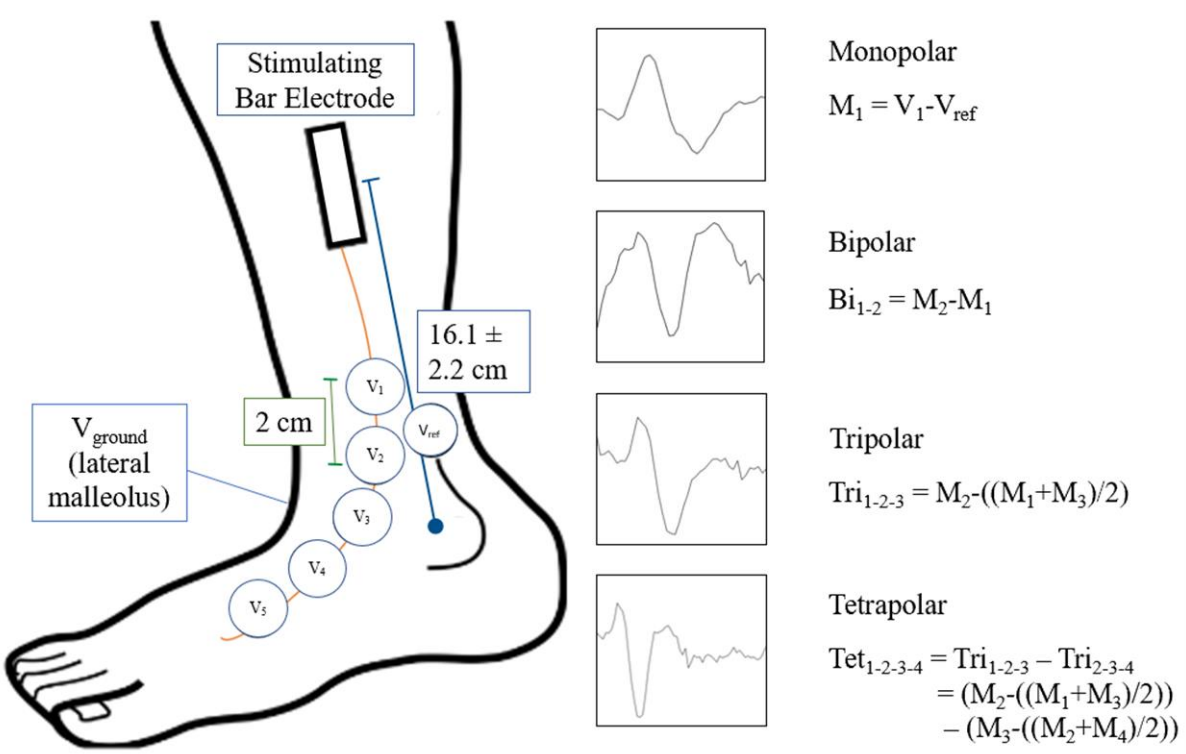
Topic: I.04. Physiological Methods

Support: CGS-M NSERC
MITACS Accelerate Fellowship (EBT Medical)
Lupina Foundation
Massey College Junior Fellowship

Title: The feasibility of non-invasively measuring neural activity evoked by saphenous nerve stimulation in humans

Authors: *A. DOJNOV, S. JOHN, K. MASANI, P. YOO;
Univ. of Toronto, Toronto, ON, Canada

Abstract: Rationale and Objectives. Overactive bladder (OAB) is a large problem, affecting 14-18% of the population in Canada and the US. Saphenous nerve stimulation is an emerging therapy for treating OAB. Stimulation-evoked neural signals can produce the subjective perception of paresthesia, but this does not confirm whether the neural target has been sufficiently activated and may be difficult in patients with impaired sensory processing. An objective measurement of non-invasive nerve stimulation could provide a more accurate representation of neural activation. Unfortunately, since the saphenous nerve is a purely sensory nerve, visible confirmation cannot be accomplished by measuring muscle contraction. This study investigated the feasibility of measuring evoked neural activity evoked as sensed by several cutaneous recording electrode configurations. Methodology. In 16 healthy volunteers (11 male; age = 24 ± 2.1 years, range: 20-28; BMI = 22.5 ± 2.8), stimulation evoked neural activity was measured and analyzed for 4 recording configurations obtained using 5 electrodes located linearly on the top surface of the foot: monopolar, bipolar, tripolar, and tetrapolar (see figure). Peak-to-peak amplitude (Vpp) was measured. Results. A high signal-to-noise ratio can be achieved by averaging the neural signals from a minimum of 70 pulses (i.e., 7 seconds at 10 Hz). Vpp at maximal recruitment ranged between 2-11 μV, which is consistent with literature, with the tetrapolar configuration yielding the largest recorded signal. Different stimulation thresholds follow patterns associated with stimulation-evoked physiological phenomena and reflect expected neural recruitment behaviour. Conclusion and Significance. The results support the feasibility of using non-invasive recording electrodes to measure stimulation-evoked signals from the saphenous nerve. Further work is needed to translate these initial findings into a clinical therapeutic device.



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Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.06

Topic: I.04. Physiological Methods

Support: Polish NCN grant 2015/17/B/ST7/04123
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Title: Kernel Electrical Source Imaging (kESI) method for reconstruction of sources of electric activity in realistic brain geometries.

Authors: *D. K. WOJCIK[1], M. BEJTKA[1], J. M. DZIK[2], C. CHINTALURI[3];
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Abstract: Around 50 million people worldwide are affected by epilepsy. Despite efficiency and steady development of pharmacological treatments, every third patient suffers from intractable seizures. A surgical intervention may be the only solution in these cases. To identify the region for resection neurosurgeons implant intracranial and subdural electrodes which are used to localize the epileptogenic zone from the measured potentials. Here we present kernel Electrical Source Imaging (kESI) method of reconstruction of the brain activity underlying the measured potentials. To reconstruct the current sources we use kernel approximation methods (Potworowski et al., 2012) for the inverse problem. We model the electric field generated by the neural activity (solving the forward problem) with finite element method (FEM). We use both simple geometries (a slice, single sphere, four sphere models) as well as human brain templates and patient-data derived meshes (with possible combination of MR scans for geometry, DTI for conductivity, CT with electrodes for registration). kESI may use information from arbitrarily placed electrodes and integrate patient-specific anatomical information to increase precision of localization of epileptogenic zone for a specific patient. We validate the method with simulated sources in simple geometries where analytical solutaion are possible. We show what can and what cannot be seen with a given electrode setup for any patient (Chintaluri, Bejtka et al. 2021). The method may lead to more precise localization of seizures' origin and better surgical outcome in pharmaco-resistant epilepsy patients.

Disclosures: D.K. Wojcik: None. **M. Bejtka:** None. **J.M. Dzik:** None. **C. Chintaluri:** None.

Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.07

Topic: I.04. Physiological Methods

Title: Chemical Profiling of Human Induced Pluripotent Stem Cell Derived Sensory Neurons on High Density Multi-Electrode Arrays

Authors: *G. MCCABE[1,2], M. O'BIEN[3], P. WALSH[2], M. DAU[2], V. TRUONG[2];
[1]Univ. of Minnesota - Twin Cities, Minneapolis, MN; [2]Anatomic Inc., Minneapolis, MN; [3]MaxWell Biosystems AG, Zurich, Switzerland

Abstract: Multiple sensory neuron sub-types exist in the human dorsal root ganglion, each responding to a broad panel of pain related stimuli based on their expression of receptors. The ability to functionally characterize these sub-populations pharmacologically generates a functional and scalable human platform useful for pain drug discovery. The advent of high-

density multielectrode array (HD-MEA) technologies allows simultaneous access to the activity of thousands of individual neurons at sub-cellular resolution. Electrical signals can be tracked along neurites to enable the investigation of novel parameters, including action potential propagation through the axonal arbor. In this study, a population of human induced pluripotent stem cell (hiPSC)-derived sensory neurons were cultured on the MaxOne System for six weeks. A panel of selective pharmacological stimuli were used to characterize cell-specific ion channels and receptors in the population to classify subclasses. Differences were found in the functional expression of voltage-gated sodium and TRP channels, along with ATP and GABA receptors. Electrical parameters such as spontaneous and evoked action potentials were also studied through the course of maturation. This study demonstrates the feasibility of functionally characterizing hiPSC-derived sensory neurons on a sub-population level useful for various pain drug discovery targets.

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Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.08

Topic: I.04. Physiological Methods

Support: SONY joint Research

Title: Detection of spontaneous firing patterns and drug responses in brain organoids with each single neuron resolution

Authors: *N. MATSUDA, M. SHIBATA, I. SUZUKI;
Tohoku Inst. of Technol., Tohoku Inst. of Tehchnology, Sendai, Japan

Abstract: Brain organoids produced from human-induced pluripotent stem cells (hiPSCs) are expected to use for research on brain disease and drug evaluation. However, due to technical limitations, the number of neurons that can be acquired is small, and the measurable area is also limited. Therefore, there is a need for measurement techniques that can evaluate the electrical activity of organoids over a wide range and with high temporal resolution. In this study, CMOS-MEA with 236,880 electrodes in 28.6 mm^2 was used to measure the extracellular potentials in cerebral organoids with high spatiotemporal resolution, to identify spontaneous firing patterns with each single-neuron resolution. As a result of measuring the spontaneous activities of cerebral organoids, 22 network bursts were detected in 150 seconds, and 6 patterns of activity propagation patterns in the network bursts were identified. Then administration of 4-

aminopyridine, a convulsant, detected changes in the frequency of network bursts and the duration of network bursts. Furthermore, an increase in activity propagation patterns and propagation area in network bursts were detected. High spatiotemporal resolution measurement using CMOS-MEA enabled the simultaneous multi-cellular measurement, the evaluation of cerebral organoids themselves and drug responses based on detailed network activities.

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Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.09

Topic: I.04. Physiological Methods

Title: Maxlab live axontracking assay: label-free identification and functional characterization of axons in neuronal networks at high-throughput.

Authors: D. JÄCKEL, B. CLÉMENT, A. THAMMAVONGSA, ***Z. LI**, M. E. OBIEN; MaxWell Biosystems, Zurich, Switzerland

Abstract: Axons enable neuronal communication by propagating electrical signals within neuronal networks. Its dysfunction plays a central role in deliberating pathologies such as Parkinson's Disease and Amyotrophic Lateral Sclerosis. Therefore, access to axonal physiology is crucial for studying information processing within neuronal networks and accelerating drug development for neurological disorders.High-density microelectrode array (HD-MEA) technology enables chronic label-free *in-vitro* extracellular recordings of axonal action potentials in neurons. MaxOne (single-) and MaxTwo (multi-well) HD-MEA Systems (MaxWell Biosystems, Switzerland) simultaneously capture fast propagating action potentials along multiple axons. Here, we present the MaxLab Live AxonTracking Assay, a software which automatically detects and functionally characterizes axonal signals across hundreds of neurons within a network. We reliably identified and measured from axonal arbors in primary neuronal cultures as well as human iPSC-derived glutamatergic and motor neurons over multiple weeks. We tracked the signal propagation to deduce the conduction velocity, axonal length, and number of axonal branches. We found that the neuronal and branch propagation velocity significantly increased between DIV 13 and 20, corresponding to the maturation of the neuronal network. In conclusion, MaxLab Live AxonTracking Assay combined with HD-MEA technology enables reliable access to electrophysiological recordings of axons, providing a novel functional phenotype for neurological disease modelling and drug screening studies.

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Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.10

Topic: I.04. Physiological Methods

Title: Laser-micromachining of paddle leads for spinal cord stimulation

Authors: M. SCHUETTLER[1], H. KARKAN[2], *J. RICKERT[2];
[1]Cortec GmbH, Freiburg Im Breisgau, Germany; [2]CorTec GmbH, Freiburg, Germany

Abstract: Introduction: Spinal cord stimulation (SCS) is a rapidly growing area in the field of neuromodulation therapies. It is applied to treat chronic pain, peripheral vascular disease, refractory angina, painful diabetic neuropathy, incontinence, and other conditions. Future application will also address restoration of lower and upper limb mobility and other motor-related conditions. Electrical interfaces to the spinal cord are typically designed as percutaneous leads (contacts are arranged as electrically conductive segments of a cable) or as surgical (paddle) leads.
Methods and Materials: We developed a method of producing SCS paddle leads made from medical grade silicone and high-purity metal (Pt90Ir10 alloy) utilizing high-precision laser-micromachining: The paddle area is produced by spin-coating silicone pre-cursor on a mechanical carrier. After curing of the silicone, a metal sheet (25um thickness) is laminated. A laser cuts trenches in the metal sheet, defining the perimeter of electrode contacts, weld contacts and interconnects. Metal sheet not contributing to these elements is removed. A covering layer of silicone is deposited. In a subsequent laser ablation step, electrode contacts and weld contacts are exposed by locally removing the covering silicone layer. A trench is laser cut, defining the perimeter of the paddle, allowing the separation of the paddle from the carrier. Cables are welded to the contacts. The weld area is silicone sealed for electrical insulation. This production method is a variation of the technology used for producing intracortical grid electrodes which received 510(k) notification by the FDA in 2019.
Results: Using the new method, we produced a large range of paddle electrodes, ranging from small devices for fundamental research in rodents (2 contacts, 1mm x 10mm paddle area, 0.1mm paddle thickness) to large paddles suitable for clinical use (16 contacts, 10mm x 80mm paddle area, 2mm paddle thickness). The selection of silicones (harder or softer) enables to achieve different grades of stiffness for the paddles tailored to the requirements of their application.
Discussion: The harder silicones permit pushing the paddles up the spinal canal, while very soft and flexible paddles need to either be placed on the spinal cord through a larger window in the spine or by pulling the paddles using string-like paddle attachments.
Conclusion: A novel SCS lead production method was developed that provides large flexibility in electrode contact design (contact area as small as 0.1mm in diameter) and mechanical properties of the paddle such as stiffness and geometrical shape. Materials and methods permit the use in animals and humans.

Disclosures: **M. Schuettler:** A. Employment/Salary (full or part-time):; CorTec GmbH. **H. Karkan:** A. Employment/Salary (full or part-time):; CorTec GmbH. **J. Rickert:** A. Employment/Salary (full or part-time):; CorTec GmbH.

Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.11

Topic: I.04. Physiological Methods

Support: NeuroOne Medical Technologies

Title: A Novel Polyimide-Based Electroencephalographic Depth Electrode Array: Safety and Performance Testing

Authors: M. VOMERO, S. ONG, B. LASOTA, G. LEY, E. STOCK, S. MERTENS, D. ROSA, *A. KULLMANN, C. A. DIAZ-BOTIA;
NEUROONE, NeuroOne, Eden Praire, MN

Abstract: Introduction Stereoelectroencephalography (sEEG) is a minimally invasive procedure that uses depth electrodes stereotactically implanted into deep brain structures to map the origin and propagation of seizures in epileptic patients. **Objective** Driven by a constant need to develop new sEEG electrodes with novel materials and features, here we describe safety and performance testing of a new thin film polyimide-based sEEG electrode array. **Methods** The sEEG arrays were manufactured from polyimide films wrapped to form a 0.8 mm-diameter cylinder with 5 to 16 Platinum contacts. The devices were designed to fit within an anchor bolt that ensures implant stability and were implanted with the aid of a removable stylet. Electrochemical tests performed on the devices included electrochemical impedance spectroscopy, cyclic voltammetry, voltage transient, and a 29-day aging bath combined with electrical stimulation. Biocompatibility tests included cytotoxicity, hemolysis, sensitization, irritation, acute systemic toxicity, pyrogenicity, a 29-day implant in sheep brain and a 29-day anchor bolt implant in rabbit bone, as per ISO-10993 guidelines. Implantation accuracy was tested in a cadaver model. Planned electrode trajectories were compared with post-implantation trajectories after fusion of pre- and post-operative computer tomography images. Implantation accuracy was quantified using the Euclidean distance for entry point error (EPE) and target point error (TPE). **Results** Electrode impedance was compatible with high quality neural recordings ($248 \pm 36.9\ \Omega$ at 1 kHz, n=15) and electrodes delivered clinically relevant stimulation without leaching metals (n=32 contact pairs). Biocompatibility tests revealed absence of cytotoxicity, hemolysis, sensitization, and irritation, or pyrogenicity. Histological examination of the sheep brain tissue showed no or minimal immune cell accumulation, necrosis, neovascularization, fibrosis, and astrocyte infiltration, with no differences from the control material (n=10, 12 implants from 5 test and 4 control animals, respectively). Cellular response at the anchor bolt - bone tissue interface (n=10 implants from 5 animals) was absent or minimal, with no necrosis,

rare presence of giant cells, macrophages, narrow fibrosis band and minimal capillaries. The EPE was 1.28 ± 0.86 mm and TPE was 1.61 ± 0.89 mm, which is in the range of reports using similar electrodes and methods. **Conclusions** These results demonstrate safety and performance of a new thin film polyimide sEEG electrode array, which is now FDA cleared for < 24 h use.

Disclosures: **M. Vomero:** A. Employment/Salary (full or part-time):; NeuroOne. **S. Ong:** A. Employment/Salary (full or part-time):; NeuroOne. **B. Lasota:** A. Employment/Salary (full or part-time):; NeuroOne. **G. Ley:** A. Employment/Salary (full or part-time):; NeuroOne. **E. Stock:** A. Employment/Salary (full or part-time):; NeuroOne. **S. Mertens:** A. Employment/Salary (full or part-time):; NeuroOne. **D. Rosa:** A. Employment/Salary (full or part-time):; NeuroOne. **A. Kullmann:** A. Employment/Salary (full or part-time):; NeuroOne. **C.A. Diaz-Botia:** A. Employment/Salary (full or part-time):; NeuroOne.

Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.12

Topic: I.04. Physiological Methods

Support: Bakar Fellow
Berkeley Wireless Research Center

Title: Ear EEG: Wireless and Non-Invasive BCI with User-Generic, Dry Electrode Earpieces and Brain-State Classification

Authors: *R. KAVEH, J. DOONG, C. SCHWENDEMAN, A. PANDEY, S. FARAJI ALAMOUTI, C. YALCIN, M. GHANBARI, A. C. ARIAS, R. MULLER;
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Abstract: Recently it has been demonstrated that electroencephalography (EEG) can be recorded from inside the ear (in-ear EEG), opening the door to discreet wearable brain-computer interfaces (BCIs) for monitoring stress, sleep, and medical disorders. However, most demonstrations require individually customized earpieces and lack wireless, end-to-end ambulatory systems. To enable low-profile and comfortable BCIs, this work presents a complete, discreet, and user-generic Ear EEG system. The presented Ear EEG is based on multiple dry electrodes, a user-generic design, and a lightweight wireless interface for streaming data and device programming. Ear EEG recordings were validated across multiple EEG phenomena (alpha attenuation, auditory evoked potentials) and a 9-subject user study to perform drowsiness detection.Ear EEG utilizes a user-generic, dry-electrode earpiece validated through multiple human subject trials and manufactured using 3D printing and electroless plating. The resultant earpiece comprises rigid gold-plated electrodes and a soft skeleton, making it comfortable for long-term use without electrode degradation. Like commercial earbuds, Ear EEG uses small, medium, and large sized earpieces to enable a comfortable fit for never-before-seen users.

Measurements taken with these user-generic earpieces are acquired with a custom integrated circuit (IC) designed for dry electrode recording. The IC has high input impedance, high dynamic range, and can also perform continuous impedance sensing on the electrode to sense contact quality and inform motion artifact removal. Measurements are wirelessly transmitted to a host pc using low-energy Bluetooth for live-plotting, post-processing, and classification. Human subject measurements taken with these gold-plated, dry electrode earpieces have also been used to perform drowsiness classification using logistic regression, support vector machines (SVM), and random forest based classifiers. To estimate drowsiness detection performance across usage scenarios, these classifiers were validated with user-specific, leave-one-trial-out, and leave-one-user-out training. To our knowledge, this is the first wireless, user-generic, multi-channel, dry electrode in-ear EEG to be used for drowsiness monitoring. With user-specific training, an SVM classifier achieved a detection accuracy of 95.9%. When evaluating a never-before-seen user, the classifier achieved a 94.5% accuracy, comparable to the state-of-the-art wet electrode ear and scalp EEG systems. This system enables high-performance Ear EEG for the general population for unobtrusive, continuous, ambulatory neural recording.

