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Nanosymposium

010. Mechanisms of Neural Reprogramming

Location: SDCC 23A

Time: Saturday, November 12, 2016, 1:00 PM - 3:15 PM

Presentation Number: 10.01

Topic: A.01. Neurogenesis and Gliogenesis

Support: The Welch Foundation (I-1724)

The Mobility Foundation

NIH NS088095 and NS070981

The Decherd Foundation

Title: Direct reprogrammed adult human motor neurons for disease modeling and drug identification

Authors: M.-L. LIU[1], T. ZANG[2], Y. TANG[1], *C.-L. ZHANG[1];
[1]Mol. Biol., [2]UT Southwestern Med. Ctr., Dallas, TX

Abstract: Neurons derived from induced pluripotent stem cells (iPSCs) are increasingly employed to model human neurological diseases. However, these neurons are reset to an embryonic stage during the reprogramming process and thus inappropriate to model adult-onset neurodegeneration. In contrast, direct reprogrammed neurons without going through a stem cell stage maintain age-associated features of their origin. By combining small molecules and transcription factors for direct reprogramming, we have established a very robust and efficient approach to produce spinal motor neurons from adult human fibroblasts. The neurons converted from healthy humans maintain age-associated features and become functionally mature by firing repetitive action potentials and forming neuromuscular junctions in co-culture. In contrasts, motor neurons converted from fibroblasts of adult human patients with amyotrophic lateral sclerosis (ALS) show degenerative features, such as poor survival and inability to control muscle contraction. A candidate approach revealed that the small molecule kenpaullone can rescue most of these disease phenotypes. To identify additional small molecules for therapeutic development, we further conducted an unbiased screen of nearly two thousand compounds. The screening process and positive hits will be discussed during the meeting.

Disclosures: **M. Liu:** None. **T. Zang:** None. **Y. Tang:** None. **C. Zhang:** None.

Nanosymposium

010. Mechanisms of Neural Reprogramming

Location: SDCC 23A

Time: Saturday, November 12, 2016, 1:00 PM - 3:15 PM

Presentation Number: 10.02

Topic: A.01. Neurogenesis and Gliogenesis

Support: Swedish Parkinson Foundation (Parkinsonfonden)

European Research Council under the European Union's Seventh Framework Programme: FP/2007-2013 NeuroStemcellRepair (no. 602278)

ERC Grant Agreement no. 30971

Swedish Research Council

BAGADILICO

MULTIPARK

Title: Modeling Parkinson's disease in patient-derived neurons using direct reprogramming

Authors: *J. DROUIN-OUELLET[1], S. LAU[1], D. RYLANDER OTTOSSON[1], L. M. COLLINS[2], W.-L. KUAN[2], R. A. BARKER[2], M. PARMAR[1];
[1]Lund Univ., Lund, Sweden; [2]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Direct reprogramming of somatic cells into induced neurons (iNs) has opened up the possibility not only to use these as a source of cells for brain repair therapies but also to study disease processes in patient-derived neurons. We and others have successfully reprogrammed human fibroblasts into functional neurons, although conversion efficiency using adult human fibroblasts has remained low. We have developed a new improved conversion protocol that relies on transduction with a polycistronic vector containing Ascl1 and Brn2, as well as shRNA targeting REST and in combination with a dual SMAD signaling inhibition. Using this protocol we achieve high efficiency conversion of adult skin derived fibroblasts and are evaluating the conversion capability of skin fibroblasts from sporadic Parkinson's disease (PD) patients (n=8) and matched controls (n=8). Generated neurons exhibit mainly glutamatergic and GABAergic phenotypes and are electrophysiologically functional. Unbiased quantification of neuronal purity and yield reveals a similar propensity to convert between control (64.0% ± 11.0 neuronal purity) and PD lines (62.8% ± 13.0) after 35 days of reprogramming. However, a trend toward a increase in cell death during the reprogramming process can be seen in PD lines, which also exhibit a significantly bigger cell body. qPCR analyses of neuronal markers shows that both PD and control lines express MAP2, Synapsin1, Tau and SNCA gene expression in similar levels. These preliminary results suggest that iN cells from sporadic PD patients reprogram in a similar

fashion as that of healthy individuals and could thus serve as a tool to model intracellular pathological features associated with PD. As such, we are now assessing iN vulnerability to stressors such as rotenone and preform fibrils of αsyn. This study will provide insights into how we will need to approach disease modeling using lines from sporadic cohorts of subjects in terms of inter-individual variability and the effect of PD on the reprogramming process itself, which will serve as the basis for wider PD modeling using iNs.

Disclosures: **J. Drouin-Ouellet:** None. **S. Lau:** None. **D. Rylander Ottosson:** None. **L.M. Collins:** None. **W. Kuan:** None. **R.A. Barker:** None. **M. Parmar:** None.