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Poster

243. Electrophysiology: Human Application

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Program #/Poster #: 243.13

Topic: I.04. Physiological Methods

Support: NIH R01NS110564

Title: Complimentary in vivo and in vitro approaches to monitoring neuronal and microglial cellular response to electrical microstimulation

Authors: *N. P. WILLIAMS[1], M. PWINT[1], X. S. ZHENG[1], A. VAZQUEZ[2], T. CUI[1];
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Abstract: Over the past few decades, there has been great advancement in the use of chronically implanted microelectrode arrays (MEAs) for recording neuronal action potentials. This technology has shown great promise both clinically for brain computer interface control of robotic prostheses as well as for applications in systems neuroscience advancing our basic understanding of neural information processing. These devices have also recently been used to electrically stimulate tissue, driving action potentials in neurons which can evoke behavioral changes in animals and sensory percepts in humans. However, little is known about the effects this electrical stimulation can have on individual cells. What is know is primarily focused on the responses of neurons. Here, we have taken a dual approach to studying cellular responses to

electrical stimulation both *in vivo* and *in vitro*. We have focused on differentiating the responses of different cell types, studying not only neurons but microglia as well, which have been understudied to date compared to neurons. To assess the effects of electrical stimulation on neurons and microglia *in vivo*, we performed 2-photon imaging on dual-labeled transgenic Cx3cr1-GFP/Thy1-RGECO mice expressing GFP in microglia and a red fluorescent calcium indicator in neurons. A standard single shank 16 channel silicon microelectrode array was implanted into the cortex and electrical stimulation was delivered using pulse waveforms with a 200 µs cathodic leading phase followed by a 200 µs interpulse interval and a 400 µs anodic phase at 50 Hz for 30 seconds. We tested a range of currents from 10 µA to 150 µA. These stimulation parameters were chosen because they are in the range that has been used to evoke behavioral responses in animals and sensory percepts in humans. In addition to the expected increase in neuronal activity, we also observed changes in the surveying behavior of microglia. Along with these *in vivo* experiments, we developed an in vitro MEA system for performing fluorescence microscopy on live cells during electrical stimulation. We seeded primary rat microglia as well as neurons onto transparent MEAs, where we could measure different aspects of the cellular response to stimulation including the timecourse and spatial extent of membrane permeability changes. We found that permeability changes are induced within the current ranges tested, that these changes occur rapidly upon stimulation onset and are highly localized. This approach of studying electrical stimulation via MEAs both *in vivo* and *in vitro* provides complimentary evidence on the effects of stimulation on specific cell types, which is of both clinical as well as basic scientific value.

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Poster

243. Electrophysiology: Human Application

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Program #/Poster #: 243.14

Topic: I.04. Physiological Methods

Support: NIH Grant R24MH120037-03

Title: Nemar: a brain initiative resource for neuroelectromagnetic data, tools, and computation

Authors: *A. DELORME[1], **D. TRUONG**[1], Y. CHOONHAN[1], S. SIVAGNANAM[1], C. STIRM[1], K. YOSHIMOTO[1], R. A. POLDRACK[4], A. MAJUMDAR[2], S. MAKEIG[3]; [2]San Diego Supercomputer Ctr., [3]Inst. of Neural Computation, [1]Univ. of California San Diego, La Jolla, CA; [4]Psychology, 450 Serra Mall Bldg. 420, Stanford, CA

Abstract: To take advantage of recent and ongoing advances in intensive and large-scale computational methods, and to preserve the scientific data created by publicly funded research projects, data archives must be created as well as standards for specifying, identifying, and

annotating the deposited data. The value of and interest in such archives among researchers can be greatly increased by adding to them an active computational capability and framework of analysis and search tools that support further analysis as well as larger scale meta-analysis and large-scale data mining. The *OpenNeuro.org* archive of BIDS-formatted neuroimaging data such an archive. We are building the Neuroelectromagnetic (NEM) Data Archive and Tools Resource (*NEMAR.org*), a portal for search, inspection, and computation on OpenNeuro NEM data (EEG and MEG, iEEG). NEM data uploaded to *OpenNeuro.org* and made public is copied to NEMAR for search and visualization. Identifying and then processing selected data using high-performance computing (HPC)resources can use NEMAR and then The Neuroscience Gateway (*nsgportal.org*), a freely available, easy-to-access portal to national HPC resources that serves many computational environments and packages (MATLAB, Python, R, EEGLAB, Freesurfer, etc.). Users can submit job scripts that use NEMAR data with no need for inefficient data download and reupload. NEMAR is thereby the first example of a freely available integrated data, tools, and compute resource (DATCOR) for neuroimaging data.

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Poster

243. Electrophysiology: Human Application

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Program #/Poster #: 243.15

Topic: I.04. Physiological Methods

Support: Egg Nutrition Center

Title: Interrelationships between mismatch negativity and visual evoked potentials and cognitive, language, and motor development among toddlers

Authors: *L. ROSOK[1], L. FIFIELD[2], R. SARMA[3], S. KEYE[2], A. WALK[4], N. KHAN[1,2,3]; [1]Neurosci. Program, [2]Kinesiology and Community Hlth., [3]Div. of Nutritional Sci., Univ. of Illinois Urbana-Champaign, Urbana, IL; [4]Psychology, Eastern Illinois Univ., Charleston, IL

Abstract: Visual evoked potentials (VEPs) and mismatch negativity (MMN), two event-related potential (ERP) outcomes, have been associated with visual processing and change detection abilities, respectively. Several studies have associated smaller latencies and larger amplitude differences in these components with greater cognitive function in adults. However, the characteristics of these neuroelectric outcomes in relation to cognitive, language, and motor skills in toddlers is unknown. This study aimed to examine how a neuropsychological battery consisting of cognitive, language, and motor subtests may relate to visual and cognitive ERP outcomes among toddlers. Toddlers 12-18 months old (N = 34, F = 17) participated in the study. Cognitive, language, and motor abilities were measured using the Bayley Scales of Infant and

Toddler Development IV Screening Test (BSID-IV). ERPs were recorded during a two-stimulus passive auditory oddball task, which has been shown to evoke MMN in adults (FZ and CZ electrodes), and a passive pattern reversal task to evoke VEPs (OZ electrode), namely the N75 and P100. Following initial bivariate correlations, partial correlations were conducted to assess any relationship between BSID-IV and ERP outcomes, adjusting for age as a covariate. Upon analyzing MMN variables, cognition (rs = -.353, p = .024) and fine motor (rs = -.463, p = .004) BSID-IV subsets were negatively associated with the difference (deviant – standard) in CZ positive peak latency and the difference in FZ negative peak latency, respectively (100-200 ms). Gross motor skills were positively related to the difference in FZ positive peak latency (rs = .359, p = .013*)*. VEP correlations showed that cognitive (rs = -.335, p = .033), fine motor (rs = -.340, p = .031), and gross motor (rs = -.351, p = .026) BSID-IV subsets were negatively related to OZ peak N75 mean latency.Toddlers with greater cognitive, fine motor, and gross motor skills exhibited faster visual processing speed. Further, toddlers with smaller latency differences between standard and deviant auditory stimuli had greater cognitive and fine motor skills. BSID-IV gross motor skills, alternatively, were related to larger latency differences. Larger prospective studies during early childhood are needed to confirm the findings of the present work.
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Poster

243. Electrophysiology: Human Application

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Program #/Poster #: 243.16

Topic: I.04. Physiological Methods

Title: Development of a Multi-Electrode Array (MEA) Assay for Phenotypic Drug Screening

Authors: *J. KAMMONEN, S. WILLIAMS, L. BUTLER, D. MAGNANI, J. ANTON, C. MANSAT-BHATTACHARYYA, G. GULBAHCE, R. BURLEY, M. IOVINO;
Early Discovery, Charles River, Saffron Walden, United Kingdom

Abstract: Multielectrode array (MEA) systems allow measurement of cellular activity (label-free and real-time) to detect neural network activity or cardiac functionality, as well as cell growth, death, and attachment. MEAs can be utilised in drug discovery as a non-invasive technique, which can investigate the electrical properties of isolated or cultured cells. This can provide a link from *in vitro* screening to *in vivo* testing, through safety or efficacy assays to show compound effects on cardiomyocytes or neurons, or by modelling the functional impacts of disease mutations. Here we describe the characterisation of a cortical model of iPSC derived glutamatergic and GABAergic neurons in co-culture with astrocytes (NeuCyte SynFire), and the development of a compound screening assay using the Maestro Pro MEA instrument (Axion BioSystems).

Immunocytochemistry showed the presence of both Glutamatergic (VGLUT1) and GABAergic (GABA) neuronal markers along with astrocytic markers (GFAP) in the cultured cells, which formed a dense, interconnected network. Over the course of 28 days, this neuron/astrocyte co-culture showed an increase in mean firing rate as well as the development of spontaneous oscillatory activity (network bursts) with increasing synchronicity. Disruption of this intrinsic activity could be achieved through the application of diazepam ($GABA_A$ positive allosteric modulator), which showed a decrease in mean firing rate and network burst activity with an IC_{50} comparable to literature data. Furthermore, seizurogenic activity was observed in response to bicuculline ($GABA_A$ antagonist) as shown by an increase in network burst frequency and duration. These data demonstrate the suitability of this system for compound profiling, proof of concept *in vitro* studies and assessment of seizurogenic liability in neuronal cultures.

Disclosures: **J. Kammonen:** A. Employment/Salary (full or part-time):; Charles River. **S. Williams:** A. Employment/Salary (full or part-time):; Charles River. **L. Butler:** A. Employment/Salary (full or part-time):; Charles River. **D. Magnani:** A. Employment/Salary (full or part-time):; Charles River. **J. Anton:** A. Employment/Salary (full or part-time):; Charles River. **C. Mansat-Bhattacharyya:** A. Employment/Salary (full or part-time):; Charles River. **G. Gulbahce:** A. Employment/Salary (full or part-time):; Charles River. **R. Burley:** A. Employment/Salary (full or part-time):; Charles River. **M. Iovino:** A. Employment/Salary (full or part-time):; Charles River.

Poster

243. Electrophysiology: Human Application

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Program #/Poster #: 243.17

Topic: I.04. Physiological Methods

Support: NIH SBIR 1R43NS120753-01

Title: Hardware-software systems framework using high-density neural probes and AI-driven real-time neuro-analytics and deep learning networks to optimize neural interfaces at the neural circuit level

Authors: A. GOLABCHI, A. PAEZ, C. FREWIN, R. VETTER, J. HETKE, ***D. KIPKE**; NeuroNexus Technologies, Inc., Ann Arbor, MI

Abstract: Ongoing advances in neural interface technologies are providing increasingly powerful tools for investigating neural circuits. Tool usage constitutes advanced electrophysiological workflows, with acquiring raw extracellular potentials from neural probes as the front end and executing the algorithms and visualization of curated and conditioned neural data streams as the back end. In this systems context, these front-end and back-end technologies are typically not well integrated, leading to significant workflow inefficiencies and bottlenecks. The objective of this study was to develop and characterize an integrated system (Summa

Framework) to provide a semi-autonomous solution for optimizing intra-operative workflows as a function of both underlying component technologies and application requirements. The Summa Framework was developed consistent with a canonical Internet-of-Things (IoT) architecture, such that the hardware-software neural recording system was designed as an “edge” device and signal conditioning and analytics algorithms were designed as edge and cloud computing layers. The framework automatically supports various neural interfaces over a wide design space, from active high-density SiNAPS probes with 1024 individually and concurrently addressable sites to thin-film conformal surface grids. A high-performance conductive polymer site coating (Z-coat) was developed to optimize signal transduction characteristics of diverse types of electrode sites. The FPGA-based instrumentation system implemented a firmware layer for programming the probes and acquiring high-bandwidth real-time neural data streams. A comprehensive software platform was developed to provide semi-autonomous system control, from probes at the front end to short-latency (real time to several minutes) signal analysis, visualization, and reporting at the back end. An AI layer was implemented to provide assistive system control. A cloud layer was implemented for automatic system provisioning and signal feature storage. Implementing the Summa Framework resulted, in part, in real-time data acquisition, spike sorting, and three-dimensional visualization of each electrode array configuration within the target tissue-interface region. The software further allows increased experiment efficiency through the identification of cell types by their electrical behavior. The AI-driven real-time system programming and data analysis significantly improved system performance across several workflow parameters, including latency-to-estimated-neuronal-ensemble and integrated signal-to-noise optimization.

Disclosures: **A. Golabchi:** A. Employment/Salary (full or part-time):; NeuroNexus. **A. Paez:** A. Employment/Salary (full or part-time):; NeuroNexus. **C. Frewin:** A. Employment/Salary (full or part-time):; NeuroNexus. **R. Vetter:** A. Employment/Salary (full or part-time):; NeuroNexus. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroNexus. **J. Hetke:** A. Employment/Salary (full or part-time):; NeuroNexus Technologies Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroNexus Technologies Inc. **D. Kipke:** A. Employment/Salary (full or part-time):; NeuroNexus Technologies, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroNexus Technologies, Inc..

Poster

243. Electrophysiology: Human Application

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Topic: I.04. Physiological Methods

Support: NSF Grant 1632738
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Title: Signal quality of tripolar visual evoked potentials

Authors: *M. V. WISE, G. P. CAPLOVITZ, M. A. CROGNALE;
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Abstract: Tripolar concentric ring electrodes (TCREs) have been designed to improve upon standard EEG methods. Standard EEG is known to be vulnerable to motion and muscle artifacts, requiring both a compliant participant and/or lengthy recording to produce high-quality signals. Conversely, TCREs utilize three conductive surfaces and advanced signal processing to remove noise from the recording in an effort to produce more robust signals. To test the efficacy of this approach in the context of the visual system, signal to noise ratios (SNRs) were compared using standard clinical visual evoked potential (VEP) stimuli (high-contrast checkerboard, 2 reversals/sec). Three conditions were assessed: large 1° checks, small .25° checks, and large 1° checks with added muscle noise (by clenching of the jaw). Recording 100 reversal events produced similar SNRs between the tripolar and the standard signals. SNR was also measured as a function of recording time to determine if the TCREs provided an advantage for shorter recording periods. Again, there was no advantage provided by the TCREs even for the shorter recording periods, in fact the standard recording slightly outperformed the TCREs. However, a significant advantage for the TCREs was found for the added noise condition, in accord with prior reports of a benefit for TCREs under conditions of high noise such as seizure detection and motor-evoked responses. These findings suggest that in studies of the visual system, under difficult (high-noise) conditions the TCREs resolve signals better than standard recording, whereas under optimal recording conditions, TCREs provide no significant advantage over standard methods of recording the VEP.

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Poster

243. Electrophysiology: Human Application

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Topic: I.04. Physiological Methods

Support: NIH Grant DP2-EB029757
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Title: Human Brain Mapping with Multi-Thousand Channel PtNRGrids Resolves Spatiotemporal Dynamics

Authors: *Y. TCHOE[1], A. BOURHIS[1], D. R. CLEARY[1], B. STEDELIN[5], J. LEE[2], K. J. TONSFELDT[3], E. C. BROWN[6], D. SILER[5], A. C. PAULK[7], J. C. YANG[7], H. OH[8], Y. RO[4], K. LEE[10], S. RUSSMAN[11], M. GANJI[9], I. GALTON[10], S. BEN-HAIM[12], A. M. RASLAN[14], S. DAYEH[13];
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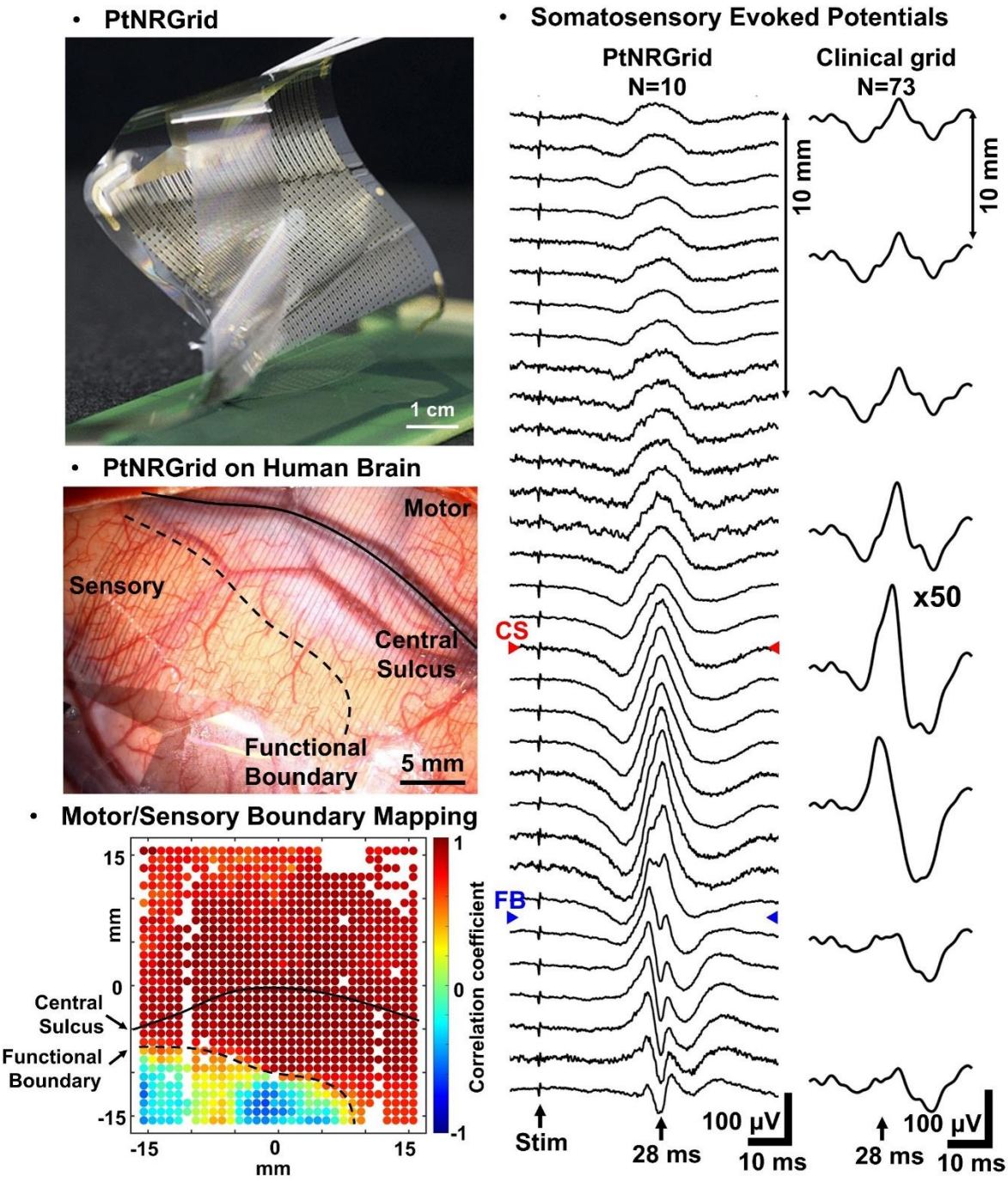
Abstract: Electrophysiological devices are critical for mapping eloquent and diseased brain regions and therapeutic neuromodulation in clinical settings and are extensively utilized for research in brain-machine interfaces. However, the existing devices are often limited in either spatial resolution or cortical coverage, even including those with thousands of channels used in animal experiments. Here, we developed scalable manufacturing processes and dense connectorization to achieve reconfigurable thin-film, multi-thousand channel neurophysiological recording grids using platinum-nanorods (PtNRGrids). With PtNRGrids, we have achieved a multi-thousand channel array of 30 μm contacts with low impedance, providing unparalleled spatial and temporal resolution over a large cortical area. The 7” long cortical electrodes together with our sterilizable compact connector overcome challenges associated with the required large separation between the sterile surgical field and the non-sterile acquisition electronics enabling reliable intraoperative recordings from patients undergoing neurosurgical resections. In the clinical setting, PtNRGrids resolved fine, complex temporal dynamics from the cortical surface in an awake human patient performing grasping tasks. HGA showed distinctive neural correlates of hand movements. Furthermore, we recorded phase reversal boundaries during motor mapping to precisely localize the central sulcus in sub-mm scale resolution. These results with our high-density grids offer an unprecedented view of the functional organization and coordination of motor function over large brain regions in the human cortex. Additionally, the PtNRGrids identified the spatial spread and dynamics of epileptic discharges in a patient undergoing epilepsy surgery at 1 mm spatial resolution, including activity induced by direct electrical stimulation. Collectively, these findings demonstrate the power of the PtNRGrids to transform clinical mapping and research with brain-machine interfaces and highlights a path toward novel therapeutics.



Disclosures: **Y. Tchoe:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Have equity in Precision Neurotek Inc. that is cofounded by the team to commercialize PtNRGrids for intraoperative mapping.. **A. Bourhis:** None. **D.R. Cleary:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Have equity in Surgical Simulations. **B. Stedelin:** None. **J. Lee:** None. **K.J. Tonsfeldt:**

E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Have equity in Surgical Simulations LLC. **E.C. Brown:** None. **D. Siler:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Have equity in Surgical Simulations LLC. **A.C. Paulk:** None. **J.C. Yang:** None. **H. Oh:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); competing interests not related to this work including equity in FeelTheTouch LLC. **Y. Ro:** None. **K. Lee:** None. **S. Russman:** None. **M. Ganji:** None. **I. Galton:** None. **S. Ben-Haim:** None. **A.M. Raslan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Has an equity and is a cofounder of CerebroAI. Have equity in Precision Neurotek Inc.. F. Consulting Fees (e.g., advisory boards); Received consulting fees from Abbott Inc. and Biotronik Inc. **S. Dayeh:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Have equity in Precision Neurotek Inc. Have competing interests not related to this work including equity in FeelTheTouch LLC.. F. Consulting Fees (e.g., advisory boards); Was a paid consultant to MaXentric Technologies..

Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.20

Topic: I.04. Physiological Methods

Support: Tiny Blue Dot Foundation

Title: Transcranial magnetic stimulation-electroencephalography detects fluctuations in level of consciousness in patients with severe brain injuries

Authors: *M. FECCHIO[1], J. N. KELEMEN[1], M. K. CAMBARERI[1], R. M. MARUJO[1], W. R. SANDERS[1], A. MEYDAN[1], Y. G. BODIEN[1,3], B. L. EDLOW[1,2];
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Abstract: After severe brain injury, a patient's level of consciousness is typically assessed at the bedside by attempting to elicit behavioral responses such as command-following. However, because the behavioral examination can be confounded by deficits of sensory, executive, and motor functions, advanced electrophysiologic markers of consciousness have been proposed. The Perturbational Complexity Index (PCI), a measure of brain complexity that is based on the analysis of EEG responses to Transcranial Magnetic Stimulation (TMS-EEG), has high sensitivity (94%) for detecting consciousness in patients who have recovered to a minimally conscious state (MCS). It remains to be determined whether TMS-EEG measurements of PCI

reliably detect fluctuations in the level of consciousness as patients transition between wakefulness and sleep. We tested the sensitivity of PCI for detecting changes in arousal in patients with severe brain injuries as they transitioned from an awake state (i.e., eyes open) to a drowsy/sleeping state (i.e., eye closure for approximately 20 minutes). We enrolled three patients with a severe brain injury and disorder of consciousness (DoC) in a four-day inpatient research study at Massachusetts General Hospital. We completed behavioral assessments (i.e., Coma Recovery Scale-Revised [CRS-R]) and conducted TMS-EEG measurements targeting frontal and parietal cortical regions. We used the Nexstim Navigated Brain Stimulation system (NBT 2), which enables precise neuronavigation and reproducible TMS targeting. EEG signals were recorded with a 62-electrode cap (EASYCAP GmbH) connected to a TMS-compatible amplifier (BrainAmp DC, Brain Products, GmbH). PCI values were calculated by applying the automatic procedure described in Casarotto et al., 2016. All three patients experienced a severe traumatic brain injury (age from 22 to 35 years, 1 male, days post-injury range from 457 to 2166). CRS-R assessment suggested a vegetative state (VS) in two patients and a MCS in one patient. Both patients in VS had previously documented behaviors consistent with MCS. In all 3 patients, the maximum PCI value was above the operational threshold (PCI > 0.31) for capacity for consciousness. While suprathreshold complexity values were recorded during behavioral wakefulness, PCI dropped below the threshold during periods of prolonged (> 20 minutes) eye closure. These preliminary results suggest that PCI detects fluctuations in arousal in patients with severe brain injuries. These observations thus provide the basis for further investigation into using PCI to evaluate the efficacy of therapies aimed at promoting recovery of arousal/wakefulness.

Disclosures: **M. Fecchio:** None. **J.N. Kelemen:** None. **M.K. Cambareri:** None. **R.M. Marujo:** None. **W.R. Sanders:** None. **A. Meydan:** None. **Y.G. Bodien:** None. **B.L. Edlow:** None.

Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.21

Topic: I.04. Physiological Methods

Support: Brain Canada Grant, BC MIRI2015-3758

Title: Considerations for optimizing investigative brain stimulation during intraoperative mapping.

Authors: *M. K. MCINTOSH, *R. LEVY;
Queen's Univ., Kingston, ON, Canada

Abstract: Electrical stimulation is used to localize therapeutic zones and prevent unnecessary functional damage during neurosurgery. While mapping intraoperatively, it is common practice

to stimulate with a current above the presumed threshold for response. Suprathreshold current is used to ensure higher elicitation probability. It is generally held that the upper limit for eliciting response is defined only by cellular damage or hardware limitations. However, computational models predict that there is an upper threshold to field evoked potential (fEP) elicitation which is beneath the threshold for damage. To test this prediction, two non-human primates were implanted with electrodes in 3 common clinical targets, the amygdala, hippocampus and entorhinal cortex. Combinations of stimulation parameters were systematically varied with pulse widths between 50-200 s and current amplitudes between 1-500 A. Responses were detected with a custom algorithm and then manually inspected to reduce the likelihood of contamination by motion artifact. A total of 6480 fEPs were used for analysis. Results indicate that increasing stimulation current amplitude beyond a threshold can cause diminishment or elimination of fEPs. This indicates that, in a clinical setting, stimulation current should not be increased arbitrarily and should instead be delivered within a range for response specific to the network or region of interest. Results additionally indicate that consecutive micro-stimulation pulses, even when separated by 10 second inter-pulse intervals, result in apparent habituation of the tissue and reduction of fEP peak magnitude. This reduction was most prominent in the entorhinal cortex, reaching 100% during multiple sessions. The maximum number of stimulation pulses that could be used for accurate mapping varied per region and was between 1 and 20. Viewing investigative stimulation from multidisciplinary lens allows for the practical interpretation of response waveforms and for the prediction of which parameters will optimize their elicitation. Taken together, these results have important implications in the use of intraoperative mapping stimulation to make clinical decisions.

Disclosures: **M.K. McIntosh:** None. **R. Levy:** None.

Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.22

Topic: I.04. Physiological Methods

Support: ERC-StG 759651

Title: Spectral connectivity fingerprint in the resting human brain: a MEG study

Authors: *O. MADDALUNO[1,2], R. GUIDOTTI[3], A. VETTORUZZO[4], L. MARZETTI[5,6], G. CISOTTO[7], V. BETTI[1,2];
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Abstract: Intrinsic functional connectivity reflects spontaneous neuronal fluctuations that are functionally coupled and organized in networks (the so-called resting-state networks, RSNs) in the absence of active tasks. Functional magnetic resonance imaging (fMRI) studies suggest that the coherent spontaneous activity accounts for variability in event-related BOLD responses, and it predicts inter-individual differences in brain responses during task performance. Recent evidence shows that spontaneous activity patterns are related to inter-individual differences in cognition, personality traits, and behavioral performance. In the present research, we investigated how intrinsic functional connectivity predicts the inter-individual difference in task-evoked connectivity in the alpha and beta bands, the neurophysiological correlates of RSNs.We analyzed MEG data from the Human Connectome Project collected from 51 subjects (age range 22-35 yo) during visual fixation and during a motor task (*i.e.*, finger tapping or toe squeezing). We computed the leakage-corrected band-limited power (BLP) correlation across 164 node regions - parcelled into 10 networks - in alpha (6.3-16.5 Hz), low beta (12.5-29 Hz), and high beta band (22.5-39 Hz). Then, we used a univariate model to explore if the variance of functional connectivity can be explained in terms of the band, subject, and task. Finally, we used a leave-one-out approach to predict task-evoked connectivity patterns from the connectivity at rest. We evaluated how accurate is the model in predicting the single subject's connectivity pattern using the *identification rate* (*ID rate*). Our results show that 1) the frequency band and the subject account for most of the explained variance (41.13% and 22.32%, respectively), while the task explains only 6.02% of the variance. 2) ID rate values are higher (above 58%) in the Motor Network (MN) and the Visual Network (VIS) in alpha and low beta bands for both tasks (*i.e.*, finger tapping and toe squeezing). In all the other RSNs and frequency bands, the ID rate was lower than 50%. Our findings show that inter-individual variability modulates functional connectivity. Moreover, we demonstrate that from intrinsic connectivity patterns, it is possible to draw inferences about the connectivity patterns during a motor task, at the level of the single subject, in specific RSNs (MN and VIS) and frequency bands. This evidence supports the existence of a MEG connectivity fingerprint already present at rest. This fingerprint is unique for each individual and can accurately predict the connections reorganization during a motor task.

Disclosures: O. Maddaluno: None. **R. Guidotti:** None. **A. Vettoruzzo:** None. **L. Marzetti:** None. **G. Cisotto:** None. **V. Betti:** None.

Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.23

Topic: I.04. Physiological Methods

Support: NSF Grant 1831962
NSF Grant 1827847

Title: Development of Efficient Electrical Impedance Tomography System for Fast Brain Imaging Applications

Authors: *H. V. TRAN[1], M. KIM[2], T. C. LE[3], H. YOON[3];
[1]Ctr. for Materials Res., [2]Physics, [3]Dept. of Engin., Norfolk State Univ., Norfolk, VA

Abstract: Fast Electrical Impedance Tomography (EIT) has high potential for imaging fast nueral activity for understanding brain function and diagnosing neurological disorders and diseases. In this research, the prototype of the EIT system and various phantoms have been developed and validated. This EIT system consists of three main parts: EIT image reconstruction algorithm, hardware module - alternating current injection and induced potential measurements, and a phantom with an electrode array on the boundary of the sensing domain. The EIT hardware module is an all-in-one measurement system board that includes a current source, multiplexing module, ADCs and a control module that results in fast, accurate, and real-time imaging of impedance change inside of the brain. Graphic user interface allows easy selection of the number of electrodes that are setup between 8, 16, and 32 and arrangement of electrode array among 16×1, 8×1, and 4×4. A reconstruction algorithm has been modified to adapt various sets of electrode arrangements and numbers. Phantoms with these parameters have been developed to assess the performance of EIT systems while providing electrical and mechanical properties similar to the human brain. To validate our system, various objects with different electrical impedances, size, and materials were placed inside of our phantom and measured. In addition, Monte Carlo simulations accompanied by ground experimentation were conducted to improve the image reconstruction algorithm. We also utilized human neural phantoms that featured a skull structure and tissue matter that reflect the composites of a human brain to validate our system and provide information to correct the EIT recontruction algorithms. Therefore, this prototype EIT system successfully visualizes fast, real-time changes of impedance distribution in our neural phantom, deeming promising results for conducting human trials.

Disclosures: **H.V. Tran:** None. **M. Kim:** None. **T.C. Le:** None. **H. Yoon:** None.

Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.24

Topic: I.04. Physiological Methods

Support: NIH R01NS096008
NSF PECASE 1553482

Title: Chronic Biomarker Identification Of Tic Onset In Centromedian Thalamus For Closed Loop Deep Brain Stimulation In Tourette Syndrome

Authors: *J. GOMEZ[1], A. GUNDUZ[2], M. S. OKUN[1], K. FOOTE[1];
[2]Biomed. Engin., [1]Univ. of Florida, Gainesville, FL

Abstract: Tourette Syndrome (TS) is a neuropsychiatric disorder characterized by repetitive and involuntary motor and phonic tics. Most cases present symptoms that start and stop during childhood, but for some people, symptoms remain and can get progressively worse during adulthood. Deep Brain Stimulation (DBS) could offer an alternative therapy when symptoms become refractory and preliminary results have shown up to a 60% average decrease in motor tics with continuous DBS to the Centromedian (CM) Nucleus of the Thalamus. However, the paroxysmal nature of this disorder leads us to believe that patients would benefit from closed-loop DBS therapy. We are testing this by implanting four patients (3 males, 1 female) with bilateral macro electrodes targeting the CM and subdural electrodes targeting M1, both connected to an RC+S neurostimulator from Medtronic, a device capable of streaming signals and electrically stimulate the region of interest. Patients also underwent preoperative MRI and postoperative CT scans, used for electrode localization. After implantation, patients visit the center periodically, where we ask them to rest, freely tic, and voluntarily move their hands when shown a cue, while we collect data from their implanted device and wearable sensors in their upper extremities and neck. Later, we align and mark down the data, to separate and average a ~±3 second window spectrogram before and after they tic. We have collected electrophysiological recordings from each visit, up to 24 months after surgery until now. We have identified low-frequency biomarkers (3-10 Hz) after tic onset in this cohort, shown as increased activity in dark red after the tic onset shown as a black line. We have also been able to start closed-loop DBS in one subject thus far using only this LFP signal. This allows obtaining similar benefits to continuous stimulation with potentially fewer side effects and longer battery life. There are still many challenges left to find the optimal settings for each patient, due to the variable presentation of the tics and the psychiatric components of TS. In a future study, we plan to not only stimulate CM but also the anterior globus pallidus interna (aGPi), whose limbic components could potentially prove beneficial to the psychiatric comorbidities of TS. Identifying physiological features in both nuclei can help us understand the underlying neuronal mechanisms and pathophysiology of this disease and could guide the development of future therapies for TS, accommodating the specifics of each patient.

Disclosures: **J. Gomez:** None. **A. Gunduz:** None. **M.S. Okun:** None. **K. Foote:** None.

Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.25

Topic: I.04. Physiological Methods

Support: ARC Future Fellowship FT160100077
Alfred Deakin Postdoctoral Research Fellowship

Title: Assessment of Age and Sex Effects on EEG-Derived Microstates in a Neurodevelopmental Cohort

Authors: *A. T. HILL, F. J. BIGELOW, J. A. G. LUM, P. G. ENTICOTT;
Cognitive Neurosci. Unit, Deakin Univ., Melbourne, Australia

Abstract: When analysed over very brief time periods (~60-120 ms), the spatial topography of the electroencephalogram (EEG) displays a series of discrete quasi-stable activation patterns known as 'microstates.' Each distinct microstate represents a transient period of global network activity, with shifts between different microstates over time indicative of large-scale changes in functional network organisation. Here, we used a microstate-based approach to explore neurodevelopmental changes in brain network activity across early-to-middle childhood. 139 typically developing children aged between 4-12 years (67 female; average age = 9.41 years, SD = 1.95) underwent two minutes of eyes-closed resting-state EEG recorded using a 64-channel Geodesic Sensor Net. Following pre-processing, the data were assigned to four separate microstate classes using a polarity-invariant (modified k-means) clustering algorithm. Prior to statistical analysis, any extreme outliers (>1.5*IQR) were removed from the microstate data. Multiple linear regressions were then run to assess if age and biological sex could predict the following microstate parameters: coverage, duration, and occurrence (overall regression model Bonferroni corrected for the four microstate classes). The four microstates obtained in the present study closely matched the canonical classes 'A', 'B', 'C', and 'D' frequently reported in the literature and together explained 67.2% of the total global variance in the data. The overall regression models for coverage, duration, and occurrence were significant for all microstate classes (all $p < .05$ [Bonferroni corrected]), with the exception of microstate B. Examination of the main effects for each regression model indicated that, irrespective of the specific microstate parameter, there was always a significant main effect of sex (all $p < .05$), but not age ($p > .05$), with females consistently having higher coverage, duration, and occurrence values for microstates A and C; while, conversely, males demonstrated higher values for microstate D. These results indicate clear sex-related alterations in microstate dynamics during the neurodevelopmental period spanning early-to-middle childhood when using a large dataset of neurotypical participants. Future work could extend these analyses to examine network activity patterns in individuals with specific neurodevelopmental disorders.

Disclosures: A.T. Hill: None. **F.J. Bigelow:** None. **J.A.G. Lum:** None. **P.G. Enticott:** None.

Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.26

Topic: I.04. Physiological Methods

Title: Investigating the effects of attention on afferent inhibition via transcranial magnetic stimulation

Authors: *K. RAMDEO[1], R. S. REHSI[1], S. D. FOGLIA[2], C. V. TURCO[3], S. L. TOEPP[1], J. W. PICKERSGILL[1], A. J. NELSON[1];

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Abstract: Attention can alter transcranial magnetic stimulation (TMS) evoked afferent inhibition, a measure of cortical inhibition that follows somatosensory input. Measures of afferent inhibition are emerging as valuable tools for clinical assessment of sensorimotor function. However, the reliability of afferent inhibition remains relatively low, limiting its value in the clinic. Attention focused on the somatosensory input is capable of eliciting a deeper magnitude of afferent inhibition. However, it is unknown whether afferent inhibition, with attention directed to somatosensory input, will improve the reliability of these measures. This is important as it suggests that methodology can improve the reliability of this measure thereby increasing the opportunity for translation to the clinic. The goal of this study was to assess the influence of attention on afferent inhibition circuits, short afferent inhibition (SAI), and long afferent inhibition (LAI) and determine whether attention modulation would increase the reliability of afferent inhibition. Thirty adult individuals participated in 3 conditions that were identical in their physical parameters and varied only in the focus of directed attention. During the 'somatosensory focused' condition, participants were asked to direct attention towards a tactile stimulation being delivered to the right forearm. The stimulation was delivered as either 1, 2 or 3 pulses. When the user felt 3 pulses they were instructed to press a button located near the left hand. During the 'visual focused' condition, attention was directed towards a visual stimulation that consisted of a black screen with a white circle fluctuating in intensity. When a change is observed the user was asked to respond with a button press.During the 'no focus' condition, the user was asked not to respond to any changes (visual or somatosensory). A fourth condition, 'rest' was such that all physical external stimuli were absent. Twenty SAI and LAI, evoked by peripheral nerve stimulation at the median nerve paired with a TMS pulse to the M1 region in the left hemisphere, were recorded during each condition with an additional 20 test stimuli. Reliability was measured by repeating conditions at 3 time points to assess intrasession and intersession reliability. Results indicate the focused attention state elicits the greatest inhibition. Reliability assessments are pending further analyses. This research will expose the influence of attention, and its impact on the reliability of afferent inhibition. By quantifying these influences, this research will make significant strides to inform the design of TMS research in sensorimotor integration.

Disclosures: K. Ramdeo: None. **R.S. Rehsi:** None. **S.D. Foglia:** None. **C.V. Turco:** None. **S.L. Toepp:** None. **J.W. Pickersgill:** None. **A.J. Nelson:** None.

Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.27

Topic: I.04. Physiological Methods

Title: Investigating the Intrasession Reliability of Short- and Long- Afferent Inhibition

Authors: *R. S. REHSI[1], K. RAMDEO[1], S. D. FOGLIA[2], S. L. TOEPP[1], J. W. PICKERSGILL[1], C. V. TURCO[3], A. J. NELSON[1];
[1]Kinesiology, [2]Sch. of Biomed. Engin., McMaster Univ., Hamilton, ON, Canada; [3]Fac. of Med. and Dent., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Afferent Inhibition is the reduction in motor output when Transcranial Magnetic Stimulation (TMS) of the motor cortex is preceded by peripheral nerve stimulation. Afferent inhibition is composed of two circuits of Short-Latency (SAI) and Long-Latency Afferent Inhibition (LAI) depending on the inter-stimulus interval between the TMS and nerve stimulation. Relative reliability refers to the ability of a measure to identify individuals on repeated testing, and absolute reliability is the repeatability of scores through repeated testing. Current literature has highlighted only the intersession relative and absolute reliability of SAI and LAI, but measures of the intrasession reliability are also needed. This study aims to quantify the relative and absolute intrasession reliability of SAI and LAI, and to identify the minimum number of trials needed to obtain a reliable measure. Thirty individuals (21.17 ± 2.84 years; 17 Females) participated in a single 3-hour session. Each session consisted of three repeated measures of SAI and LAI separated by 30-minute rest periods. TMS was delivered to the hotspot of the right first dorsal interosseous muscle (FDI) over the left motor cortex, and nerve stimulation to the right median nerve at the wrist. LAI was elicited by stimulating the median nerve prior to the TMS pulse at an ISI of 200ms and SAI at an ISI normalized to the N20 latency of the participant + 2ms. EMG signal was recorded from the right FDI. Relative and absolute reliability was assessed across the three sessions. To identify the minimum number of trials required to reliably elicit SAI and LAI, relative reliability was assessed at running intervals of every 5 trials through measures of the intraclass correlation coefficient. LAI displayed excellent reliability when only 5 trials were included, whereas SAI required 20 trials to achieve high relative reliability. Both SAI and LAI also had similarly high levels of measurement error, as reflected by the high levels of Standard Error of Measurement and Smallest Detectable Change (SDC). However, the measures can be used for group change as indicated by moderate levels of the SDC at the group level. This is the first study to quantify the intrasession reliability of SAI and LAI, allowing for the determination of whether intrasession changes in other studies are due to interventions or the expected variation due to measurement error. Further, these results can be used to inform future work exploring the diagnostic utility of these measures in clinical populations.

Disclosures: R.S. Rehsi: None. **K. Ramdeo:** None. **S.D. Foglia:** None. **S.L. Toepp:** None. **J.W. Pickersgill:** None. **C.V. Turco:** None. **A.J. Nelson:** None.

Poster

243. Electrophysiology: Human Application

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 243.28

Topic: I.04. Physiological Methods

Support: ANR-18-CE92-0048
ANR-11-IDEX-0007

Title: The macaque VIP functional connectivity patterns imply areal expansion into three parietal homologues in humans

Authors: *W.-A. SHENG[1], S. CLAVAGNIER[1], W. VANDUFFEL[2], S. BEN HAMED[1];
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Abstract: The intraparietal sulcus (IPS) is involved in multisensory integration, and the ventral intraparietal area (VIP) in the IPS fundus responds to visual, vestibular, tactile and auditory signals, with tactile receptive fields over-representing the face. In macaques, VIP has been divided cytoarchitonically into medial and lateral parts, however, no functional specialization has been associated up to now to these two divisions. In contrast, task fMRI data identifies a functional gradient along the anterior-posterior axis, where the anterior part shows visual-tactile properties and face preference, while the posterior part is activated by visual large-field dynamic stimuli. This is well in line with our recent description of the human VIP complex containing three VIPs revealed by reviewing human studies attempting to identify a potential VIP homologue.
The above description is inferred based on comparing task fMRI in two species and is limited by task design, activation sites and number of subjects involved. In order to further delineate the topographical functional gradients within VIP, we probe the functional connectivity along the anterior-posterior axis using resting state fMRI data from 10 awake macaques (voxels of 1mm3). VIP occupies 13 slices along the anterior-posterior axis and is divided into three parts (anterior, middle and posterior), each containing 16 to 20 voxels.
The connectivity from the three parts VIPs match with the known anatomical connectivity pattern identified by previous tracer studies. The posterior VIP has the strongest link to motion detection (V2, V3, V6, MST, FST) and eye movement areas (FEF, 7m, LIP, V3A), which corresponds to the human posterior VIP bordering the lateral intraparietal area (LIP) specialized also for eye movement and deducing heading perception from vision. The middle VIP is a weaker version of the posterior VIP pattern connecting to almost the same areas with less connection strength, while the responses to smooth pursuit eye movements and optic nystagmus have been found between the lateral and the posterior VIPs in humans. The anterior VIP connects to tactile/proprioception (area 2, 3, 5, M1, S2, LIP, F4, 7b) and peripersonal space areas (AIP, F5, MIP, V6A), which is congruent with the human anterior VIP with an appreciable topographical organization of both face tactile and visual maps.
In conclusion, functional connectivity from resting state fMRI data fills the gap between task fMRI and anatomical connectivity, and confirms a functional specialization between posterior and anterior macaque VIP. We propose that this functional specialization is the precursor to that we describe in human VIP complex.