Nanosymposium

010. Mechanisms of Neural Reprogramming

Location: SDCC 23A

Time: Saturday, November 12, 2016, 1:00 PM - 3:15 PM

Presentation Number: 10.03

Topic: A.01. Neurogenesis and Gliogenesis

Support: DFG Postdoctoral Fellowship

Title: A repressive pro-neuronal master regulator

Authors: ***M. MALL**[1], M. KARETA[1], S. CHANDA[1], X. GE[1], H. AHLENIUS[3], T. SÜDHOF[2], M. WERNIG[1];
[1]Inst. for Stem Cell Biol. & Regenerative Med., [2]Dept. of Mol. and Cell. Physiol., Stanford Univ., Stanford, CA; [3]Lund Stem Cell Ctr., Lund Univ., Lund, Sweden

Abstract: Neurogenesis involves a complex interplay between morphogens and transcription factors to initiate genetic programs that promote neuronal differentiation and subtype specification. However, at the same time unwanted genetic programs have to be shut down. In addition, signaling pathways such as Notch repress proneural transcription factors and neuronal differentiation to maintain progenitor cells. This raises the intriguing question how differentiating neurons can escape this inhibition and turn of unwanted transcription programs to enable neurogenesis.
Studying the conversion of fibroblasts to neurons we found that the neuronal reprogramming factor Myt1l can access most of its physiologic targets in fibroblasts and acts predominantly as repressor through recruitment of the Sin3/HDAC complex to silence many non-neuronal programs including the fibroblast-specific transcriptome. One of the repressed pathways is Notch by silencing of several members, explaining how newborn neurons can escape Notch activation during normal development. Based on our findings, we propose that active and sequence-specific

repression mechanisms exist to generally suppress many unrelated lineage programs enabling cell fate choice and stability involved in development and disease.

Disclosures: **M. Mall:** None. **M. Kareta:** None. **S. Chanda:** None. **X. Ge:** None. **H. Ahlenius:** None. **T. Südhof:** None. **M. Wernig:** None.

Nanosymposium

010. Mechanisms of Neural Reprogramming

Location: SDCC 23A

Time: Saturday, November 12, 2016, 1:00 PM - 3:15 PM

Presentation Number: 10.04

Topic: A.01. Neurogenesis and Gliogenesis

Support: Philip and Sima Needleman Graduate Student Fellowship

UMS SCIDRP

NIH DP2

Title: Expression of brain enriched microRNAs opens the neurogenic potential of adult human somatic cells

Authors: ***D. ABERNATHY**, M. MCCOY, W. KIM, A. YOO;
Developmental Biol., Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Advances in our understanding of genetic pathways that specify neuronal cell fates during neuronal development have directly enabled directed differentiation of pluripotent stem cells into specific neuronal subtypes. This knowledge has been further leveraged to directly convert (reprogram) fully differentiated somatic cells into neurons. However, the efficiency in converting primary adult human somatic cells - the ideal source of cells for patient specific disease modeling and regenerative therapy – into functionally mature neurons remains to be improved. Here, we demonstrate that the brain-enriched microRNAs alone, miR-9/9* and mir-124 (miR-9/9*-124), robustly induce a neurogenic state in young and old human fibroblasts. These microRNA-induced neurons are characterized by over 80% of cells positive for the neuronal markers TUBB3, MAP2 and NeuN. In monoculture, a majority of cells are capable of firing single and multiple action potentials. In order to demonstrate the potential of guiding the miR-9/9*-124-induced state guiding towards a specific neuronal subtype, we screened a pool of transcription factors (TFs) known to promote motor neuron (MN) identity during development. We identified two TFs capable of directing miR-9/9*-124 conversion into MNs with a high efficiency, whereas these TFs alone were not sufficient to reprogram primary human adult fibroblasts into neurons. Converted motor neurons stain positive for the hallmark MN markers

MNX1 and CHAT, fire multiple action potentials and exhibit spontaneous activity in mono-culture, demonstrating that our conversion method generates mature, excitable cells even in the absence of glial and other neuronal support. Co-culturing with human myotubes revealed that the converted MNs are able to form neuromuscular junctions. Transcriptional profiling of neurons that were reprogrammed by miR-9/9*-124 alone revealed a dramatic upregulation of hallmark neuronal genes with a concurrent downregulation of fibroblast genes, whereas MN-inducing TFs resulted in further enrichment of MN-specific genes. Our results demonstrate the potential of utilizing miR-9/9*-124 as a foundation for subtype-specific direct conversion and demonstrate the potency of microRNAs in overcoming cell fate barriers.

Disclosures: D. Abernathy: None. **M. McCoy:** None. **W. Kim:** None. **A. Yoo:** None.