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Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.01

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Direct conversion of urine-derived cells into motoneurons by defined transcription factors

Authors: H. NAGAI, M. SAITO, ***H. IWATA**;
Takeda Pharmaceut. Co. Limited, Fujisawa, Japan

Abstract: Direct cell-type conversion of somatic cells into the cell-types of interest is attracting great attention since it circumvents rejuvenation and preserves hallmarks of cellular aging (unlike iPSC), more suitable for modeling diseases with strong age-related and epigenetic contributions. Common cell source for direct conversion has been fibroblasts, however, it requires highly invasive skin biopsy. Alternative cell source is urine-derived cells (UDC), which are available by non-invasive procedures. Here we report induced motor neurons (iMN) are generated from UDC by transducing transcription factors involving MN differentiation. iMN showed neuronal morphology, upregulation of pan-neuron and MN markers, indicating that UDC can be converted to MN. This technology will allow to understand disease pathogenesis, progression, discover biomarkers and drugs for MN-related at population level.

Disclosures: H. Nagai: A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **M. Saito:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **H. Iwata:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited.

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.02

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Functional and pharmacological evaluation of iPSC-derived astrocytes generated by a rapid differentiation method

Authors: *Y. WADA[1], Y. KAGA[1], T. SAMESHIMA[1], M. KILANDER[2], T. ARATANI[1], R. YAMOTO[1], T. HOSOYA[1];
[1]Ricoh Company, Ltd., Ricoh Company, Ltd., Kawasaki, Japan; [2]Elixirgen Scientific, Elixirgen Scientific Inc., Baltimore, MD

Abstract: The Quick-Tissue™ technology (Elixirgen Scientific, Inc.) is a transcription factor-based method for rapid differentiation of induced pluripotent stem cells (iPSCs) into desired cell

types. The method generates a pure population of astrocytes (Quick-Glia™ Astrocyte) within 28 days, which is about three times faster than the conventional methods. Astrocytes provide various supportive functions to neurons and contribute to the regulation of the central nervous system. Recent studies have revealed that astrocytes play important roles in the brain function and nervous system diseases. The neuron-astrocyte co-culture system is an *in vitro* tool to evaluate the supportive functions and therefore is an attractive test bed for drug screening, toxicological assays, and disease research. In the present study, we evaluate whether the Quick-Glia™ Astrocytes provide supportive functions that are similar to that of *in vivo* astrocytes and whether their co-culture system is suitable for HTS assays. We co-cultured iPSC-derived excitatory neurons (Quick-Neuron™ Excitatory) with the astrocytes and characterized their drug responses using calcium imaging and multielectrode arrays (MEAs). In the calcium imaging analysis, the neurons that were co-cultured in 384-well plate responded to antagonists and agonists for receptors of various neurotransmitters as expected, indicating that the co-culture system is suitable for the analysis of drug effects on those receptors. The co-cultured neurons exhibited stronger synchrony and higher frequency of calcium spikes compared to neuronal cultures without astrocytes, suggesting supportive functions of the astrocytes. The cells co-cultured on MEA plates showed similar results. These results suggest that iPSC-derived astrocytes generated by the Quick-Tissue™ technology provide neuron-supportive functions that are similar to those of *in vivo* astrocytes and that they are useful for HTS pharmacological assays in the 384-well plate format. Thus Quick-Glia™ Astrocyte will likely contribute to the acceleration of future drug discovery.

Disclosures: Y. Wada: None. **Y. Kaga:** None. **T. Sameshima:** None. **M. Kilander:** None. **T. Aratani:** None. **R. Yamoto:** None. **T. Hosoya:** None.

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.03

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Characterization of Robust and Consistent Excitatory Human Induced Neurons for Custom Disease Modeling and Drug Discovery

Authors: R. BRADLEY, J. MA, ***A. FATHI**, C. HOGAN, S. SCHACHTELE, S. HILCOVE, J. LIU;
Fujifilm Cell. Dynamics Inc., Madison, WI

Abstract: Human iPSC-derived neurons have been used for many years to model development, function, aging and disease etiology in vitro. NGN2 forward reprogramming is a robust approach to generate induced neurons with low variability. As a leader in iPSC technology and innovation, FUJIFILM Cellular Dynamics, Inc. will present data on the generation, characterization, and use of iCell Induced Neurons for disease modeling. Specifically, induced neurons differentiated from

a Granulin (GRN) knockout iPSC line, as well as from its isogenic control iPSC, were manufactured at scale for disease research applications. Optimization of the cryopreservation, media formulation and required supplements demonstrate highly repeatable results from large single batched lots which increase reliability and run-to-run consistency of assays. iCell Induced Neurons can also be cultured along with iCell Astrocytes and iCell Microglia to create disease and developmentally relevant organ-on-a-chip models without the need for optimization or protocol development.

Disclosures: **R. Bradley:** A. Employment/Salary (full or part-time):; Fujifilm Cellular Dynamics Inc. **J. Ma:** A. Employment/Salary (full or part-time):; FUJIFILM CELLULAR DYNAMICS INC. **A. Fathi:** A. Employment/Salary (full or part-time):; FUJIFILM CELLULAR DYNAMICS INC. **C. Hogan:** A. Employment/Salary (full or part-time):; Fujifilm Cellular Dynamics Inc. **S. Schachtele:** A. Employment/Salary (full or part-time):; Fujifilm Cellular Dynamics Inc. **S. Hilcove:** A. Employment/Salary (full or part-time):; Fujifilm Cellular Dynamics Inc. **J. Liu:** A. Employment/Salary (full or part-time):; FUJIFILM CELLULAR DYNAMICS INC.

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.04

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: A Carbon Nanotube Array for In-Vitro Neural Diseases Study through a Size-based Extracellular

Authors: *Y.-T. YEH[1], M. TERRONES[2];
[1]The Pennsylvania State Univ., Pennsylvania State Univ., University Park, MD; [2]The Pennsylvania State Univ., Pennsylvania State Univ., University Park, MA

Abstract: Extracellular Vesicles (EV) contains information that represents status of the host cells at the moment of their excretions. Preliminary study shows that EV is an important biomarker for different neural diseases and cancer metastasis. To have a better understanding of EVs, the first key step is to isolate EVs from the extracellular matrix. However, since a population of EVs is heterogeneous mixture, this challenges existing technologies to obtain an effective isolation. Currently, Currently, based on their biogenesis and sizes, EVs are divided into three groups, including exosomes (30-100 nm in diameter), microvesicles (100- 1000 nm in diameter), and apoptotic bodies (800 - 5000 nm in diameter). In this report, we developed a handheld platform that captures and separates a mixture of EVs according to their sizes. After capture, this platform is compatible with downstream analysis, such as cell culture and genomic sequencing. In briefly, we synthesized a nanostructured array made of aligned carbon nanotubes (CNTs). This porous array has a gradient of inter-tubular distance ranging from 20-800 nm, which covers the sizes for both exosomes and microvesicles. We applied this technology to study

communications of neuroglia from a mouse model. We built a CNT array with 100 and 500 nm inter-tubular distance to capture and separate EVs that are excreted from primary cell culture of the glia cells. Our results showed that both exosomes and microvesicles are successfully isolated and separated by CNT arrays with different inter-tubular distance. Furthermore, we integrated this platform and established an *in vitro* model to study neurotransmission. After EVs isolation, this biocompatible CNT arrays serves as a primary culture substrate that allows us to study and monitor how EVs are uptaken by other neuron cells as recipients. Our preliminary results show feasibilities of study transmissions of EVs between neuroglia to neurons, by using SH-SY5Y cells as a model of a recipient. At the moment, we are applying this technology to answer the following questions: 1) What contents (e.g. genome and protein expression) of an EV population are excreted from different types of neurons, 2) whether the EV profiles are altered when an external stimulus (e.g. heat, electrical pulse, and viral infection) is applied, and 3) If so, what is the difference. We believe this technology is an effective approach to analyze EVs.

Disclosures: Y. Yeh: None. **M. Terrones:** None.

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.05

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Identification of synaptic plasticity modulator through phenotypic screening in rat and human neurons

Authors: *H. NAGAI, M. KAISHIMA, T. HIDAKA, T. YUKAWA, M. MARUYAMA, M. KAMATA, T. ANDO, H. IWATA;
Takeda Pharmaceut. Co. Limited, Fujisawa, Japan

Abstract: The synapse plays a fundamental role in the information processing by the brain, and synaptic plasticity is the cellular basis of learning and memory. It is therefore not surprising that synaptic dysregulation, or synaptopathy, has been implicated in the underlying mechanism of diseases such as neuropsychiatric disorders, neurodevelopmental disorders, and neurodegenerative diseases. Thus, a drug that promotes synaptogenesis and synaptic plasticity and function is highly warranted as a disease-modifying treatment. To identify such compounds, we performed high-throughput, high-content phenotypic screenings in both rat primary neurons and human induced pluripotent stem cell (iPSC)-derived neurons. Here we report the identification of potential synaptic plasticity modulators that increased the densities of dendritic post-synaptic puncta, as well as co-localizing post- and pre-synaptic puncta indicative for synapse formation. Through compound profiling, we identified a couple of chemotypes showing post-synaptic puncta-increasing effects without inducing the expression of post-synaptic marker. We also performed transcriptomic analysis and found that one of the chemotypes induced different gene expression pattern from BDNF and forskolin. By using CopyCATS software

(inSili.com LLC), we were able to identify more potent tool compounds from parental hits. These results suggest that such a phenotypic screening would provide an attractive drug candidate against synaptopathies as well as chemical tools to unravel the mechanisms underpinning synaptic plasticity.

Disclosures: **H. Nagai:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **M. Kaishima:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **T. Hidaka:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **T. Yukawa:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **M. Maruyama:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **M. Kamata:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **T. Ando:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **H. Iwata:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited.

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.06

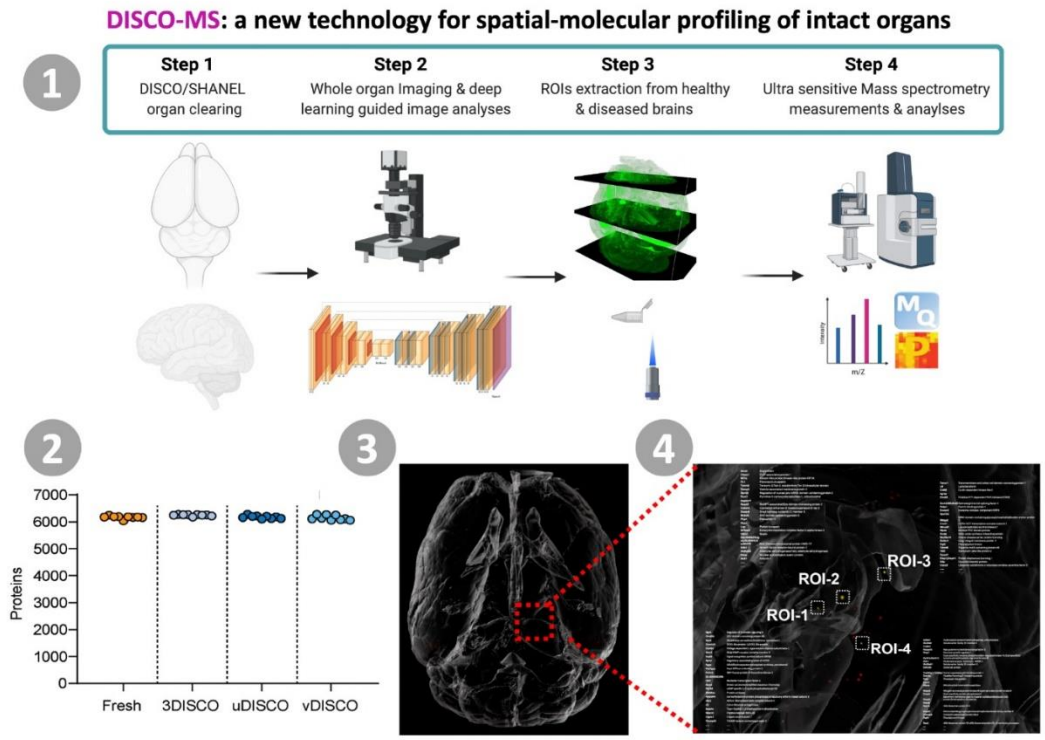
Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Proteomics of spatially identified tissues in whole organs and organisms

Authors: H. BHATIA[1], A.-D. BRUNNER[2], F. ÖZTURK[1], S. KAPOOR[1], *F. HELLAL[1], F. THEIS[1], M. MANN[2], **A. ERTURK**[1];
[1]iTERM, Helmholtz Munich, Neuherberg, Germany; [2]Dept. for Proteomics and Signal Transduction, Max-Planck Inst. of Biochem., Munich, Germany

Abstract: Spatial molecular profiling of complex tissues is essential to investigate cellular function in physiological and pathological states. However, methods for molecular analysis of biological specimens imaged in 3D as a whole are lacking. Here, we present DISCO-MS, a technology combining whole-organ imaging, deep learning-based image analysis, and ultra-high sensitivity mass spectrometry. DISCO-MS yielded qualitative and quantitative proteomics data indistinguishable from uncleared samples in both rodent and human tissues. Using DISCO-MS, we investigated microglia activation locally along axonal tracts after brain injury and revealed known and novel biomarkers. Furthermore, we identified initial individual amyloid-beta plaques in the brains of a young familial Alzheimer's disease mouse model, characterized the core proteome of these aggregates, and highlighted their compositional heterogeneity. Thus, DISCO-MS enables quantitative, unbiased proteome analysis of target tissues following unbiased imaging of entire organs, providing new diagnostic and therapeutic opportunities for complex diseases, including neurodegeneration.
The work is available at bioRxiv:
https://www.biorxiv.org/content/10.1101/2021.11.02.466753v1

Graphical Abstract



Highlights

1. DISCO-MS combines tissue clearing, whole-organ imaging, deep learning-based image analysis, and ultra-high sensitivity mass spectrometry

2. DISCO-MS yielded qualitative and quantitative proteomics data indistinguishable from fresh tissues

3. DISCO-MS enables identification of rare pathological regions & their subsequent molecular analysis

4. DISCO-MS revealed core proteome of plaques in 6 weeks old Alzheimer's disease mouse model

Disclosures: H. Bhatia: None. **A. Brunner:** None. **F. Özturk:** None. **S. Kapoor:** None. **F. Hellal:** None. **F. Theis:** None. **M. Mann:** None. **A. Erturk:** None.

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.07

Title: WITHDRAWN

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.08

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: A high-throughput fluorescence and automated electrophysiology screening cascade to support discovery of novel TRPML1-targeting therapeutics

Authors: *P. MADAU, L. HUTCHISON, C. BROWN, L. GERRARD, D. DALRYMPLE, I. MCPHEE, D. PAU;
SB Drug Discovery, Glasgow, United Kingdom

Abstract: TRPML1 is a non-selective cation-permeable channel, primarily located on the membranes of late endosomes and lysosomes of all mammalian cell types. TRPML1 plays a key role in maintaining lysosomal calcium homeostasis, autophagy and modulation of oxidative stress. Mutations which result in defective TRPML1 are thought to contribute to a wide range of conditions including lysosomal storage disorders and neurodegenerative disorders such as Alzheimer's and Parkinson's disease. It is thought that increasing the activity of TRPML1 may help to promote autophagy, leading to clearance of protein / ROS build-up in specific neurons impacted by these disorders. While a small number of synthetic compounds have been shown to modulate TRPML1, a lack of specific pharmacological tools has hampered further investigation of the exact role of TRPML1 in normal lysosomal function and its role in associated pathological processes.

Developments in ion channel screening technologies have allowed rapid assessment of large numbers of compounds against novel ion channel drug targets. Using recombinant HEK cell lines, we have successfully designed fluorescence-based screening and electrophysiology assays for TRPML channels which show excellent reproducibility and platform-to-platform correlation using standard reference compounds. These assays were employed in a screening paradigm to identify and characterize novel small molecule modulators of TRPML1 using a 48,000 compound small molecule library. Both primary screening assay and hit confirmation assays (fluorescence and electrophysiology) yielded high success rates and showed excellent reproducibility. From the initial screen, a number of novel compounds (both activators and inhibitors) were identified which were successfully confirmed as TRPML1 modulators using automated patch clamp electrophysiology.

Successful development of robust hit-identifying TRPML assays such as these will help advance our knowledge of this exciting target and its role in normal physiology and disease, as well as enabling high-throughput screening and data-driven drug discovery to facilitate novel therapeutic advances.

Disclosures: P. Madau: None. **L. Hutchison:** None. **C. Brown:** None. **L. Gerrard:** None. **D. Dalrymple:** None. **I. McPhee:** None. **D. Pau:** None.

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.09

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: A high-throughput screening cascade for the identification and characterization of novel TRPM2 modulators.

Authors: *J. KEWNEY[1], A. DICKSON[1], D. SWIFT[2], D. BAKOWSKI[2], M. MIRZA[2], D. DALRYMPLE[1], I. MCPHEE[1];
[1]SB Drug Discovery, Glasgow, United Kingdom; [2]Sygnature Discovery, Nottingham, United Kingdom

Abstract: Transient receptor potential melastatin 2 (TRPM2) is a calcium-permeable, non-selective cation channel belonging to the TRP superfamily of ion channels. TRPM2 acts as a sensor of reactive oxygen species and is found in almost all tissues suggesting important roles in numerous aspects of normal physiology.
In the brain, where it is most abundant, TRPM2 is ubiquitously expressed and found in microglia, astrocytes and neurons, with emerging evidence for a role in neuroinflammation. Aberrant function of TRPM2 has also been associated with a number of neurological diseases including Alzheimer's disease, Parkinson's disease, stroke and neuropathic pain, raising the possibility that intervention with TRPM2 modulators could provide new therapeutic opportunities. However, the lack of specific pharmacological tools has hampered further investigation into the exact role of TRPM2 in normal physiology and role in associated pathological processes.
Using a blend of high throughput fluorescence and automated electrophysiology assays, we have developed a screening platform to identify, verify and characterize novel TRPM2 antagonists. TRPM2 channels were activated by either Hydrogen Peroxide (calcium assay) or a combination of free internal calcium plus ADP-ribose (automated electrophysiology assay). This TRPM2 screening platform was used to interrogate a small molecule compound library with view to identifying novel starting points for TRPM2 drug discovery. Hit compounds were verified using automated electrophysiology and selectivity further assessed using an extensive panel of assays against other TRP channel family members.
The results confirm the successful development of a TRPM2 screening platform capable of rapidly identifying novel pharmacological tools with which to further investigate the role of this exciting target in normal physiology and disease.

Disclosures: J. Kewney: None. **A. Dickson:** None. **D. Swift:** None. **D. Bakowski:** None. **M. Mirza:** None. **D. Dalrymple:** None. **I. McPhee:** None.

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.10

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: In Utero electroporated neurons for high throughput screening of compounds regulating dendrite development

Authors: *A. M. SOKOLOV, A. F. BORDEY;
Dept Neurosurg., Yale Sch. of Med., New Haven, CT

Abstract: Several neurodevelopmental disorders are associated with pathogenic neuronal dysmorphogenesis. In particular, increased mTOR activity in several genetic disorders lead to pathogenic soma and dendrite overgrowth that contributes to abnormal connectivity and epileptogenesis. These abnormalities can be reduced by mTOR blockers, but there is a need to identify novel small molecules that rescue dendrite overgrowth without size-effects *in vivo*. To address this need, we developed a high-throughput phenotypic assay to examine drug efficacy on diseased neuron dendrite morphogenesis. Our approach is based on sequential strategies using *in utero* electroporation (IUE) to label and increase mTOR activity in a specific neuronal population *in vivo*, resulting in sparse labeling of diseased neurons upon plating. This is followed by automatic cell imaging and morphological analysis. IUE of a plasmid encoding a constitutively active mTOR activator Rheb fused to tdTomato led to increased mTOR activity in layer II/III cortical pyramidal neurons. Following neonatal microdissection of the cortex and seeding into 96-well plates, neurons were treated with several compounds, including the mTOR blocker Torin1. Automated imaging with an InCell Analyzer 2200 and analysis using a pipeline in CellProfiler confirmed that Torin1 decreased neurite overgrowth. Importantly, this protocol allows up to 31 compounds to be screened per plate in two weeks plus a control, highlighting the robustness and efficiency of our approach for high-throughput screening of candidate therapeutics on wildtype or diseased primary neurons. By using different electroporation plasmids, this approach can be applied to other neurological disorders associated with abnormal cell morphogenesis.

Disclosures: A.M. Sokolov: None. **A.F. Bordey:** None.

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.11

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NHMRC Ideas Grant No. 2013285

Title: Development and optimisation of a bionic array directed gene electrotransfer (BaDGE) system for neural applications.