Nanosymposium

010. Mechanisms of Neural Reprogramming

Location: SDCC 23A

Time: Saturday, November 12, 2016, 1:00 PM - 3:15 PM

Presentation Number: 10.05

Topic: A.01. Neurogenesis and Gliogenesis

Title: Origin and development of different astroglial phenotypes in the cerebellum

Authors: *V. CERRATO[1,2], E. PARMIGIANI[3], K. LETO[1,2], E. FUCÀ[1,2], M. FIGUERES-OÑATE[4], L. LÓPEZ MASCARAQUE[4], A. BUFFO[1,2];
[1]NICO (Neuroscience Inst. Cavalieri Ottolenghi), Orbassano, Italy; [2]Dept of Neurosci. Rita Levi Montalcini, Univ. of Turin, Turin, Italy; [3]Dept. of Biomedicine, Univ. of Basel, Basel, Switzerland; [4]Cajal Inst., Madrid, Spain

Abstract: The cerebellum is anatomically and functionally a complex structure. This is reflected by an extraordinary heterogeneity of neuronal and astroglial phenotypes, with distinct morphological and spatial features. While the mechanisms of neuronal diversification have been partially clarified, astrogliogenesis remains poorly explored. In this study, we investigated the genesis and the development of the repertoire of astroglial phenotypes. In order to study the lineage relationships among different astroglial phenotypes, we performed in vivo clonal analysis of embryonic ventricular progenitors using Star Track plasmids. We found clones containing both cortical and white matter (WM) astrocytes, separately or as members of the same family. The clone composition indicated the existence of four major embryonic progenitor types producing either granular layer (GL) or WM astrocytes, or a mixed progeny composed of Bergmann glia (BG) and GL astrocytes. In parallel, analysis in Confetti mice revealed that, postnatally, radial progenitors in the PCL divide in situ to generate both BG and GL astroglia. Moreover, early in embryonic development, another progenitor type produces big heterogeneous

clones that comprise WM, GL and BG astrocytes. Afterwards, this capability is significantly reduced. Of note is the fact that this reduction parallels the increase in fractions of homogeneous clones, suggesting a progressive fate restriction as development proceeds. Furthermore, in WM-GL-BG clones, astrocytes in the WM are overall fewer than their cortical counterparts. Double-thymidine and birthdating analyses showed that differences in the number of cells of each astrocyte subtype within a single heterogeneous clone are associated with layer-specific proliferative behaviour of precursors during the entire first postnatal week. In particular, the cells in the WM stop proliferating earlier than those in the cortex and those of the PCL (i.e. immature BG) reveal the highest amplification rate. Additionally, BG display different proliferative rhythms in crowns and fissures during early postnatal development, suggesting a contribution to cerebellar foliation.
In conclusion, our study demonstrates that cerebellar astrogliogenesis occurs from distinct embryonic progenitors, with a progressive shift from multipotency to fate-restriction that occurs according to a well-defined spatiotemporal pattern. Notably, besides the commonly known origin of cerebellar astrocytes from the ventricular neuroepithelium, here we highlight the existence of a secondary progenitor pool, represented by radial cells in the PCL capable of generating both BG and GL subtypes.

Disclosures: **V. Cerrato:** None. **E. Parmigiani:** None. **K. Leto:** None. **E. Fucà:** None. **M. Figueres-Oñate:** None. **L. López Mascaraque:** None. **A. Buffo:** None.

Nanosymposium

010. Mechanisms of Neural Reprogramming

Location: SDCC 23A

Time: Saturday, November 12, 2016, 1:00 PM - 3:15 PM

Presentation Number: 10.06

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01NS088095

NIH R01NS070981

Title: Boosting *In vivo* reprogrammed neurons in the injured adult spinal cord

Authors: ***L. WANG**[1], Z. SU[2], W. TAI[1], X.-M. XU[3], C.-L. ZHANG[1];
[1]Mol. biology, Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [2]Neurosci. Res. Ctr. of Changzheng Hosp., Second Military Med. Univ., Shanghai, China; [3]Stark Neurosciences Res. Inst., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Spinal cord injury (SCI) often leads to a permanent loss of neurons and the disruption of neuronal circuitry with a consequence of paralysis and long-term disability. However, the adult mammalian spinal cord lacks any intrinsic neurogenic capacity rendering it incapable of self-repair through neuron regeneration. New neurons were recently shown to be generated through *in vivo* reprogramming; nonetheless, the low number of these newly born neurons highly limits their potential clinical use in the future. In this study, we aimed to boost the number of reprogrammed neurons by understanding the reprogramming process induced by SOX2 in the adult spinal cord. We conducted a series of *in vivo* screens through gene overexpression or knockdowns. These genes include important factors for neurogenesis, cell reprogramming, and signaling pathways. A specific signaling pathway was identified to be critically involved in controlling SOX2-mediated *in vivo* reprogramming in the adult spinal cord. As a consequence, the overall production of *in vivo* reprogrammed neurons was greatly boosted in the adult spinal cord with mild or severe injuries. Subtype analyses revealed a predominant glutaminergic phenotype, although GABAergic and other subtypes were also readily detectable. A detailed examination of this boosted reprogramming process will be presented during the meeting. Together, our ability to successfully produce a large population of long-lived and diverse subtypes of new neurons in the adult spinal cord provides a cellular basis for regeneration-based therapy for SCI.

Disclosures: **L. Wang:** None. **Z. Su:** None. **W. Tai:** None. **X. Xu:** None. **C. Zhang:** None.

Nanosymposium

010. Mechanisms of Neural Reprogramming

Location: SDCC 23A

Time: Saturday, November 12, 2016, 1:00 PM - 3:15 PM

Presentation Number: 10.07

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant MH083911

NIH Grant AG045656

Charles H. Smith Endowment Fund

Title: Small molecules efficiently reprogram human astroglial cells into functional neurons

Authors: ***L. ZHANG**[1], J. YIN[2], H. YEH[2], N. MA[2], G. LEE[2], X. CHEN[3], Y. WANG[3], P. JIN[4], L. LIN[4], L. CHEN[4], G. WU[2], G. CHEN[2];
[1]biology, The Pennsylvania State Univ., State College, PA; [2]biology, [3]Biochem. and Mol. Biol.,

Pennsylvania State University, State College, PA; [4]school of medicine, Emory Univ., Atlanta, GA

Abstract: Mammalian central nervous system (CNS) possesses very limited self-repair capability as very few newborn neurons are constantly generated during the adulthood. Regeneration of functional neurons under the CNS injured or diseased condition remains a major challenge for functional recovery. To generate neurons locally, one big reservoir can be glial cells. In response to CNS injury, glial cells including astrocytes are activated to proliferate and become hypertrophic to occupy the injured CNS area, forming scar-like tissue in the chronic stage after injury. Previous work including our own (Guo et al., Cell Stem Cell, Best of 2014), have demonstrated that reactive astrocytes can be in vivo reprogrammed into functional neurons in mouse brain and can potentially be utilized for brain repair. However, this glia-to-neuron conversion largely depends on virus-based gene delivery and requires complex brain surgery procedures. To make such glia-to-neuron reprogramming technology more applicable for future clinical trial, here we report a convenient chemical-based reprogramming method. We demonstrate that sequential exposure to a cocktail of small molecules can successfully reprogram human astroglial cells into neuron-like cells in 8 days with conversion efficiency around 67%. These human astrocyte-converted neurons can survive for more than 4 months in culture and form functional synaptic networks, as shown by robust synchronous burst activities. We further demonstrate that both transcriptional and epigenetic regulations play critical roles in astrocyte-to-neuron reprogramming. After injected into the lateral ventricle of mouse brains, the small molecule-reprogrammed human neurons can survive for at least one month and migrate out of the ventricles to integrate into local brain circuits. Our study opens a new avenue using small molecules to reprogram reactive glial cells into functional neurons for brain repair.

Disclosures: **L. Zhang:** None. **J. Yin:** None. **H. Yeh:** None. **N. Ma:** None. **G. Lee:** None. **X. Chen:** None. **Y. Wang:** None. **P. Jin:** None. **L. Lin:** None. **L. Chen:** None. **G. Wu:** None. **G. Chen:** None.

Nanosymposium

010. Mechanisms of Neural Reprogramming

Location: SDCC 23A

Time: Saturday, November 12, 2016, 1:00 PM - 3:15 PM

Presentation Number: 10.08

Topic: A.01. Neurogenesis and Gliogenesis

Title: Unraveling the molecular underpinnings of ascl1 and sox2 mediated human brain pericyte-to-neuron lineage conversion

Authors: M. KAROW[1,2], G. CAMP[3], S. PERON[2], A. PATASKAR[4], C. SCHICHOR[5], V. TIWARI[4], B. TREUTLEIN[3], *B. BERNINGER[2];
[1]LMU unich, Munich, Germany; [2]Adult Neurogenesis and Cell. Reprogramming, Inst. of Physiological Chem., Univ. Med. Ctr. of the Johannes Gutenberg Univ., Mainz, Germany; [3]Dept. of Evolutionary Genet., Max Planck Inst. for Evolutionary Anthrop., Leipzig, Germany; [4]IMB, Mainz, Germany; [5]LMU, Munich, Germany