Authors: *K.-Y. LAI, A. AL ABED, G. VON JONQUIERES, J. L. PINYON, G. D. HOUSLEY, N. H. LOVELL;
UNSW, Randwick, Australia

Abstract: Gene electrotransfer is a powerful technique in which a pulsed electric field is used to drive DNA for therapeutic constructs onto cell membranes, with endocytotic uptake for expression and protein translation. Traditionally the DNA solution is first injected into the target region, then plate or needle electrodes are positioned across the target region for delivery of electric pulses. However, these approaches are highly invasive and dependent on the geometry of the target tissue and typically require high voltages to achieve the necessary field strengths. Here we present a novel system, 'Bionic array Directed Gene Electrotransfer' (BaDGE®) for conducting gene electrotransfer in which delivery of the DNA solution and both electrodes are achieved in a single device - a pair of offset concentric needles forming a linear electrode array. Because of the compact design and ability to produce focused asymmetric electric fields, with lower charge transfer requirements, the system can be used for precision targeting of deep brain structures for gene electrotransfer. One planned application is the delivery of plasmid DNA expressing neuromodulating molecules to the internal globus pallidus for the treatment of Parkinson's disease. To optimise our gene electrotransfer system for neural applications we applied a technique known as quasimonopolar current steering to control the size and shape of the electric field to match the volume of gene transfection to the target structure. We compared the effects of splitting the return current between a near (bipolar) and distant (monopolar) electrode on the resulting electric field. The fields were determined through the mapping of voltages produced by the BaDGE® device in an in vitro conductive model of the brain, validated through the transfection of HEK293 cells with a green fluorescent protein biomarker. By varying the quasimonopolar ratio we were able to shift the centroid of the transfection volume between along the length of the BaDGE® probe as well as produce volumes ranging from spherical (greatest depth of transfection) through pyriform to cylindrical (greatest volume of transfection). Future work includes investigating the dispersion of fluorescently labelled DNA in an animal brain model, and the usage of sucrose in the injection solution to modify the local tissue electrical resistance. These studies serve to demonstrate the effectiveness of our BaDGE® system for brain applications and will additionally be used as a basis for computational models for gene electrotransfer in the human brain.

Disclosures: K. Lai: None. **A. Al Abed:** None. **G. Von Jonquieres:** None. **J.L. Pinyon:** None. **G.D. Housley:** None. **N.H. Lovell:** None.

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.12

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: UW-Madison School of Pharmacy Start-up Fund

Title: Development of cross-reactive antibodies for the identification and treatment of synthetic cannabinoid receptor agonist toxicity

Authors: A. WOROB, ***C. WENTHUR**;
Sch. of Pharm., Univ. of Wisconsin - Madison, Madison, WI

Abstract: Synthetic cannabinoid receptor agonists (SCRAs) are compounds which mimic the pharmacology of psychoactive components in cannabis. These compounds are structurally diverse, inexpensive, commercially available and difficult to identify with modern analytical methods, making them highly accessible for recreational use. Suspected SCRA toxicity, which can present with a breadth of cardiovascular, gastrointestinal and neurological disturbances, is currently addressed through symptom management followed by toxicological screening that often occurs long after patient discharge.Here, we report the development of four cross-reactive anti-SCRA bioconjugate vaccines as a platform for developing improved diagnostic and therapeutic interventions against SCRA intoxication, using SCRA-resembling small molecule haptens that combine common subregional motifs occurring within and across different generations of SCRA molecules. Using a combination of multiplexed competitive ELISA screening and chemoinformatic analyses, it was found the antibodies resulting from vaccination with these bioconjugates demonstrated their ability to detect multiple SCRAs with a Tanimoto minimum common structure score of 0.6 or greater, at concentrations below 8 ng/mL. The scope of SCRAs detectable using these haptens was found to include both bioisosteric and non-bioisosteric variants within the core and tail subregions, as well as SCRAs bearing valine-like head subregions, which are not addressed by commercially available ELISA screening approaches. Vaccination with these bioconjugates was also found to prevent the changes in locomotion and body temperature that were induced by a panel of SCRAs at doses of 1 to 3 mg/kg. Further refinement of this genericized hapten design and cross-reactivity prioritizing approach may enable the rapid detection of otherwise cryptic SCRAs that arise during overdose outbreaks, and could ultimately lead to identification of monoclonal antibody species applicable for overdose reversal.

Disclosures: A. Worob: None. **C. Wenthur:** None.

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.13

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Development of a novel highly sensitive mBDNF sandwich ELISA

Authors: *S. SUGINO[1], M. KOJIMA[3], F. AKUTSU[3], M. TAMURA[2], K. ENDO[2], N. TAKAHIRO[2];
[1]FUJIFILM Wako Pure Chem. Corp., Tokyo, Japan; [2]FUJIFILM Wako Pure Chem. Corp., Hyogo, Japan; [3]FUJIFILM Wako Shibayagi Corp., Gumma, Japan

Abstract: BDNF, a member of the neurotrophin family of growth factors, is involved in neurogenesis and synaptogenesis, and plays key roles in numerous signaling pathways associated with nervous system disorders ranging from depression, autism and schizophrenia, in addiction to heart disease, diabetes, gout, periodontal disease, stress, and cognitive effects associated with exercise. BDNF is synthesized as the precursor proBDNF, which undergoes intra or extracellular cleavage to produce mature BDNF (mBDNF). Being able to distinguish between the two forms is imperative, as both proBDNF and mBDNF are found in the brain and the periphery, and exert opposite effects by binding to p75 neurotrophin receptor and tyrosine kinase receptor B receptors, respectively. However, most commercially available BDNF ELISA kits fail to specifically detect mBDNF in mouse serum and plasma, compromising studies targeting these proteins. To address this problem, we used an N-terminal end-specific anti-mBDNF antibody to develop a highly sensitive ELISA. The new ELISA showed very low reactivity (<0.5%) to proBDNF and other NGF family proteins (human NGF, NT-3 and NT-4). Moreover, by using biotin-labeled, streptavidin- conjugated horseradish peroxidase and a chemiluminescent substrate, we were able to achieve a high sensitivity of 0.2 pg/ml. The ELISA was tested using brain lysates from BDNF KO mice, resulting in very low levels of mBDNF detection compared to wild-type mice lysates [1,820 - 3,864 pg/mL]. This indicates that our new ELISA was superior in both sensitivity and specificity, to other conventional methods already commercially available. In dilution linearity and spike-recovery tests, the new ELISA also showed good recovery rates (84.8-110%) from mouse, rat and human plasma and serum samples. Furthermore, mBDNF could be detected in the serum of wild-type [6.96 - 9.01 pg/mL] and autism model mice [12.4-18.1 pg/mL], and human saliva samples [0.296 - 4.09 pg/mL], the latter being a result that other conventional methods have not yet achieved. In conclusion, our novel ELISA is expected to be a useful tool in BDNF basic research and mental diseases research.

Disclosures: **S. Sugino:** A. Employment/Salary (full or part-time):; FUJIFILM Wako Pure Chemical Corporation. **M. Kojima:** A. Employment/Salary (full or part-time):; FUJIFILM Wako Shibayagi Corporation. **F. Akutsu:** A. Employment/Salary (full or part-time):; FUJIFILM Wako Shibayagi Corporation. **M. Tamura:** A. Employment/Salary (full or part-time):; FUJIFILM Wako Pure Chemical Corporation. **K. Endo:** A. Employment/Salary (full or part-time):; FUJIFILM Wako Pure Chemical Corporation. **N. Takahiro:** A. Employment/Salary (full or part-time):; FUJIFILM Wako Pure Chemical Corporation.

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.14

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Korea Institute of Toxicology Grant No. 1711159827

Title: A Novel approach for Glioblastoma Cell line-derived Xenograft Model in Mice by Intracerebroventricular Injection Using 3D-Printed Mouse Head Fixation Holder

Authors: *J. KIM[1], J.-H. NOH[2], J.-H. SONG[3], J. LEE[4], H. KIM[1], S. PARK[1], K.-H. HAN[5]; [1]Plasbio Inc., Jeonju, Korea, Republic of; [2]Keyprime Researh, Cheongju, Korea, Republic of; [3]Dept. of pharmacy, Kangwon national university, Chuncheon, Korea, Republic of; [4]Korea zoonosis research institute, Iksan, Korea, Republic of; [5]Korea institute of toxicology, Deajeon, Korea, Republic of

Abstract: Glioblastoma is the most aggressive of all brain tumors and difficult to treat. Since the animal Cell line-derived xenograft (CDX) model of glioblastoma represents the physiological specificity of cancer, it can be used as a tool to study tumor biology and therapeutic effects. In glioblastoma xenograft disease models, it is mainly used as a method of transplanting cells by intracerebroventricular (I.C.V.) or intrathecal as the route of administration. However, there are many problems to be solved, such as lack of speed, technical difficulties, and expensive equipment. In response to these troubles, we have designed a novel mouse head fixation holder (MFH) via 3D scanning and 3D printing technology. 3D scanning technology that provides accurate head measurement data and 3D printing technology that can produce various shapes through CAD were applied to this study. We describe a simple, exact, inexpensive and rapid method for I.C.V. injection without stereotaxic surgery in mice. First of all, the MFH was designed based on the mouse head 3D scanning data and all the components were coordinated in CAD file using Fusion 360 (AUTODESK). The G-code was generated using the slicing software Preform (Formlabs). 3D printing was fabricated with stereolithography (SLA) of FORM2 3D printer and photosensitive polymer (resin). To evaluate the effectiveness of the MFH, trypan blue and Cy 5.5 were directly injected into the SCID mouse's lateral ventricle. Compared with hands on and MFH significantly increased the probability these dye solutions were administered into the brain ventricle. Hematoxylin & Eosin and Immunohistochemistry-DAB staining images of brain sections after human glioblastoma likely cell line U87MG injection showed that MFH had the same injection accuracy and CDX model generation ability as stereotaxic apparatus. These results indicate that a novel MFH can make it very effective, easy and economical for researchers to generate CDX model of glioblastoma. Furthermore, 3D printing technique provides a reproducible, flexible, simple and cost-effective method for researchers to produce the equipment needed to quickly adopt neurological experiments.

Disclosures: J. Kim: None. **J. Noh:** None. **J. Song:** None. **J. Lee:** None. **H. Kim:** None. **S. Park:** None. **K. Han:** None.

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.15

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Combining spatial analysis and multispectral imaging for greater insights into the tumor-immune microenvironment in glioblastoma

Authors: *A. HAGGERTY[1], N. MAMMADOVA[2], M. E. BARISH[3];
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Abstract: Glioblastoma (GBM) is an aggressive and almost universally fatal brain cancer. Glioblastoma tumor masses are highly vascularized and infiltrated with cells mediating both innate and adaptive immunity. The presence and organization of various immune cells within the tumor microenvironment varies between patients and within individual tumors. This variability in the immune landscape influences responses to different treatment modalities, including immunotherapies. Understanding reciprocal immune-tumor cell interactions will enhance design and implementation of novel therapies. Currently, review of GBM patient tissues by neuropathologists is done visually using single or low-plex biomarker panels whose complexity is constrained by limitations of traditional immunohistochemistry, including the lack of well characterized primary antibody clones, limited antibody selection due to species-dependent workflows, and inaccuracies such as false signal intensities introduced by autofluorescence (AF) and spectral overlap from traditional immunofluorescence imaging methods. Multiplex immunofluorescence (mIF) and multispectral imaging (MSI) allows for fast, accurate imaging on a single tissue section of up to eight fluorophores (using Phenoimager (formerly Phenoptics) technology), cell type-specific AF spectra, and DAPI/Hoechst. It has been shown in other cancer types (i.e. lung cancer, breast cancer, melanoma) that mIF plus spatial biology techniques is necessary to determine cellular densities and interactions in tissue context to better predict outcomes and stratify patient populations. Here, we have modified pre-validated mIF kits that use Opal fluorophores together with the PhenoImager HT (formerly Vectra Polaris) Automated Quantitative MSI system on human formalin-fixed, paraffin embedded GBM tissue samples to further characterize immune-tumor microenvironments, with consideration for spatial distributions of interacting cells and extended neighborhood. Pre-optimized immunofluorescent kits targeting immune cell subsets, checkpoint inhibitors, and other biomarkers have been shown to provide clinically relevant information in other cancer types (such as lung cancer and melanoma). Our study indicates that a combination of higher plex mIF combined with spatial phenotyping allows for better identification of unique patient samples. Widespread use of these reagents, coupled with spatial analysis and complex phenotyping, would better characterize the complex immune landscape of tumors and enhance design and implementation of therapeutics for GBM.

Disclosures: **A. Haggerty:** A. Employment/Salary (full or part-time):; Akoya Biosciences. **N. Mammadova:** A. Employment/Salary (full or part-time):; Akoya Biosciences. **M.E. Barish:** None.

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.16

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Inhibition of neuronal activity using a novel chemogenetic platform developed for the treatment of neurological disorders

Authors: *K. BENTHALL, J. IAFRATI, S. DODSON, C. XIE;
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Abstract: Rationale: CODA Biotherapeutics is developing a chemogenetics-based platform to treat neurological disorders. With a focus on indications caused by neuronal hyperexcitability, CODA will use AAV-mediated gene therapy to deliver inhibitory ligand-gated ion channels (CODA receptors) to affected neuronal subpopulations. CODA receptors are comprised of the nicotinic α7 receptor ligand binding domain fused to a glycine receptor ion-pore domain. They are engineered to have decreased sensitivity to acetylcholine while retaining sensitivity to select clinical stage compounds, which act on the receptor to inhibit neuronal activity. Optimization of receptor properties is achieved through site-directed mutagenesis of the parental construct (P1). Resulting variants are screened through a series of assays to assess receptor pharmacology and function.
Methods: The parental receptor P1, the first-generation variant V1, and the second-generation variant V2 were characterized using whole-cell patch clamp electrophysiology. The dose-response relationship and current amplitudes of TC-5619 (TC) and acetylcholine (ACh) were assessed in CODA receptor-expressing HEK293 (HEK) cells and in primary neonatal mouse hippocampal (mHC) neurons. Effects of CODA receptor activation on membrane properties and action potential generation were measured in mHC neurons as well as in hippocampal brain slices from adult mice injected with an AAV expressing a CODA receptor. For all experiments, non-transduced and scramble-transduced controls were included and an n value of at least 5 cells was obtained.
Results: TC binding to CODA receptors elicits chloride currents with EC_{50} of less than 50 nM for P1, V1, and V2 in HEK cells and mHC neurons. In contrast, V1 and V2 are >10x less sensitive to ACh than the parental receptor P1. Assessment of the EC_{90} TC current in HEK cells revealed that P1 demonstrates the largest peak current followed by V2 and then V1. Further, we find that activation of the CODA receptors with an EC_{90} concentration of TC suppresses neuronal excitability in mHC neurons compared to baseline (BL), measured as a decrease in input resistance (P1: BL=174 MΩ, TC=58 MΩ; V1: BL=190 MΩ, TC=36 MΩ) and an increase in rheobase (P1: BL=87 pA, TC=246 pA; V1: BL=46 pA, TC=354 pA). Similar results are seen in brain slices from transduced animals. Thus activation of CODA receptors is sufficient to produce inhibitory currents and silence action potential firing in neurons.
Conclusion: Optimization of engineered CODA receptors for desired properties can increase the safety and effectiveness of CODA Biotherapeutics' chemogenetics-based therapeutic approach for neurological disorders.

Disclosures: K. Benthall: A. Employment/Salary (full or part-time):; Employee. **J. Iafrati:** A. Employment/Salary (full or part-time):; Employee. **S. Dodson:** A. Employment/Salary (full or part-time):; Employee. **C. Xie:** A. Employment/Salary (full or part-time):; Employee.

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.17

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: DFG
ERC
Einstein Foundation Berlin

Title: Rapid expression using vesicular stomatitis and semliki forest virus in vivo and in vitro

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Abstract: The delivery of transgenes into neurons via viral vectors has become an indispensable tool in the study of neurons and neuronal networks. Typically, transgene expression occurs within days to weeks after in vivo viral injection, thus imposing a limit on experiment duration and on our ability to rapidly probe and manipulate cortical circuits. To overcome this limitation we previously introduced an ‘all in one go’ protocol that used Semliki Forest viral vector (SFV) to express - gcamp, gfp, rcamp, and opsins - and perform electrical recording, imaging and optogenetic manipulations within 24 hours after in vivo or ex vivo injection in rodents. With SFV expression of vectors begins in 4 hrs in culture and ex vivo brain slices. With VSV expression of vectors in culture begins even earlier. In ex-vivo brain slices pyramidal neurons and interneurons express GFP within 6 hours. Neurons expressing vectors were physiologically normal. With the goal of performing circuit mapping in human ex vivo preparations we have begun to co-inject VSV-GFP and SFV-RFP in vivo and in culture and show that rodent neurons co-express both vectors within 24 hours. Thus, the use of SFV and VSV dramatically reduces experiment time and can be extended to a variety of brain preparations from other species -- potentially including human ex vivo brain slices -- where *in vivo* injection is not feasible.

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Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.18

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Gene Therapy to Treat Fragile X Syndrome

Authors: N. K. MORRILL[1], A. JOLY-AMADO[2], Q. LI[2], ***K. NASH**[3];
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Abstract: Fragile X Syndrome (FXS) is the most prevalent form of inherited intellectual disability. FXS patients have a reduction in Fragile X Mental Retardation Protein (FMRP) expression. FMRP is critical for synaptic plasticity, spatial learning, and memory. We have previously demonstrated a reduction in the extracellular matrix protein Reelin in *Fmr1* knock-out (KO) mice, but more importantly, we demonstrated that protein supplementation with the central fragment of Reelin could improve *Fmr1* KO cognitive deficits. Here we explore the potential of a novel construct of Reelin repeats 3 and 6, termed R36, which is small enough to be used in a recombinant adeno-associated virus (rAAV) vector. We show that Reelin signaling enhancement via a single intracerebroventricular injection of R36 protein can profoundly rescue cognitive deficits in hidden platform water maze and fear conditioning, as well as hyperactivity during the open field maze for *Fmr1* KO mice. Additionally, we demonstrate the same recovery of cognitive deficits in behavioral assays following 2 months of expression of R36 when *Fmr1* KO mice were given a single intracerebroventricular injection of a rAAV expressing R36. Our data suggest that a gene therapy approach directed at increasing Reelin signaling via the R36 construct, could offer a novel therapeutic approach for improving cognition in FXS.

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Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.19

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: AAV capsid optimization for transduction of the striatum in rodents and non-human primates using a barcoded library approach yields a novel AAV9-based engineered variant with enhanced transcriptional capabilities

Authors: *J. B. SMITH, A. GILES, E. FIRNBERG, B. S. HOLLIDGE, S. A. YOST, J. M. EGLEY, W. H. HENRY, S. KARUMUTHIL-MELETHIL, O. DANOS, Y. LIU, J. T. BRUDER, A. C. MERCER;
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Abstract: Adeno-associated viral (AAV) vectors are increasingly used for gene therapy in the central nervous system (CNS) and there is a need for identifying optimal capsids for specific targets. In this study, we evaluated the tropism and transcriptional expression of a library of 118 AAV capsids following intraparenchymal delivery to the striatum in mice and non-human primates (NHPs). This library consisted of approximately equal concentrations of 56 NAV® Platform vectors, 49 engineered AAV variants, and 13 commonly used AAVs. Each AAV vector was produced individually with a unique cis plasmid expressing GFP under the control of the universal CAG promoter and a unique barcode sequence allowing for measurement of the relative abundance of each capsid's genomes (DNA) and transcripts (RNA) in different tissues using next-generation sequencing (NGS). The library was administered intraparenchymally to the putamen of cynomolgus macaques via MRI-guided injection with convection enhanced delivery, as well as the striatum of C57BL/6 mice via stereotaxic injection. These studies allowed for the direct comparison of each capsid in the pool within each animal and translation of findings between species. We identified an AAV9 variant, labeled BC029, with an engineered homing peptide inserted after residue S454 capable of achieving significantly higher transgene expression compared to AAV9, AAV8, AAV5, AAV2, and AAV1. Specifically, NGS analysis of tissue punches at the injection site (i.e., putamen) revealed that BC029 produced more transcript copies than AAV9 (4x-fold), AAV8 (29x-fold), AAV5 (17x-fold), AAV2 (55x-fold), and AAV1 (7x-fold). BC029 further demonstrated similar properties in brain regions projecting to the striatum (substantia nigra, thalamus, and cortex), producing 10-fold more RNA than AAV9. These findings correspondingly translated to mice, where we observed higher BC029 transcript expression in striatum, thalamus, and cortex than AAV9, AAV8, AAV5, AAV2, and AAV1. In a second set of studies, a mixture of AAV9 and BC029 expressing GFP and tdTomato, respectively, were injected into the striatum of mice and NHPs. Results confirmed the enhanced transcriptional efficacy of BC029 over AAV9, and histological analysis revealed similar cellular tropism (e.g. primarily neurons with a small population of astrocytes). Together these studies provide a useful guide for AAV capsid selection across mammalian species, as well as demonstrate the utility of a novel, rationally-engineered capsid with enhanced transgene expression over traditional serotypes that could be employed in both research and clinical applications.

Disclosures: **J.B. Smith:** A. Employment/Salary (full or part-time):; REGENXBIO Inc. **A. Giles:** A. Employment/Salary (full or part-time):; REGENXBIO Inc. **E. Firnberg:** A. Employment/Salary (full or part-time):; REGENXBIO Inc. **B.S. Hollidge:** A. Employment/Salary (full or part-time):; REGENXBIO Inc. **S.A. Yost:** A. Employment/Salary (full or part-time):; REGENXBIO Inc. **J.M. Egley:** A. Employment/Salary (full or part-time):; REGENXBIO Inc. **W.H. Henry:** A. Employment/Salary (full or part-time):; REGENXBIO Inc. **S. Karumuthil-Melethil:** A. Employment/Salary (full or part-time):; REGENXBIO Inc. **O. Danos:** A. Employment/Salary (full or part-time):; REGENXBIO Inc. **Y. Liu:** A. Employment/Salary (full or part-time):; REGENXBIO Inc. **J.T. Bruder:** A. Employment/Salary (full or part-time):; REGENXBIO Inc. **A.C. Mercer:** A. Employment/Salary (full or part-time):; REGENXBIO Inc..