Abstract: Reprogramming of brain-resident glia and non-neural cells emerges as a novel approach toward brain repair (Heinrich et al., Nat Cell Biol, 2015). We have recently shown that forced expression of Sox2 and Ascl1 can lineage-convert cells of pericyte origin isolated from the adult human brain into induced neurons (Karow et al., Cell Stem Cell, 2012), while the same factors can reprogram NG2 glia into DCX-positive neurons in the lesioned adult mouse cerebral cortex in vivo (Heinrich et al., Stem Cell Reports, 2014).
In this study we addressed whether the functional synergism of Sox2 and Ascl1 in pericyte-to-neuron reprogramming is based on a molecular synergism. Towards this end, we performed genome-wide RNA-Sequencing of FAC-sorted pericytes undergoing Ascl1- and Sox2-induced reprogramming. Brain pericytes were obtained from three different individuals and transduced with retroviruses encoding Ascl1, Sox2 or both factors as experimental group and reporter-only as control. Given the protracted reprogramming of adult brain pericytes, cells were FAC-sorted at day 2 and 7 to determine early changes in gene expression. Surprisingly, our transcriptome analysis revealed that Ascl1, a transcription factor postulated to act as pioneer factor in fibroblast reprogramming (Wapinski et al., Cell, 2014) fails to transactivate its targets in pericytes. While Sox2 alone did not significantly change gene expression, co-expression of both factors resulted in the significant transactivation of neurogenesis genes, including of those that have been characterized before as Ascl1 direct targets (Raposo et al., Cell Reports, 2015). Moreover, consistent with many of the Sox2/Ascl1 expressing cells becoming GABA-positive, co-expression of these transcription factors resulted in the induction of master regulators of interneuron generation. This data suggest that Sox2 co-expression has a massive influence on Ascl1-dependent gene expression.
This work was supported by the German Research Foundation (DFG), Federal Ministry of Education and Research (BMBF), the Bavarian State Ministry of Sciences, Research and the Arts (ForIPS).

Disclosures: **M. Karow:** None. **G. Camp:** None. **S. Peron:** None. **A. Pataskar:** None. **C. Schichor:** None. **V. Tiwari:** None. **B. Treutlein:** None. **B. Berninger:** None.

Nanosymposium

010. Mechanisms of Neural Reprogramming

Location: SDCC 23A

Time: Saturday, November 12, 2016, 1:00 PM - 3:15 PM

Presentation Number: 10.09

Topic: A.01. Neurogenesis and Gliogenesis

Support: FNRS

Fond Léon Frédéricq

Télévie

Title: human bone marrow harbor cells with neural crest-associated characteristics

Authors: *S. WISLET[1], C. COSTE[2];
[1]Univ. of Liege, Liege, Belgium; [2]Univ. of Liège, Liège, Belgium

Abstract: Adult neural crest stem cells (NCSC) are of extraordinary high plasticity and promising candidates for a use in regenerative medicine. Several locations like skin, adipose tissue, dental pulp or bone marrow have been described in rodent. However, very few information is available concerning their correspondence in human tissues. The main objective of this study was therefore to characterize NCSC from adult human bone marrow. In this purpose, we compared human bone marrow stromal cells to human adipose tissue and dermis, already described for containing NCSC. We performed comparative analyses in terms of gene and protein expression, as well as functional characterizations. It appeared that human bone marrow, similarly to adipose tissue and dermis contains $NESTIN^{+}$ / $SOX9^{+}$ / $TWIST^{+}$ / $SLUG^{+}$ / $P75^{NTR+}$ / $BRN3A^{+}$/ $MSI1^{+}$/ $SNAIL1^{+}$ cells and were able to differentiate into melanocytes and Schwann cells. Moreover, when injected into chicken embryos, all those cells were able to migrate and follow endogenous neural crest migration pathways. Altogether, the phenotypic characterization and migration abilities strongly suggest the presence of neural crest-derived cells in human adult bone marrow.

Disclosures: S. Wislet: None. **C. Coste:** None.

Nanosymposium

011. APP Processing and Metabolism

Location: SDCC 33C

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 11.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Functional impact of rare SORL1 mutations observed in early-onset Alzheimer's Disease

Authors: *I.-M. RUDOLPH[1], S. CAGLAYAN[1], O. M. ANDERSEN[2], T. E. WILLNOW[1]; [1]Max Delbrueck Ctr. For Mol. Med., Berlin, Germany; [2]Dept. of Biomedicine, Aarhus Univ., Aarhus, Denmark