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.20

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Differences in biodistribution of AAV9 and AAV6 in the central nervous system

Authors: *T. Y. YANG, T. H. KIM, C. CHAU, S. YELLAI, I. CHENG, S. T. PERUZZARO; Charles River Labs., South San Francisco, CA

Abstract: Adeno-associated virus (AAV) is a leading method and effective tool for gene therapy because of its low immunogenicity and strong neuronal tropism. To date, 12 AAV serotypes have been identified with each serotype preferentially targeting a subset of tissues throughout the body. Serotype 9 (AAV9) is one of the most widely used serotypes because of its ability to bypass the blood-brain barrier and transduce astrocytes and neurons throughout the central nervous system (CNS). Serotype 6 (AAV6) has high transduction in skeletal muscles and astrocytes. Both serotype and route of delivery (i.e., intravenous, intraparenchymal, lumbar intrathecal, and cisternal) differentially influence biodistribution and expression. In this study, we investigate the biodistribution differences of two AAV serotypes, AAV9-eYFP and AAV6-mCherry, after bilateral and unilateral intracerebroventricular (ICV), intrathecal (IT), cisterna magna (ICM), epidural, and subpial injections in both Sprague Dawley rats and C57Bl/6 mice. Both serotypes are administered to the same animal for each route of administration. Four to six-weeks after AAV administration, brain tissue, spinal cord, liver, and heart are collected for analysis. Immunohistochemistry and quantitative PCR is used to visualize and compare transgene expression between the two serotypes and among the delivery routes. From this, we can gain a better understanding of how AAV9 and AAV6 biodistribution differ and overlap and how this may be modulated by the route of delivery into the CNS.

Disclosures: T.Y. Yang: None. **T.H. Kim:** None. **C. Chau:** None. **S. Yellai:** None. **I. Cheng:** None. **S.T. Peruzzaro:** None.

Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.21

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Kinetics and durability of transgene expression in the striatum of mice delivered by intraparenchymal injection of rAAV9

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Abstract: Adeno-associated viral (AAV) vectors are being used to deliver transgenes to the brain for therapeutic use and for neuroscience research. Therefore, understanding the kinetics of AAV-delivered transgene expression in the brain is vital for both translational disease studies as well as basic neuroscience studies. Furthermore, an understanding of the kinetics and durability of transgene expression is critical to experimental design and interpretation of data. Here, we directly characterize the temporal profile of transgene expression after bilateral injections of rAAV9 encoding GFP under the ubiquitous promoter CAG into the striatum of mice. When brains were collected, one hemisphere was processed for histological analyses and the other hemisphere was used to extract DNA and RNA from the striatum for ddPCR analyses. Using this approach, we were able to monitor the timecourse of vector DNA as it rapidly decreased over the first week following injection and established stable episomes. GFP expression was detectable by luminance of native GFP fluorescence within the first week after rAAV9 administration, which increases until 3 weeks followed by a decrease that plateaus at 3 and 6 months. As with protein expression, mRNA was produced within the first week and plateauing 3 months after rAAV9 administration; promoter strength, assessed by the mRNA-to-DNA ratio, followed a similar trend. Expressing transgenes specifically in neurons is another important variable for therapeutic use and for neuroscience research. We found that CamKII was a weak neuron-specific promoter and synapsin (hSyn) was a strong neuron-specific promoter. However, hSyn had slower kinetics of expression with its highest expression at 3 months after intrastriatal administration. Importantly, transgene expression driven by the hSyn promoter at 6 months was not repressed as has previously been reported (Jackson et al., 2016). Therefore, using hSyn as a promoter for transgene expression provides neuron-selective expression, but may require a longer time after vector administration to achieve steady levels relative to CAG. These studies demonstrate that promoter strength and kinetics are critical factors to consider for experimental design in neuroscience research.

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Poster

244. Gene, Protein, and Cell Based Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 244.22

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Discovery of Novel Drug Candidates for The Treatment of Neurological Disorders Through NINDS Blueprint Neurotherapeutics Network (BPN)

Authors: *P. LAENG[1], S. RAEISSI[1], M. HACHICHA[1], M. PELLEYMOUNTER[1], R. ROOF[1], E. MICHELOTTI[2], C. TAYLOR-BURDS[1], O. O'NEIL-MATHURIN[1], C. BONDAR[1], R. RANGARAJAN[1], B. KLEIN[1], M. OSHINSKY[1], V. WHITTEMORE[1], C. CYWIN[1];
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Abstract: Although the success rate of novel treatments for CNS diseases is low, the perceived difficulties have not limited the enthusiasm of academic and industry scientists to undertake novel drug discovery approaches for the treatment of neurological disorders. To de-risk and boost the drug discovery and development process in the neuroscience field, the division of translational research (DTR) within NINDS, and in collaboration with other NIH-institutes, introduced a series of translational programs to promote neuroscience drug discovery and development efforts to mitigate the current pipeline gaps. In this presentation, we show examples of how the Blueprint Neurotherapeutics (BPN) funding and cooperative mechanism, resources and knowhow have contributed to translate academic and industry discoveries in basic disease biology into development of novel drug candidates for IND approval and advance toward clinical testing.

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Poster

245. Tools and Resources for Data Standardization and Data Sharing

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 245.01

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Common Fund's SPARC program, OT3OD025349
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NIH Common Fund's SPARC program, OT2OD030541

Title: Collaborative, sustainable, and FAIR neurosciences in bioelectronic medicine: the SPARC Data Resource Center

Authors: *E. IAVARONE[1], E. NEUFELD[1], J. B. WAGENAAR[2], P. HUNTER[3], M. E. MARTONE[4], B. DE BONO[3], M. HEAL[5], N. KUSTER[1,6];
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Abstract: Stimulating the autonomic nervous system (ANS) to modulate organ function holds enormous therapeutic promise, but the underlying mechanisms are poorly understood. The NIH SPARC program aims to improve targeted therapies by providing access to high value datasets, maps, tools, and predictive simulations to support the development of safe, and effective neuromodulation devices. Over 100 teams world-wide have worked on ANS mapping,

manipulation/observation tools, and therapeutic translation. Such a large initiative requires infrastructure to ensure that the vast amounts of generated data are interpretable, integrated, sustainable preserved, and FAIR (Findable, Accessible, Interoperable and Reusable). For these reasons, and to facilitate collaborative open science, SPARC has established infrastructure to: i) store, organize, and disseminate data, ii) establish standards and provide curation and knowledge management services, iii) generate interactive visualizations of nerve-organ anatomy and function, and iv) provide integrated modeling and data analysis.The SPARC Portal hosts data and computational studies, which can be found through knowledge-base and map-driven search, viewed, downloaded, or executed. It provides unified access to: 1) Pennsieve, a cloud-based data management platform to curate and publish large scientific datasets; 2) the Knowledge Graph that links data with knowledge about ANS anatomy, physiology, and connectivity and supports search; 3) technology for image segmentation, co-registration, annotation, and visualization; 4) the ApiNATOMY framework to define relationships between anatomy components and the nervous system; 5) Flatmap/Scaffold views that provide multi-scale visualization of ANS connectivity and data relationships; and 6) o²S²PARC, a cloud-based, open, extensible computational platform that serves to predict the effects of ANS neuromodulation on organ function.The SPARC Portal currently hosts over 100 experimental and computational datasets from 30+ anatomical structures and organ systems. Another 150 datasets will soon become available. Many of these are linked to computational tools such as Jupyter Notebooks on the o²S²PARC platform, which allows to interactively explore them in real time. Example applications include a treatment planning pipeline for spinal-cord stimulation and the use of SPARC image-data as part of an AI- and biophysics-based computational nerve-stimulation and sensing model.The integration of these SPARC platform resources will accelerate progress in the field of bioelectronics medicine, the wider life-sciences and beyond.

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Poster

245. Tools and Resources for Data Standardization and Data Sharing

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 245.02

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant U24 NS120057

Title: Tools for Linking NWB Neurophysiology Data to Ontologies and Digital Identifiers

Authors: M. AVAYLON[1], *R. LY[1], O. RUEBEL[2];
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Abstract: Neuroscience datasets generated from different sources often lack important metadata that provide context and definitions for terminology used in the data. As a result, researchers are

often unable to perform their own analysis of existing data. In order to have a synergistic, FAIR data ecosystem that supports data reuse, it is essential to have a standardized method to create and manage linkages between data terms and external references, such as online ontologies or digital identifiers, and to have these linkages be integrated with a data standard. Neurodata Without Borders (NWB) is a neurophysiology data standard that lays out an interoperable standard for sharing and storing data, while also providing a variety of analysis tools. Focused towards community-driven development, NWB has seen widespread adoption across neuroscience labs.
Integrated within NWB, we present the "Neuroscience External Resources Data Standard" (NERD) to enable researchers to link terms within neurophysiology data (e.g., data from intracellular and extracellular electrophysiology experiments), stored as NWB files, to external resources. To reduce data redundancy and make data queries efficient, NERD stores both these assets and a reference to the corresponding NWB data object internally in a collection of interlinked denormalized tables. NERD is expected to be used with memory intensive datasets and files (e.g., NWB files). To avoid reading and rewriting these large files to make annotation updates, NERD is stored as a separate, tailored HDF5 file that is read automatically by the PyNWB API when reading the associated NWB file.
This framework is a first step in providing the neuroscience community with valuable metadata tools within the NWB data standard.

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Poster

245. Tools and Resources for Data Standardization and Data Sharing

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 245.03

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant U24 NS120057

Title: Support for proprietary formats and best practices in the NWB ecosystem

Authors: *C. BAKER[1], S. WEIGL[2], R. H. MARTINEZ[3], J. SPRENGER[4], O. RUEBEL[6], B. DICHTER[5];
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Abstract: Neurodata Without Borders (NWB) is a data standard that packages neurophysiology data with the metadata necessary for reanalysis. The NWB format allows data to be human- and machine-readable, and allows data to be aggregated across many labs. As NWB sees growing adoption, helping a community with very diverse data converge on a standard is a challenge with many nuances. Here we discuss NWB Conversion Tools (NCT) and NWB Inspector.

Researchers often need to convert their data from common acquired proprietary formats such as Intan, Neuropixels, TIFF, ABF, etc. It can be challenging to map these different data formats, often with specialized APIs, to NWB. To address this challenge, we have developed NCT, a library for automatically extracting data and metadata from each of these formats and writing this data to NWB. NCT now supports 46 proprietary formats spanning extracellular, intracellular, and optical electrophysiology. For each of these formats, NCT applies chunking and lossless compression, which reduces data volume by on average of ~30%.
Through inspecting published NWB files, we have found patterns of mistakes and opportunities for improvement. Inspecting this data by hand has several drawbacks: it is time-intensive, it is variable, and it necessarily occurs after data submission. To help data publishers produce the best data possible, we have developed a collection of 'Best Practices' to serve as additional guidelines beyond the base schema and NWB Inspector, a tool for detecting deviations from these guidelines. In contrast to the NWB validator, which checks for strict schema adherence, NWB Inspector looks at the values, shapes and layout of data and applies heuristics to ensure compliance with the Best Practices. The checks are divided into 3 categories: critical, best practice violations, and best practice suggestions. It also supports dynamic configuration to support alternative or stricter guidelines, such as those enforced by the DANDI Archive. Finally, NWB Inspector generates human- and machine-readable reports that communicate what adjustments would align the data with NWB Best Practices.
NCT and NWB Inspector are open-source, have thorough documentation and testing, and are effective tools for any lab interested in adopting NWB.

Disclosures: **C. Baker:** F. Consulting Fees (e.g., advisory boards); Sr. Neuro-data Scientist. **S. Weigl:** F. Consulting Fees (e.g., advisory boards); Jr. Neuro-data Scientist. **R.H. Martinez:** F. Consulting Fees (e.g., advisory boards); Jr. Neuro-data Scientist. **J. Sprenger:** F. Consulting Fees (e.g., advisory boards); Sr. Neuro-data Scientist. **O. Ruebel:** None. **B. Dichter:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Founder of CatalystNeuro.

Poster

245. Tools and Resources for Data Standardization and Data Sharing

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 245.04

Topic: I.07. Data Analysis and Statistics

Support: NIMH:1RF1MH123220-01
NIMH/NIH: R24MH116922
NIMH/NIH: U24MH114827

Title: Ontologies to support BRAIN Initiative Cell Census Network (BICCN) data and future expansion

Authors: *P. L. RAY[1], S. Z. TAN[2], P. M. BAKER[1], T. GILLESPIE[3], H. KIR[2], L. NG[1], D. OSUMI-SUTHERLAND[2], C. THOMPSON[1];
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Abstract: The BRAIN Initiative Cell Census Network (BICCN) represents an ambitious and groundbreaking work in cataloging cell types in the mouse, human, and non-human primate brain. The initial phase of this project focuses on the primary motor cortex, identifying cell types using single-cell transcriptomic, morphological, electrophysiological, and anatomical data to characterize these cell types. The BICCN's impact and ambition is reflected in the volume of the data generated during this project. This presents a challenge for data management especially given the increasing impact of FAIR (Findability, Accessibility, Interoperability, and Reusability) sharing principles. Ontologies are critical infrastructure that play a key role in the searchability and integration of these data, providing a framework for data and driving categorization of novel cell types. One of the goals of the BICCN is to catalog the cell types of the brain, and the foremost task for ontology development in support of this goal has been building a cell types ontology to define and represent cell types directly from data generated through the BICCN. The resulting Brain Data Standards Ontology (BDSO) is an extension of the Cell Ontology (CL) which assists in cell type annotation and supports a web application (Cell Types Explorer). A semi-automated pipeline is used to link cell type taxonomies to marker gene profiles from datasets generated by the BICCN, thus integrating data from multiple sources. The Electrophysiology Stimulation (ESTIM) ontology describes 1-dimensional electrophysiology stimulation patterns and waveforms. The ontology integrates with the NWB schema for stimulus description and referencing external resources such as registries. The ESTIM ontology leverages existing ontology classes from common reference ontologies and contains 422 classes for annotation of electrophysiological data. The Techniques and Methods for Neuroscience (TMN) ontology helps neuroscientists communicate about their experiments and results by defining a set of terms for techniques, modalities, devices, and related experimental entities. The TMN ontology is built using the Ontology for Biomedical Investigations (OBI) description framework and leverages other ontologies from the OBO Foundry ontologies to ensure that proper classification and descriptions of the experimental protocols involved in a large project like the BICCN are available for reuse. Finally, we have cross-referenced and mapped the Allen Brain Atlas to the Uber-Anatomy Ontology (UBERON) so that specific cell location, projection, and anatomy is integratable across species in a centralized and scalable fashion.

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Poster

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Program #/Poster #: 245.05

Topic: I.07. Data Analysis and Statistics

Support: NIMH grant U24MH114827

Title: A Guide to Five years of Data from the BRAIN Initiative Cell Census Network (BICCN)

Authors: *C. THOMPSON[1], A. E. BANDROWSKI[3], K. BAKER[1], P. M. BAKER[2], P. BISHWAKARMA[1], T. FLISS[1], P. L. RAY[5], L. KRUSE[1], L. NG[5], T. TICKLE[6], M. E. MARTONE[4], M. J. HAWRYLYCZ[7];
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Abstract: The BRAIN Initiative Cell Census Network (BICCN) is a five year effort to integrate molecular, anatomical and functional data for describing cell types in mouse with preliminary work in primates. Single cell approaches are applied with an emphasis on rapid dissemination of data to the public before publication of associated articles. Of the 57 grants in the BICCN, over 30 of them focused on generation of foundational cell typing datasets. While most data are for the mouse brain, over 23 species are profiled using more than 40 different imaging, sequencing, and neurophysiological recording techniques. The BICCN recently published a landmark survey of cell types in primary motor cortex using multimodal data from single cell transcriptomics, ATAC-seq and DNA methylation, spatial transcriptomics, and Patch-seq. This analysis was a template and framework for profiling of the entire mouse brain and an opportunity to extend this work to human and non-human primates in the upcoming consortium effort, the BRAIN Initiative Cell Atlas Network (BICAN).
The BRAIN Cell Data Center (BCDC) serves as a coordinating center and provides a community resource for organizing and accessing data, information, knowledge and associated tools about the cellular organization of the brain. The BCDC maintains a portal of information including descriptions of community standardized pipelines developed for the BICCN. The current BCDC infrastructure includes a cloud-based data ecosystem for ingesting and hosting data for the BICCN Data Catalog, a searchable catalog of BICCN data accessible at several BRAIN-supported data archives. To support FAIR data and interoperability, we developed the Allen Institute Ontology (AIO) as an application ontology that underlies the BICCN Data Catalog and utilizes several ontologies from the Open Biological and Biomedical Ontologies (OBO) in the context of the Basic Formal Ontology (BFO) as well as more recent extensions we have developed. Furthermore, our ontology and triple store use a suite of W3C standards that include RDF, RDFS, OWL, SPARQL, and SHACL to store, query and validate data. These data provide a significant resource for cell type characterization in the brain.

Disclosures: C. Thompson: None. **A.E. Bandrowski:** None. **K. Baker:** None. **P.M. Baker:** None. **P. Bishwakarma:** None. **T. Fliss:** None. **P.L. Ray:** None. **L. Kruse:** None. **L. Ng:** None. **T. Tickle:** None. **M.E. Martone:** None. **M.J. Hawrylycz:** None.

Poster

245. Tools and Resources for Data Standardization and Data Sharing

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Program #/Poster #: 245.06

Topic: I.07. Data Analysis and Statistics

Support: NSF grant 1840218,2114202

Title: Using Open Science Chain to Protect the Integrity and Provenance of Research Data

Authors: *S. SIVAGNANAM;
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Abstract: In neuroscience large amounts of experimental and imaging data are produced in a wide variety of formats and are utilized to develop data-driven computational models for elucidating neuronal and network functions. Investigators whose research involves sophisticated processing of large amounts of these multimodal data require techniques to ensure its integrity, especially when trying to build upon prior research work done by other scientists. When the scientific dataset evolves or is reused in workflows creating derived datasets, the integrity of the dataset, its metadata, and the provenance needs to be securely preserved while providing assurances that they are not accidentally or maliciously altered during the process.
Open Science Chain (OSC) provides a cyberinfrastructure platform where the integrity information about scientific dataset is stored and managed in a consortium blockchain. Other researchers can independently verify authenticity of scientific data using the information stored in the blockchain and provide feedback when the data cannot be validated. OSC allows researchers to store the cryptographic hash of the data as a manifest in the blockchain along with the metadata. When updates are made to a dataset or data collection in OSC, all metadata changes, including the SHA256 checksum for every file in that data collection, are tracked in the blockchain, enabling users to view a detailed, immutable history of that dataset over time. Researchers can also link external repositories (e.g., GitHub) and other datasets contributed to OSC to create a detailed workflow of their scientific experiment, linking multiple sources of data and computational code used in their published results.
To lower the complexity barrier for use of this technology and to promote adoption, a web-based portal is developed with seamless user interfaces for interacting with the underlying blockchain. A python-based command line utility is also available that can be used from within a researcher's environment or integrated into research workflows. Neuroscience researchers, especially those involved in collaborative research will benefit from using the OSC to track the dataset that maybe generated and maintained at various locations. Members of research labs will benefit from tracking the provenance of data produced and referenced during different stages of research by various members. OSC aims to enhance data sharing and reproducibility in the neuroscience community by increasing the confidence of the scientific results.

Disclosures: S. Sivagnanam: None.

Poster

245. Tools and Resources for Data Standardization and Data Sharing

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Program #/Poster #: 245.07

Topic: I.07. Data Analysis and Statistics

Support: DFG grant 316803389, CRC 1280 "Extinction Learning", INF project

Title: From daily storage to data publication - Managing research data collaboratively across institutions

Authors: *M. PACHARRA[1], S. LINN[1], J. FRENZEL[2], N. O. C. WINTER[2], T. OTTO[3]; [1]Biopsychology, CRC 1280 "Extinction Learning", [2]IT.SERVICES, [3]Cognitive Psychology, Ruhr Univ. Bochum, Bochum, Germany

Abstract: Research data management (RDM) is part of the foundations of rigorous neuroscience research and key for fast and effective cooperation within collaborative research projects. A critical step in the RDM is to agree on common standards for data storage and metadata. However, standardizing research workflows, creating machine-readable metadata and using metadata to find research objects are often daunting tasks for researchers, if repository infrastructure is just being established.
This case report describes how the interdisciplinary Collaborative Research Center (CRC) 1280 “Extinction Learning” that includes more than 70 researchers at four different institutions addresses these issues: The participating researchers agreed on a common metadata scheme and a folder structure to store research data. To enable the implementation of this scheme within active research, open-source applications were developed that (1) store metadata as local files together with the research data (MetaDataApp), and (2) make metadata searchable (DatabaseApp). In this way, neuroscientific data and metadata from more than 2000 human subjects and lab animals are shared within the CRC.
Based on this preliminary work, workflows within an open-source platform were developed that allow bulk and daily ingest of research data and metadata as well as the creation of new metadata. Incorporating dedicated internal review workflows will allow researchers to exchange information within the CRC, archive the data according to guidelines of good scientific practice and make CRC data publicly available according to the FAIR principles. The roll-out of the future repository solution and sustainable implementation of RDM within the CRC is accompanied by establishing a CRC policy for the handling of research data.