Abstract: The early-onset form of Alzheimer's Disease (EOAD) is a rare condition caused by autosomal-dominant inheritance of familial disease genes. So far, three loci have been shown to cause EOAD, encompassing mutations in the amyloid precursor protein (*APP*) and genes encoding for subunits of the γ-secretase *PSEN1* and *PSEN2*. However, recent analysis in EOAD patients carrying neither *APP* nor *PSEN1/2* mutations identified *SORL1* as the top-scoring disease gene with five previously unknown mutations that were not retrieved in healthy individuals (Pottier *et al.*, Mol. Psychiatry, 2012). *SORL1* encodes for SORLA, a neuronal sorting receptor known to decrease Aβ levels by two distinct mechanisms. It acts as a trafficking factor that directs APP to the Golgi compartment, protecting it from being proteolytically processed by secretases at the cell surface and in endosomes. In addition, SORLA binds newly produced Aβ and moves it to lysosomes for catabolism. We have shown previously that the EOAD *SORL1* mutation G511R disrupts the ability of SORLA to sort Aβ to lysosomes, resulting in increased Aβ levels (Caglayan *et al.,* Sci. Transl. Med., 2014). To investigate the functional impact of additional EOAD *SORL1* mutations, we generated SY5Y cell lines overexpressing wild-type or the N924S, N1358S, and G1618D mutant variants of SORLA and determined the level of APP processing products in these cell lines. The N924S and G18681D SORLA variants showed normal activity and reduced Aβ levels to a similar extent as the wild-type receptor. In contrast, cells overexpressing N1358S SORLA showed increased Aβ40 and Aβ42 levels as compared to wildtype cells, resembling a SORLA null phenotype. Expression levels and subcellular localization of the N1358S variant were similar to that of the wildtype receptor. Also, the analysis of interaction by BIACore and FLIM/FRET showed similar affinities of APP binding to wildtype and mutant SORLA. Currently, we conduct an unbiased screen of the interactome of wildtype and N1358S SORLA variants to elucidate the molecular mechanism by which the N1358S mutation impacts the protective function of SORLA on amyloidogenic processes and potentially causes EOAD.

Disclosures: I. Rudolph: None. **S. Caglayan:** None. **O.M. Andersen:** None. **T.E. Willnow:** None.

Nanosymposium

011. APP Processing and Metabolism

Location: SDCC 33C

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 11.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BrightFocus Foundation

NIH-NIA 2P50AG005131-31

Title: Retromer complex stabilization reduces pathogenic APP processing and tau phosphorylation in a hiPSC models of sporadic alzheimer's disease.

Authors: *J. E. YOUNG[1,3], D. BERMAN[4], L. FONG[2], S. A. SMALL[5], G. PETSKO[6], L. S. B. GOLDSTEIN[2];
[1]UCSD, LA Jolla, CA; [2]UCSD, La Jolla, CA; [3]Pathology, Univ. of Washington, Seattle, WA; [4]Pathology & Cell Biol., [5]Neurology, Radiology & Psychiatry, Columbia Univ., New York, NY; [6]Neurol. and Neurosci., Weill Cornell Med., New York, NY

Abstract: The retromer complex is an important factor in the traffic of amyloid precursor protein (APP) through the endosomal network, where it regulates APP cleavage primarily by directing APP away from endosomes and reducing processing by beta and gamma secretases. Stabilization and enhancement of retromer and retromer interacting proteins may be beneficial for AD by reducing pathogenic APP cleavage. Conversely, this protein complex harbors variants that may increase AD risk (1). We have previously generated human induced pluripotent (hiPSC) lines from 13 patients and controls from the UCSD Alzheimer's Disease Research Center (ADRC) and documented relevant AD phenotypes from stem-cell derived neurons (2). In the current study, we used this cohort of human cell lines to test the effects of pharmacological retromer stabilizing chaperones, which have previously been shown to reduce APP processing in mouse primary neurons (3). We have found that, in stem cell-derived human neurons, treatment with the retromer stabilization compound R33 increases VPS35 levels and decreases APP processing in both SAD patient neurons and controls. Overexpression of SORL1, a protein that binds both APP and tethers it to the retromer complex similarly reduces APP processing in these neurons. Interestingly, and for the first time, we also show that this compound reduces phosphorylation of Tau on threonine 231. The reduction of phospho-Tau is highly correlative with the reduction in pathogenic APP processing, but, interestingly, can also occur in APP null cells, suggesting that retromer can also affect tau phosphorylation independently of APP. Our results reinforce the validity of targeting the retromer complex for therapeutic development in AD and also suggest dual regulation of phospho Tau by retromer, involving both APP dependent and independent mechanisms, in human neurons.

Abstract references: 1. Vardarajan et al., 2012. *Neurobiology of Aging.* 2. Young et al., 2015. *Cell Stem Cell.* 3. Mecozzi et al., 2014. *Nature Chemical Biology.*

Disclosures: J.E. Young: None. **D. Berman:** None. **L. Fong:** None. **S.A. Small:** None. **G. Petsko:** None. **L.S.B. Goldstein:** None.

Nanosymposium

011. APP Processing and Metabolism

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Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 11.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: European Research Council Advanced Grant BeyOND No. 335692

Title: Alzheimer's disease associated VPS10P-domain receptors SORCS1 and SORCS3 cooperate to regulate amyloid-beta peptide level in the brain

Authors: *H. JU, A. SUBKHANGULOVA, A. MALIK, T. BREIDERHOFF, T. E. WILLNOW;
Max Delbruck Ctr. For Mol. Med., Berlin, Germany

Abstract: The vacuolar protein sorting 10 protein domain (VPS10P-D) receptors are type-I transmembrane proteins, which direct target proteins to their destined location in secretory or endocytic compartments of neuronal and non-neuronal cell types. In mammals, this gene family encompasses five receptors, termed sortilin, SORLA, SORCS1, SORCS2, and SORCS3. Importantly, many genetic studies have associated VPS10P-D receptors with the pathogenesis of Alzheimer’s disease (AD). The underlying molecular mechanisms have best been elucidated for SORLA and sortilin that control amyloid-beta peptide levels in the brain by modulation of APP processing and by clearance of amyloid-beta peptides, respectively. However, the molecular basis of the association of other VPS10P-D receptors with onset and progression of AD is poorly understood. Here, we studied the role of SORCS1 and SORCS3 in amyloidogenic processes using several mouse models of AD. Because SORCS1 and SORCS3 are encoded by two closely linked genes on human chromosome 10q23-q25, and share a high degree of sequence similarity, we reasoned a possible functional redundancy of both receptors. Thus, we studied mice with either single of dual receptor deficiencies generated by sequential targeting of the murine *Sorcs1* and *Sorcs3* loci. Consistent with our assumption, levels of amyloid-beta peptide were unchanged in mice with individual *Sorcs1* or *Sorcs3* gene defects as compared to control animals when introduced into the PDAPP or 5xFAD strain of APP transgenic mice. However, the levels of amyloid-beta peptides in cortex and hippocampus were significantly reduced in animals lacking

both receptors. This phenotype was most obvious in female animals, in line with the gender specific effects of these receptors observed in other paradigms before. Dual receptor deficiency did not impact the levels of soluble (s) APP-alpha or sAPP-beta in the brain suggesting a distinct function for both receptors in modulation of amyloid-beta peptide production or catabolism. Current proteomics approaches are directed at elucidating the molecular mechanism whereby SORCS1 and SORCS3 cooperate to enhance amyloid-beta peptide levels in the brain and to promote AD-related processes.

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Nanosymposium

011. APP Processing and Metabolism

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Presentation Number: 11.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 010124

Penn Institute on Aging ADC Pilot Program

NIH AG043503

Dana Foundation

Title: Primary age-related tauopathy: a genetic model of amyloid resilience?

Authors: *C. MCMILLAN[1], E. B. LEE[2], K. JEFFERSON-GEORGE[1], V. M. VAN DEERLIN[2], D. WOLK[1];
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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease that is characterized neuropathologically by inclusions of both neurofibrillary tangles (NFTs) and amyloid plaques (Aβ). In contrast, a recently defined neuropathological condition, primary age-related tauopathy (PART), is defined by the accumulation of NFTs in the absence of Aβ. Age is a significant risk factor for NFT and Aβ accumulation since it is rare that aging individuals >70 years-old have neither pathology, and therefore it is unclear why some individuals develop AD and others develop PART. We consider two potential hypotheses: the "risk" hypothesis suggests that individuals with AD have two risk factors for developing both NFTs and Aβ relative to PART; the "resilience" hypothesis suggests that individuals with PART have a protective factor that

minimizes the risk of Aβ burden. Case-control genome-wide association studies have identified single nucleotide polymorphisms (SNPs) associated with AD risk relative to controls, but "controls" are often poorly defined and may include individuals who are also at risk of AD, or have preclinical AD pathology. By investigating SNP genotypes in AD relative to PART we can specifically address the risk and resilience hypotheses. We evaluated 225 individuals with a neuropathological diagnosis of PART (N=77) or AD (N=178). All individuals were genotyped for 20 SNPs previously identified through genome-wide association studies as risk factors for AD. Genotyping was performed with brain DNA using a Fluidigm-based protocol. To evaluate whether SNP genotypes were associated with reduced risk of Aβ pathology we first evaluated logistic regressions models (PART vs. AD) with a dominant coding for each SNP (0 or 1+ risk alleles) while controlling for age at death. We then also compared minor allele frequency (MAF) for significant associations relative to MAF in a reference control dataset, 1000genomes. We observed that 2 SNPs were associated with reduced risk of Aβ: rs4938933 (OR=2.70; p=0.0005; PART MAF = 0.14; AD MAF = 0.48) and rs983392 (OR=2.50; p=0.0012; PART MAF = 0.15; AD MAF = 0.48). A comparison to control references revealed a MAF of 0.38 and 0.23, respectively. Together, MAFs for these SNPs is significantly reduced in PART relative to both AD and controls. We interpret these findings as potential evidence for Aβ resilience in PART. Both SNPs are in high linkage disequilibrium and located in the *MS4A* gene cluster. Members of the *MS4A* gene family have been suggested to play an important role in immunoresponse. While the biological basis of these associations requires additional research, our results provide a candidate genetic mechanism for Aβ resilience in aging.