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Poster

245. Tools and Resources for Data Standardization and Data Sharing

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Program #/Poster #: 245.08

Topic: I.07. Data Analysis and Statistics

Support: NIH R24EB029173

Title: NIH funded NITRC's triad of services: software, data, compute

Authors: *G. A. ASCOLI[1], T. NITRC[2], D. N. KENNEDY[3];
[1]George Mason Univ., George Mason Univ., Fairfax, VA; [2]TCG, Washington, DC; [3]U. Massachusetts Med., U. Massachusetts Med., Worcester, MA

Abstract: NeuroImaging Tools and Resources Collaboratory (NITRC) is a neuroimaging knowledge environment for MR, PET/SPECT, CT, EEG/MEG, optical imaging, clinical neuroinformatics, computational neuroscience, and imaging genomics tools and resources. Initiated in 2006 through the NIH Blueprint for Neuroscience Research, NITRC's mission is to foster a user-friendly knowledge environment for the neuroinformatics community. By continuing to identify existing software tools and resources valuable to this community, NITRC's goal is to support its researchers dedicated to enhancing, adopting, distributing, and contributing to the evolution of neuroinformatics analysis software, data, and compute resources. Located on the web at www.nitrc.org, the Resources Registry (NITRC-R) promotes software tools and resources, vocabularies, test data, and databases, thereby extending the impact of previously funded, neuroimaging informatics contributions to a broader community. NITRC-R gives researchers greater and more efficient access to the tools and resources they need, better categorizing and organizing existing tools and resources, facilitating interactions between researchers and developers, and promoting better use through enhanced documentation and tutorials—all while directing the most recent upgrades, forums, and updates. All services freely downloadable, NITRC-R offers over **1,300** public resources; NITRC-Image Repository (NITRC-IR) offers 17 data projects, **11,559 subjects,** and **13,282 imaging sessions**, and NITRC Computational Environment (NITRC-CE) provides cloud-based computation services downloadable to local machines or via commercial cloud providers such as Amazon Web Services. In summary, NITRC is an established knowledge environment for the neuroimaging community where tools and resources are presented in a coherent and synergistic environment. NITRC is a trusted source for the identification of resources in this global community. With over **12,400 citations** on Google Scholar, NITRC has supported over **71,000 registered users,** served up **12.3 million total**, and of that, **10.4 million data** downloads, to over **1.9 million users** generating **3.9 million sessions**. In addition to untold downloaded Virtual Machines, NITRC-CE currently supports over **310 subscriptions** on AWS Marketplace running over **611,400 compute hours**. We encourage the neuroinformatics community to continue providing valuable resources, design and content feedback and to utilize these resources in support of data sharing requirements, software dissemination and cost-effective computational performance.

Disclosures: G.A. Ascoli: None. **T. Nitrc:** None. **D.N. Kennedy:** None.

Poster

245. Tools and Resources for Data Standardization and Data Sharing

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Program #/Poster #: 245.09

Topic: I.07. Data Analysis and Statistics

Title: A novel platform for open source neuroscience research

Authors: *E. POLLOCK, D. OROZCO COSIO, D. MCCALL, J. WANG;
Data Mgmt. for Open Sci., Cambridge, MA

Abstract: Numerous computational methods have been developed in recent years to analyze large, complex datasets in neuroscience. However, the code libraries implementing those methods often rely on particular hardware specifications and software dependencies, and the time, effort, and resources required to implement researcher-generated code without technical support can pose significant barriers to research. Furthermore, neuroscience datasets often contain multiple data modalities, and although standards for organizing and formatting these datasets exist, the adoption of data standards is low and uneven across subfields. Thus, even when the datasets for a given publication are made available, they are often inaccessible due to a lack of human-readable metadata. Together, these problems with code and data accessibility mean that analysis pipelines are often inflexible and difficult to compare or replicate. Making data and code more accessible and reusable can increase the reproducibility of findings and help to accelerate the cycle between experiment and analysis.
Towards this goal, we introduce a research exchange platform that combines several features to provide a novel solution to the problem of data and code accessibility, called Ontologic. First, Ontologic guides users through the process of annotating important metadata in their datasets and packaging their analysis code for public use. These actions lower the barriers to adopting data standards and translating between them; they also reduce the technical expertise needed to publish and implement open source computational tools. Second, the platform allows users to arrange standardized tools and input data into a modular analysis pipeline, represented as a directed graph. Results are therefore associated with a unique pipeline, which can be easily shared, studied, and reproduced by others. Third, the platform provides cloud computing infrastructure for running complex analyses, negating the need to own and maintain local resources. We illustrate these features in a case study involving the analysis of electrophysiology data.

Disclosures: **E. Pollock:** ; Employment and Ownership Interest: Data Management for Open Science. **D. Orozco Cosio:** ; Employment and Ownership Interest: Data Management for Open Science. **D. McCall:** ; Employment and Ownership Interest: Data Management for Open Science. **J. Wang:** ; Employment and Ownership Interest: Data Management for Open Science..

Poster

245. Tools and Resources for Data Standardization and Data Sharing

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 245.10

Topic: I.07. Data Analysis and Statistics

Support: LDRD 151345

Title: N2a: a language and software tool for large-scale modeling

Authors: *F. ROTHGANGER;
Sandia Natl. Labs., Albuquerque, NM

Abstract: Sharing models and data is a prerequisite for progress toward a full understanding of brain function. The Neuroscience Information Framework (NIF) does this for many forms of descriptive data. Interchange languages such as NeuroML/LEMS provide a simulator-agnostic description of models, and repositories such as NeuroML-DB and ModelDB make them searchable and accessible.
However, exchanging models and data is not sufficient. It is also necessary to assemble those shared models into larger functional units, ultimately reaching the level of an entire nervous system. To create abstract descriptions of function, the modeling system must be capable of crossing all the scale levels, and component models should be expressed in a form suitable for automated analysis.
N2A ("Neurons to Algorithms") is an effort toward these challenging goals. It treats models as data rather than code. This declarative approach describes a model as a set of attributes and equations, without specifying a step-by-step procedure for simulation. It emphasizes the relationships between values within a model and the relationships between models in a larger functional unit.
The declarative approach allows one model to directly extend and modify another, simply by referencing the parent model and declaring new values for specific attributes and equations. A model may also incorporate other models as components, allowing the assembly of arbitrarily deep systems. We will demonstrate an open-source implementation of N2A.

Disclosures: F. Rothganger: None.

Poster

245. Tools and Resources for Data Standardization and Data Sharing

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Program #/Poster #: 245.11

Topic: I.07. Data Analysis and Statistics

Support: ERC starting grant #639272
Research Council of Norway #274306

Title: Nansen - a generalized data pipeline and visualisation toolbox for two-photon imaging data

Authors: *E. HENNESTAD[1], A. LANDE[1], K. G. VERVAEKE[2];
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Abstract: Two-photon microscopy is widely used to investigate brain functions. This technique produces vast amounts of images that require advanced and labour-intensive processing steps to extract neuronal signals. The complete analysis pipeline typically includes many steps such as brain motion correction (image registration), delineating neural structures of interest (image segmentation), and methods to infer neuronal spiking from the extracted signals. An increasing number of toolboxes have appeared that deal with these individual steps, but few of these provide a complete analysis pipeline. This often leaves the users with a significant amount of custom programming to incorporate a diverse set of toolboxes into their data analysis pipeline. Furthermore, these toolboxes often provide limited functionality for visualization and quality control. To tackle this problem, we have developed the "Neuroscience ANalysis Software ENsemble" (NANSEN). This package contains modules for building a complete processing pipeline and apps for exploring data using interactive graphical user interfaces. Toolboxes that are widely used by the neuroscience community, such as NoRMCorre, CaImAn and Suite2P are included as plugins in Nansen. Because each toolbox has strengths and weaknesses for a specific step in the analysis pipeline, users can compare the different toolboxes side by side to learn which one works best for their specific data. Also, as new toolboxes are published there is a standardised framework in place for adding them to Nansen. Importantly, to facilitate sharing and re-use data, users can also work with Neurodata without Borders (NWB) files, as well as create sharable projects based on their custom files and data.The aim of Nansen is to streamline the data management and processing for new users, to provide powerful visualization tools for existing and advanced users, and act as a platform where users can share data projects as well as analysis & processing methods.

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Poster

245. Tools and Resources for Data Standardization and Data Sharing

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Program #/Poster #: 245.12

Topic: I.07. Data Analysis and Statistics

Support: This project has been made possible in part by a grant from the Chan Zuckerberg Initiative DAF, an advised fund of Silicon Valley Community Foundation.

Title: Addgene's AAV data hub: An open-source resource for technical AAV expression data

Authors: *J. NASSE, D. BOURGES, B. PYHTILA, E. PERKINS, H. ZIRKLE, M. FAN;
Addgene, Watertown, MA

Abstract: The use of AAV vectors in neuroscience research continues to rise along with the complexity of the genetic tools they deliver. As the complexity of these vectors increases, the

knowledge required to effectively use these tools also increases. This can result in scientists spending months performing optimizing and troubleshooting experiments prior to addressing the scientific question at hand and depleting often limited resources. Additionally, troubleshooting or purely technical data is rarely published or shared with the greater scientific community where it can be used for planning AAV experiments. One factor contributing to the problem is a lack of appropriate outlets for publishing purely technical data. To address this problem, Addgene initiated an open-source, community driven, AAV Data Hub (DH) to house this technical data. Our DH receives over 10,000 views per year and currently hosts 140 data entries from 5 species, 45 viral tools, and 8 serotypes that can be used by scientists planning AAV experiments. Despite the initial success of this initiative, challenges still remain in obtaining high-quality data and in convincing scientists of the value in sharing their technical AAV data. As such, we surveyed scientists that had previously submitted data to the DH or had received AAV vectors from Addgene to ascertain what measures we could take to improve this resource. This survey identified several key areas for improvement. First, the primary reason for scientists not depositing data stems from a lack of knowledge about the DH (51.5% of respondents) and what type of data, for example negative data (11.7%), is accepted. Second, respondents indicated they have concerns around submitting unpublished (23.3%), poor quality (28.3%), or negative (11.7%) data. When asked what would increase interest in submitting data, citable DOIs and public recognition represented 67.6% of respondents. Financial incentives (35.1%), easier data submission (24.3%) and data embargoes (24.3%) were also indicated. In response to these survey results, Addgene has improved the functionality of the AAV DH and made it easier to submit data through our online DH webpage. We are also initiating minimum data standards and the ability to embargo a submission until publication. The DH improvements were developed using FAIR principles allowing for machine readability, and the code will be released as open-source. We are also now providing citable DOIs through DataCite at no cost to the depositor so data can be found and scientists are recognized for their work. By coming together scientists can help each other save valuable time and funds. Please help your colleagues by sharing your AAV data today!

Disclosures: **J. Nasse:** None. **D. Bourges:** None. **B. Pyhtila:** None. **E. Perkins:** None. **H. Zirkle:** None. **M. Fan:** None.

Poster

246. Electrical Stimulation: New Techniques

Location: SDCC Halls B-H

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Program #/Poster #: 246.01

Topic: I.08. Methods to Modulate Neural Activity

Support: NSERC

Title: Open MEA: an open-source multielectrode array platform for closed-loop neuroelectronics

Authors: *G. O'LEARY[1], I. KHRAMTSOV[1], R. RAMESH[1], A. PEREZ-IGNACIO[1], P. SHAH[2], H. MORADI[4], R. GENOV[3,5], T. A. VALIANTE[6,5];
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Abstract: Neuroelectronic interfaces have the potential to revolutionize the treatment of medical disorders and augment physiology. Implantable devices such as pacemakers and deep brain stimulators have already been deployed to control diseases including Parkinson's Disease and epilepsy. These devices typically operate by delivering electrical stimulation at pre-programmed intervals (known as open-loop stimulation). Recent advances in machine learning and low-power integrated circuits have brought the emergence of personalized medical devices that monitor the user's state and stimulate in response to measured biological activity (known as closed-loop stimulation). However, the development of neural-interfacing devices has been hampered by the complexity of proving their efficacy and safety, and our limited understanding of disease-relevant circuitry. While *in vivo* implantation must ultimately be performed, *in vitro* testing enables the preliminary assessment of functionality and device-tissue interaction dynamics using organotypic slices or cell cultures. This is typically performed using multielectrode arrays (MEAs). However, existing MEA systems have functional limitations and have closed-source designs that hinder their use by researchers who wish to prototype enhancements or make modifications for specific use-cases. This paper introduces OpenMEA, an open-source platform for closed-loop bioelectronics research. OpenMEA provides full access to all the components necessary for a benchtop in vitro laboratory including electrophysiological recording and stimulation electronics, a perfusion system for extracellular fluid and chemical delivery, physical designs for multielectrode arrays and microfluidic wells, and user interface software. The system is demonstrated with the electrical recording and stimulation of epileptogenic human and rodent brain slices. The aim of OpenMEA is to provide more equitable access to bioelectronic research and development tools to accelerate the deployment of devices for the treatment of disorders and beyond.

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Poster

246. Electrical Stimulation: New Techniques

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Program #/Poster #: 246.02

Topic: I.08. Methods to Modulate Neural Activity

Support: DARPA Grant N65236-19-C-8017

Title: Temporal Interference Stimulation Is Exhibited Differently Between Pyramidal And Parvalbumin Expressing Neurons

Authors: *S. CALDAS MARTINEZ[1], A. L. BARTH[3], P. GROVER[2];
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Abstract: Motivation. Temporal Interference (TI) stimulation proposes the idea of being able to stimulate the deep brain without superficial stimulation at kHz frequency. TI stimulation tries to leverage the observation that while high-frequency sinusoids modulated by a low-frequency sinusoid cause neural stimulation i.e. at the on-target, pure sinusoids of the same maximum amplitude do not i.e. at the off-target. This observation opens the door to a whole new world of possibilities in neurostimulation strategies. Hence, a complete understanding of how the TI phenomenon occurs at the neuronal level is essential. In this work, we examine the responses to TI stimulation of two different neuron-types: regular-spiking Pyramidal (Pyr) neurons and fast-spiking ParValbumin (PV) expressing neurons of cortical layer 2/3 (L2/3). Methods/Approach. We performed whole-cell current-clamp recordings of acute mouse brain slices (postnatal day (P18-30)). KiloHertz-frequency modulated and pure sinusoids were separately applied using a bipolar probe approximately 100um distant from the patched neuron. A neuron was said to exhibit TI stimulation when it was consistently stimulated by the modulated sinusoids but not by the corresponding pure sinusoid having the same peak amplitude. Results. Unexpectedly, our experimental results show that these two neuron-types exhibit different responses to TI stimulation. On one hand, 73% (out of 30) L2/3 Pyr neurons exhibit TI stimulation:they are only consistently stimulated by modulated sinusoids (and not pure sinusoids). On the other hand, 76% (out of 25) L2/3 PV neurons do not exhibit TI stimulation: they are consistently stimulated by pure sinusoids at peak amplitudes at which also modulated sinusoids cause it to be consistently stimulated. Further, indistinctly on the type of sinusoid, L2/3 PV neurons have 3x lower peak amplitude threshold for consistent firing than L2/3 Pyr neurons. Conclusions. We provide experimental results that L2/3 PV neurons exhibit TI with significantly lower tendency than L2/3 Pyr neurons and at lower peak amplitude thresholds. A by-product of this observation is that during TI stimulation, both Pyr and PV L2/3 neurons are stimulated on-target (where modulated sinusoids are observed), meanwhile only L2/3 PV neurons are stimulated off-target (where pure sinusoids are observed) changing the current understanding of TI stimulation.

Disclosures: S. Caldas Martinez: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; DARPA No. N65236-19-C-8017. **A.L. Barth:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; DARPA No. N65236-19-C-8017. **P. Grover:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; DARPA No. N65236-19-C-8017..

Poster

246. Electrical Stimulation: New Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 246.03

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant NS120922-01A1 and AG069912-02
Pennsylvania Department of Health (Project Number: 4100087331)
University of Pittsburgh Momentum Fund

Title: Full length APP modulates the properties of the axon initial segment and modulates neuronal network activity in vitro

Authors: *F. MA, H. AKOLKAR, K. HERRUP;
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Abstract: Alzheimer's disease (AD) is the most common cause of dementia in the elderly. Though accounting for less than 5% of its total prevalence, familial forms of AD caused by mutation in one of three known disease genes, *PS1*, *PS2* and *APP*, have been described. The *APP* gene encodes the amyloid precursor protein, whose proteolytic product (the Aβ peptide) occupies a central role in the AD literature due to its presence in the amyloid plaques found in AD brains. Curiously, beyond serving as an Aβ precursor, the normal function of the full-length APP protein remains largely unknown. We have previously discovered an important role for APP in modulating the length and position of the axon initial segment (AIS). Our new work explores the changes in neuronal spiking activity resulting from changes in APP levels. We established cultures of mouse cortical neurons on high density multielectrode-array (HD-MEA, Maxwell Biosystems) with electrodes spaced 17 μm apart, center-to-center. Such cultures become electrically active after 10 days in vitro (DIV10). We found that the number of neurons where spikes could be detected and the amplitudes of their action potentials remained constant after DIV12. The spontaneous firing rate, however, slowly increased through at least DIV21. These trends however change when cells were modified to overexpress APP at DIV14 by transduction with APP expressing lenti virus. In such manipulated cultures, the firing rate and the number of active neurons dropped significantly. We have further analyzed the cultured neural networks to identify axonal tracks and compared the different features such as speed of axon potential propagation and length of formed axons in the presence or absence of APP overexpression. Further, using extracellular electrical stimulations using the HD-MEA, we will study the changes in the generation of action potentials due to APP overexpression. Wild type human APP as well as APP carrying a familial APP mutation (APP_{Swe}) will be compared. We have demonstrated the slowing of network activity following virally induced APP expression, and will compare these findings with MEA cultures of neurons from the R1.40 AD mouse model. Cells from this mouse carry an APP_{swe} transgene that expresses human APP at 3-times the levels of the endogenous mouse *APP* gene. These findings with APP overexpression will be contrasted with the behavior of neurons after shRNA knockdown of APP. Taken together, our findings suggest that neuronal activity alters the levels of APP which in turn modulates the properties of the AIS in such a way as to maintain network homeostasis. The implications of these findings for the interpretation of *APP* as an AD disease gene will be discussed.

Disclosures: F. Ma: None. **H. Akolkar:** None. **K. Herrup:** None.

Poster

246. Electrical Stimulation: New Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 246.04

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH SPARC OT2 OD025340

Title: Validated computational models of autonomic nerve stimulation

Authors: *N. PELOT[1], E. MUSSELMAN[1], D. MARSHALL[1], M. HUSSAIN[1], A. UPADHYE[2], O. BUYUKCELIK[2], A. SHOFFSTALL[2], W. GRILL[1];
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Abstract: Background: Devices are under development to stimulate the autonomic nervous system with electric currents to treat diseases including rheumatoid arthritis and heart failure. However, mechanistic understanding is often lacking. We are developing and validating computational models to enable robust design of therapies that are efficient, effective, and selective. **Methods**: We implemented anatomically-realistic, biophysical computational models of vagus nerve stimulation (VNS) in rat, pig, and human. We optimized stimulation parameters to activate target and avoid off-target fibres. **Results**: We validated our models against in vivo and clinical responses. The modelled and in vivo rat VNS strength-duration responses corresponded well for myelinated fibres, but the models overestimated thresholds for unmyelinated fibres. The modelled and in vivo pig VNS thresholds corresponded well for large and small myelinated fibres. The modelled human VNS thresholds for large and small myelinated fibres corresponded well to clinical laryngeal muscle and heart rate responses, respectively. We modelled human VNS using 2D segmented histology that we extruded longitudinally to create pseudo-3D models and compared thresholds to models with true three-dimensional (true-3D) morphology from segmented microCT images. The pseudo-3D thresholds were in a similar range as the true-3D models, but the true-3D thresholds varied more across fibres within a given fascicle. We optimized stimulation amplitudes for pig and human VNS with a six-contact cuff electrode. We successfully achieved selective activation using both biophysical models and a recurrent neural network. **Conclusions**: Our computational models provide a validated approach for analysis and design of autonomic nerve stimulation therapies.

Disclosures: N. Pelot: None. **E. Musselman:** None. **D. Marshall:** None. **M. Hussain:** None. **A. Upadhye:** None. **O. Buyukcelik:** None. **A. Shoffstall:** None. **W. Grill:** None.

Poster

246. Electrical Stimulation: New Techniques

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Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 246.05

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH SPARC OT2 OD025340

Title: Computational Modeling of Human Vagus Nerve Stimulation using Three-Dimensional Nerve Morphology

Authors: *D. P. MARSHALL[1], O. BUYUKCELIK[2], A. UPADHYE[2], A. SHOFFSTALL[2], W. M. GRILL[1], N. PELOT[1];
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Abstract: Vagus nerve stimulation (VNS) is a promising treatment for a range of medical conditions. However, inter-subject variability of efficacy remains high, and successful results from animal models often fail to translate to humans. Effective therapeutic stimulation is limited by side effects from activation of off-target fibers. Computational modeling enables study of VNS mechanisms and optimization of stimulation protocols.
Prior VNS modeling used 2D extrusion modeling (2DEM), where a representative histological section was extruded into the third dimension to generate pseudo-3D nerve morphology. However, human vagus nerve morphology changes rapidly along its length, with an average of ~14 fascicle merge or split events over the 8 mm that separates the electrode contacts of a typical clinical cuff. 2DEM assumes a constant cross section along the length of the nerve and may not accurately capture fiber responses to VNS.
We implemented and simulated true-3D models (3DM) of the human cervical vagus nerve (cVN; n = 6; 5 cm in length). We segmented volumetric microCT images of human cVN samples and built finite element models of VNS using a clinical cuff electrode. We modeled activation thresholds for mammalian myelinated axons and compared patterns of activation in 2DEM and 3DM for each of the 6 nerve samples. The range of activation thresholds across fiber locations and the strength-duration response were comparable between the 2DEM and 3DM for a given nerve. However, 2DEM did not accurately predict the spatial distribution of thresholds over the nerve cross section; 3DM thresholds exhibited higher intra-fascicle variability and lower inter-fascicle variability than 2DEM thresholds. Further, thresholds in 2DEM were more strongly correlated with perineurium thickness and fascicle diameter than in 3DM. 3DM thresholds were negatively correlated with fiber tortuosity, whereas 2DEM represents fibers as straight lines.
We quantified the accuracy of activation thresholds from 2D extrusion models of VNS as compared to microCT-based true-3D models. The targets of interest for optimizing VNS therapy involve selective activation of different fiber diameters (types) and fibers in different locations. We showed that 2D extrusion modeling can robustly predict the overall population response of the nerve fibers but can be inaccurate for predicting spatial targeting.

Disclosures: D.P. Marshall: None. **O. Buyukcelik:** None. **A. Upadhye:** None. **A. Shoffstall:** None. **W.M. Grill:** None. **N. Pelot:** None.

Poster

246. Electrical Stimulation: New Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 246.06

Topic: I.08. Methods to Modulate Neural Activity

Title: Analysis of MEG recordings by using variety of computational methods for understanding the neural mechanism underling conceptual numerical and color perception.

Authors: *A. SLEMAN[1], Y. EISENBERG[2], A. KLEKS[1], M. TEICHER[1];
[1]Bar-Ilan Univ., Ramat Gan, Israel; [2]Cornell Univ., Ithaca, NY

Abstract: Many functional neuroimaging studies have investigated the brain regions which support numerical and color perception processes. Yet, despite their central position in human cognitive importance, our understanding of the brain correlations of the processes involving is still evolving. In the first step, we developed methods to analyze the brain data: 1. ICA algorithm: a computational method for separating a multivariate signal into additive subcomponents. 2. PCA algorithm: a method of computing the principal components and using them to perform a change of basis on the data. After processing the data and analyzing the results we obtained the following: - The beginning of the response, for different representations of the same number, is found in the same area of the brain. - For different representations of the same number, the center of the reaction area is the same, but the continuation of the response varies according to the type of representation of the number. Additionally, the beginning of the response is found in the same area, however the later stages of the response vary different. - For different numbers, there are different response areas in the human brain. In particular, 1 and 5 appear in different power then 2,3 and 4. - The human brain utilizes more resources when it responds to a stimulus of the symbols, specifically seeing a group of white circles, rather than seeing a digit (such as 3), which corresponds to the same amount of circles. - The difference in response, seeing a number, and seeing white circles, was not due to the counting action of the white circles.

Disclosures: **A. Sleman:** None. **Y. Eisenberg:** None. **A. Kleks:** A. Employment/Salary (full or part-time):; IBM. **M. Teicher:** None.

Poster

246. Electrical Stimulation: New Techniques

Location: SDCC Halls B-H

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Program #/Poster #: 246.07

Topic: I.08. Methods to Modulate Neural Activity

Support: This work was also supported by NIH-NCATS CTSA KL2 TR002379 (KJM)
NIH-NIMH CRCNS R01MH122258 (DH)

Title: Canonical Response Parameterization: Quantifying the structure of responses to single-pulse intracranial electrical brain stimulation

Authors: *K. MILLER[1], K.-R. MUELLER[3], A. OJEDA VALENCIA[2], H. HUANG[2], N. GREGG[1], G. A. WORRELL[1], D. HERMES[2];
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Abstract: Single-pulse electrical stimulation in the nervous system, often called cortico-cortical evoked potential (CCEP) measurement, is an important technique to understand how brain regions interact with one another. Voltages are measured from implanted electrodes in one brain area while stimulating another with brief impulses separated by several seconds. Historically, researchers have tried to understand the significance of these evoked potential changes by visual inspection, but no general-purpose tool has emerged to understand them or describe them mathematically. We describe and illustrate a new technique to parameterize brain stimulation data, where voltage response traces are projected into one another using a semi-normalized dot product, varying the length of time from stimulation to obtain a temporal profile. The peak of the profile uniquely identifies the duration of the response. Using linear kernel PCA, a canonical response shape is obtained over this duration, and then single-trial traces are parameterized as a projection of this canonical shape with a residual noise term. This parameterization allows for dissimilar trace shapes from different brain areas to be directly compared by quantifying response duration, projection magnitudes & amplitudes, signal-to-noise ratios, explained variance, and statistical significance. Artifactual trials are automatically identified by outliers in sub-distributions of projection magnitude, and rejected. Our technique, which we call "Canonical Response Parameterization" (CRP) dramatically simplifies the study of CCEPs, but may also be applied in a wide range of other settings involving event-triggered data.

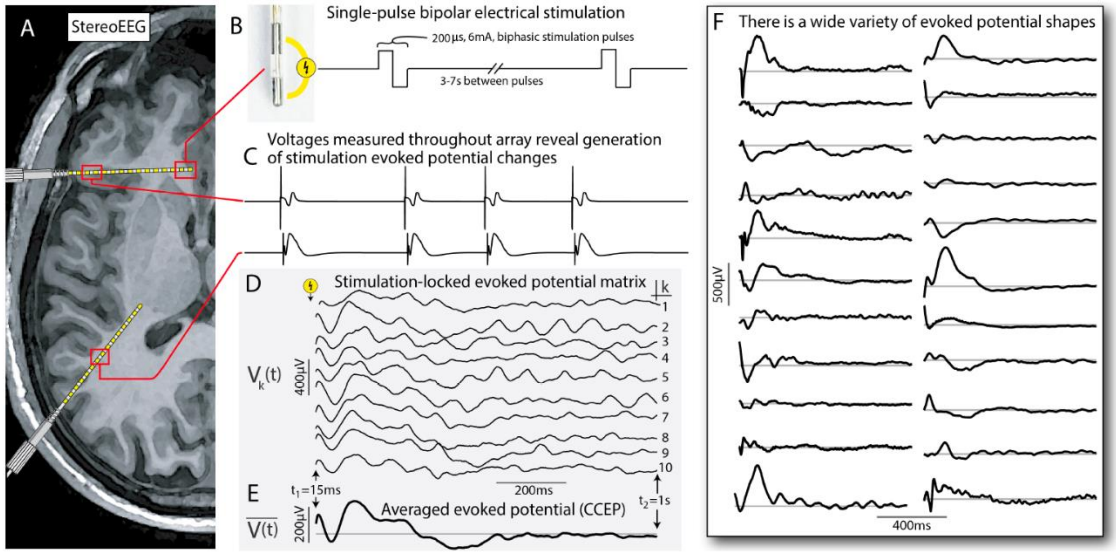

Fig 1. Single-pulse electrical stimulation with stereoelectroencephalography (sEEG). A. A cartoon schematic of an axial MRI with two sEEG leads. **B.** Single-pulse biphasic electrical stimulation is delivered through adjacent sEEG electrode contacts (200μs, 6mA), separated by 3-7s between pulses. **C.** Cartoon voltage traces that might be elicited at two different sites in response to stimulation at a third site (i.e. with a stimulation artifact followed by a characteristic evoked potential deflection). **D.** An example set of actual evoked potentials showing the stimulation-locked evoked potential matrix **V**, with columns $V_k(t)$) shown as individual traces. **E.** Average stimulation-evoked potential from (D). **F.** Examples of some of the different measured response shapes seen in these studies. These selected responses were produced from 5 stimulation sites across two patients (over the interval 15ms-1s post-stimulation, where the gray line indicates 0 μV). The variety of different shapes seen in just this small subset shows that there is no one typical form of the evoked potential ("CCEP" / "SPES") shape.

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Poster

246. Electrical Stimulation: New Techniques

Location: SDCC Halls B-H

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Program #/Poster #: 246.08

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant 1UG3NS123723-01
NIH Grant 1-DP2-EB029757-01
NIH Grant 1R01NS123655-01

Title: A Universal Model of Electrochemical Safety Limits for Electrophysiological Stimulation

Authors: *R. VATSYAYAN[1], S. DAYEH[2];
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Abstract: Electrical stimulation is a widely adopted therapy for diagnostic and therapeutical treatments with neuromodulation devices. The modalities of tissue damage have been extensively investigated empirically both *in-vivo* in animal models and in benchtop environments for conventional electrodes. However, there exists the need for a parametric study of the electrochemical safety models to predict the safety threshold independent of the electrode geometry, interface, and stimulation paradigm. In this work, we will present the stimulation limits for planar Platinum and Platinum Nanorods based flexible microelectrodes in benchtop experiments in saline, and then experimentally verify our results *in-vivo* in acute experiments in the rat cerebral cortex. We experimentally calculate the geometric correction factor, the electrolysis water window and the current safety limits from voltage transient measurements. Using four and three-electrode impedance measurements and comprehensive circuit models, we recreate the electrochemical interface in our measurements. Based on our benchtop measurements, we create a predictive equation for the cathodal excitation measured at the electrode interface as a function of the electrode dimensions, geometry, material and stimulation paradigm. We verify the accuracy of the modeled equation *in-vivo*, and using detailed electrochemical models, compare the experimentally determined safety thresholds to the stimulation protocols used clinically for stimulation, showing the necessity of determining electrochemical safety thresholds for clinical applications.

Disclosures: R. Vatsyayan: None. **S. Dayeh:** None.

Poster

246. Electrical Stimulation: New Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 246.09

Topic: I.08. Methods to Modulate Neural Activity

Title: The recruitment of afferents in response to dorsal root ganglion stimulation: A modelling study

Authors: *J. FAROOQUI[1,5,6], A. C. NANIVADEKAR[1,2,6], M. CAPOGROSSO[1,3,2], S. F. LEMPKA[7,8,9], L. E. FISHER[1,4,2];
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Abstract: Electrical stimulation of the dorsal root ganglion (DRG) is a clinically approved therapy for the management of refractory pain and a promising approach for neuroprosthetic systems to restore sensory feedback. Our lab has shown that penetrating microstimulation of the DRG can selectively activate distal nerve targets and, surprisingly, epineural macrostimulation can achieve similar selectivity. However, to develop targeted DRG stimulation approaches for focal sensory feedback, we must understand the mechanisms of selective activation. Therefore,

this work examines the recruitment properties of DRG stimulation using computational models to characterize the principles governing neural selectivity during DRG stimulation via epineural and penetrating electrodes.
To investigate the impact of neuron location, size, and structure on neural recruitment, we developed equivalent circuit models of pseudounipolar DRG neurons consisting of somas, axon initial segments, stem axons, T-junctions, and peripheral and central axons. We built a finite element method (FEM) model of the DRG (representing neural tissue, epineurium, epineural space, bone, and implanted electrodes) to calculate extracellular potentials resulting from current injection using penetrating and epineural electrodes, and populated it with neuron models representing Aα- and Aβ-afferent types according to realistic size and spatial distributions based on histology from feline lumbar DRG.
Our model indicates that the unique geometry and spatial distribution of neurons in the DRG constrains neural recruitment in response to stimulation. Epineural stimulation preferentially recruits the initial segment of the stem axon (adjacent to the cell body), while penetrating stimulation preferentially recruits T-junctions. Combined with the varying density of initial segments and T-junctions near the epineural and penetrating electrodes, these recruitment differences suggest that epineural electrodes achieve selectivity by evoking responses near the cell bodies, which are concentrated closer to the DRG circumference, instead of the fibers recruited by penetrating electrodes. Additionally, we find differences in epineural recruitment across fiber types: Aβ neurons are primarily recruited at the initial segment while Aα neurons are frequently recruited at a distal axon. These differences suggest the possibility of selecting specific sensory modalities via epineural stimulation.
Our results suggest that DRG micro-anatomy can make epineural stimulation an effective method for delivering focal sensory feedback of desired modality that appears to originate in the missing limb.

Disclosures: **J. Farooqui:** None. **A.C. Nanivadekar:** None. **M. Capogrosso:** None. **S.F. Lempka:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Abbott Neuromodulation, Medtronic PLC, Presidio Medical, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CereGate, Hologram Consultants, LLC, Presidio Medical, Inc. F. Consulting Fees (e.g., advisory boards); Abbott Neuromodulation, CereGate, Presidio Medical, Inc. **L.E. Fisher:** None.

Poster

246. Electrical Stimulation: New Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 246.10

Topic: I.08. Methods to Modulate Neural Activity

Support: ERC H2020 Grant 759998 "FeelAgain"
SNSF Grant 197271 "MOVEIT"
Innosuisse Grant 47462.1 IP-ICT

Title: Model-guided development of a novel neural interface for the selective stimulation of the autonomic nervous system

Authors: *F. CIOTTI[1], A. CIMOLATO[1], G. VALLE[1], L. SIFRINGER[2], S. RASPOPOVIC[1]; [1]Dept. of Hlth. Sci. and Technol., [2]Dept. of Information Technol. and Electrical Engin., ETH Zürich, Zürich, Switzerland

Abstract: Peripheral nerve stimulation has the potential to treat a plethora of clinical conditions, such as in the treatment of cardiovascular disease by vagus nerve stimulation or in the restoration of sexual function by sacral nerve stimulation. Its efficacy is strongly related to the performance of the adopted neural interface. State-of-the-art, highly selective interfaces require very long and complex surgeries, have poorly repeatable outcomes which are highly dependent on surgeon skills, and cause high tissue reactions. On the other hand, clinically-approved electrodes, such as extraneural leads, lack the ability to obtain desired therapeutic outcomes without tolerable off-target effects (i.e., have low selectivity). Through detailed computational models of the nerve-electrode interface, we developed a novel electrode designed for fast and repeatable implants on inter-individually varying nerve anatomies. The use of an in-silico platform allows for a high degree of optimization. Indeed, we chose each parameter with computationally inexpensive geometric models specifically developed for automatic optimization. The electrode was dimensioned for pudendal, sacral, and vagus nerves, characterized by similar sizes, but the proposed method can be applied to optimize any electrode design on any target nerve. We verified the performance of the final design against interfaces commonly used in clinical and research settings such as quadripolar leads, multipolar cuffs, and transverse intrafascicular multichannel electrodes in anatomically and neurophysiologically detailed models. In this environment, the new design has shown a favorable combination of selectivity, repeatability, and invasivity, making it a promising alternative to existing electrodes. We therefore manufactured a prototype using an original fabrication process using soft materials, i.e. a spin-coated PDMS substrate with stretchable conductive tracks and Parylene-C insulation patterned by focused ion beam. We evaluated its performance in-vitro by electrochemical characterization, showing viability for future in-vivo studies to sustain the predicted functional improvements with measured physiological outcomes.

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Poster

246. Electrical Stimulation: New Techniques

Location: SDCC Halls B-H

Time: Sunday, November 13, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 246.11

Topic: I.08. Methods to Modulate Neural Activity

Title: Sinaps bidir: a fully bidirectional, high spatio-temporal resolution, implantable cmos neural probe

Authors: *G. ANGOTZI[1], J. F. RIBEIRO[3], G. ORBAN[3], A. M. FOSSATI[4,6], M. VINCENZI[5], A. PERNA[4,6], F. BOI[2], L. BERDONDINI[7];
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Abstract: The recent advent of CMOS implantable neural probes has given neuroscience the unprecedented possibility of monitoring large brain ensembles at cellular resolution in animal models. Neuropixels and SiNAPS have become over the last few years the technological landmark for high-resolution implantable neural probes and research projects can now routinely rely on the analysis of action potentials (APs) from hundreds of neurons. Although this allows unprecedented progress in understanding the coordinated activity of large neuronal ensembles (e.g., those underlying sensory, motor and cognitive operations), a commensurate advance in the neuro devices needed for the manipulation of neural population dynamics is also necessary. With the aim of enabling active dense neural recordings and modulation of neural circuits at cellular level with sub-millisecond temporal resolution, we have designed a reconfigurable and low-power active pixel sensor circuit for realizing radically new high-density bi-directional SiNAPS CMOS probes. These probes integrate low-power microelectronic circuits to manage a large number (from hundreds to thousands) of bidirectional and flexibly configurable recording/stimulating microelectrode channels within a latency of few hundreds of microseconds. We will present preliminary results from a realized CMOS prototype that integrates 256 fully bidirectional active pixels (pitch of 30µm). Simulation and bench test results demonstrate that the proposed circuit architecture permits electrical microstimulation (>50uA without exceeding the power supply voltage limit) between any pair of single/clustered electrode-pixels, while guaranteeing concomitant LFPs and APs recordings at 20 kHz/channel from the rest of the electrode array. These results pave the way to new generations of closed-loop systems for studying neurodynamics at the mesoscale and for producing novel neuroelectronic therapeutic devices.

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Poster

246. Electrical Stimulation: New Techniques

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Program #/Poster #: 246.12

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF Grant CI-152159
NSF Career Grant 2145412

Title: Probing the Deep Brain: Using Novel Carbon Fiber Electrode Arrays to Record and Stimulate Brain Regions in Order to Better Understand Neuropsychiatric Disorders

Authors: *M. N. BAKER, S. R. SANTACRUZ;
Univ. of Texas, Austin, Austin, TX

Abstract: The burden of neurological and neuropsychiatric disorders has been increasing, with neuropsychiatric disorders being the leading cause of disability worldwide. The current route of treatment centers around pharmaceuticals and psychotherapy. These treatment options are limited, often having many adverse side effects. To better understand these disorders and how they can be more effectively treated, better tools are needed. Many common neurological disorders involve deep brain structures that are difficult to access with current electrodes. Standard metal and silicon electrodes often cause tissue damage during insertion that makes chronic recordings difficult and are typically optimized for superficial cortical recording. We propose a carbon fiber electrode array that can penetrate deep brain structures (>10mm) to record electrophysiological activity. To develop our array, we chose carbon fibers because of their flexibility and similar mechanical properties (Young's modulus) to brain tissue when compared to the conventional metals used in electrode arrays. These mechanical properties lessen the likelihood of biofouling during implantation, making it an ideal candidate for chronic implantation. Normally carbon fiber arrays are difficult to fabricate since the carbon fibers are not compatible with bulk silicon microfabrication, but we leverage methods that combines silicon microfabrication techniques with out-of-plane assembly to produce 2D, high-throughput devices. Once fabrication is complete, we perform electrical characterization of devices, including impedance spectroscopy, cyclic voltammetry, and charge injection analysis. We also analyze the mechanical durability and insertion mechanisms of the device using a phantom brain model. These tests establish that our novel carbon fiber arrays have ideal properties for neural recording, electrical stimulation, and insertion with minimal trauma. Once verified *in vivo*, the carbon nanofiber electrode arrays will be a powerful, multimodal tool that can be used in various research settings to help discover the modalities underlying neurological and neuropsychiatric disorders.

Disclosures: M.N. Baker: None. **S.R. Santacruz:** None.

Poster

246. Electrical Stimulation: New Techniques

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Program #/Poster #: 246.13

Topic: I.08. Methods to Modulate Neural Activity

Support: VA Grant I01RX001640

Title: Modulation of ensemble co-firing in motor cortex via theta-burst stimulation of motor-thalamus

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Abstract: Changes in ensemble co-firing, particularlythose associated with ongoing low frequency oscillations, is known to trackmotor recovery after stroke. Lowfrequency alternating current electrical stimulation (or direct currentstimulation) can also enhance ensemble co-firing and improve movement controlafter stroke. While such stimulation has been found to be effective, there is atranslational challenge given the high charge density required to enableeffective modulation. This is because the energy of electrical currentstimulation exponentially decays as the distance increases; the requiredstimulation power is likely well above current FDA safety guidelines to coverlarge scale motor cortical areas. In the current study, we tested whetherelectrical stimulation of the motor thalamus (Mthal) may be a viablealternative to direct modulation of cortical regions after stroke. Our approach is based on the fact that Mthalis a major pre-synaptic input to the motor cortex, including the primary andsecondary motor cortex (M1 and M2). We systematically tested the parametersthat can allow reliable modulation of ensemble co-firing in motor corticaltargets using Mthal stimulation in adult male rats. We found that singlebiphasic stimulation impulses in Mthal could modulate M1 and M2 on average.Intracortical electrophysiological recordings showed that single impulses withshorter than 1ms pulse width could drive upstates which had a jittered peakaround 30-120ms in repeated trials after stimulation onset. However, biphasicpulse train stimulation (theta-burst stimulation with 3 to 8 pulses with 10msinter-pulse interval) enhanced the modulation effect in M1 and M2 showingphase-locked response of upstate peaks at 80ms delays. We also found evidencefor higher population ensemble co-firing (and increased firing rate in M1 andM2) prior to the upstate. Increased number and larger width of pulses with1-8Hz burst stimulation could achieve reliable responses and temporal delays.The current results were performed with less than 1% of the charge-densitylimit for FDA safety guidelines (i.e., 30μC/Cm^2).These results suggest the possibility of using theta-burst stimulation in Mthalas a safer alternative to modulate cortical regions after stroke.

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