Disclosures: C. McMillan: None. **E.B. Lee:** None. **K. Jefferson-George:** None. **V.M. Van Deerlin:** None. **D. Wolk:** None.

Nanosymposium

011. APP Processing and Metabolism

Location: SDCC 33C

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 11.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/ NIA 2R01AG008200-27

NIH/NIA 2P50-AG05138-29

NINDS 2R01-NS047229-16

Title: Presenilin1 mutations impair neovascularization and increase vulnerability of brain to ischemia.

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Abstract: A large amount of evidence has linked brain vascular dysfunction to the onset of Alzheimer's Disease (AD). A strong association between cognitive decline and cerebrovascular abnormalities is supported by data that AD brains exhibit impaired brain vasculature pathology (1,2) which precedes neurodegenerative changes and cognitive decline (3,4,5). Angiogenesis, a process that modulates the structure and function of the vasculature, is controlled by growth factors including ephrinB ligands (EFNB) and EphB receptors (EPH)(6,7). EPHB4 receptor interacts with EFNB2 ligand at the surface of endothelial cells promoting cell sprouting. Sprouting is regulated by the recruitment of angiogenic complexes of Raf-1 and Rok-α kinases to the Vascular endothelial (VE)-cadherin (8). Mutations in Presenilin 1 (PS1) are responsible for most cases of familial AD (FAD). We have shown that PS1/γ-secretase promotes the EPHB4-induced cleavage of EFNB2, sprouting of endothelial cells (9) and Raf-1/ Rok-α/VE-cadherin angiogenic complexes (10). To examine whether PS1 FAD mutations affect vascular integrity and angiogenesis we examined their role in brain neovascularisation after ischemic insult. We induced ischemia using middle cerebral artery occlusion (MCAO) in wild type (WT) and knock-in mice carrying PS1 FAD mutations. Lesion was detected by DWI imaging and restoration of cerebral blood flow (CBF) in the lesion area, an indicator of neovascularization, was quantified using perfusion MRI (T7). Neovascularization was quantified in the lesion area using collagen IV staining. To identify molecular mechanisms via which PS1 FAD mutations affect brain angiogenesis we performed co-immunoprecipitation experiments measuring Raf-1/Rok-α/VE-cadherin complexes which we have found to depend on PS1/γ-secretase (10). We show that MCAO-induced brain lesion remains significantly longer in FAD brains compared to WT and that recruitment and activation of astrocytes in the lesion area lasts much longer in the PS1 FAD brains indicating a prolonged tissue scar formation compared to WT. Neovascularization and blood volume restoration in the lesion area are also significantly decreased in the PS1 FAD brains compared to WT. In addition, endothelial cells carrying PS1 FAD mutations fail to form tubes, sprouts and angiogenic complexes in response to EPHB4 *in vitro* suggesting a mechanism by which PS1 FAD mutations may impair angiogenesis. Together our data indicate that PS1 FAD brain vasculature may be compromised due to defective angiogenesis, rendering the brain more vulnerable to ischemic toxic insults leading to increased cell death and neurodegeneration.

Disclosures: Y. Yoon: None. **G. Voloudakis:** None. **N. Doran:** None. **L. Chen:** None. **N.K. Robakis:** None. **A. Georgakopoulos:** None.

Nanosymposium

011. APP Processing and Metabolism

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Presentation Number: 11.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Giunta Scholarship

Center for Chronic Disorders of Aging Grant

Adolf and Rose Levis Foundation

Title: Expression of amyloidogenic secretases implicated in neurodegeneration is altered in astrocytes following infection with Chlamydia pneumoniae

Authors: *Z. AL-ATRACHE, A. CADER, S. HINGLEY, D. APPELT;
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Abstract: The enzymatic processing of amyloid precursor protein (APP) in the central nervous system (CNS) is catalyzed by neuronal and glial α-, β-, and γ-secretases: A Disintegrin and Metalloproteinase 10 (ADAM10), βAPP Cleaving Enzyme-1 (BACE1), and Presenilin-1 (PSEN1), respectively. Amyloid-β (Aβ) products of APP cleavage are considered pathologic sequelae of both chronic neurodegenerative illnesses, such as Alzheimer's Disease (AD), and acute CNS hypoxia and traumatic injury. Therefore, APP proteases serve as indirect biomarkers for Aβ-producing neuroinflammation. Recent literature supporting the "pathogen hypothesis" of AD shows a strong association between bacterial and viral pathogens and Aβ production. Although the mechanism of pathogen-induced neurodegeneration of AD remains unclear, astrocytes, a key player of the CNS innate immune response and producer of Aβ, have been implicated. To test the hypothesis that the bacterium, *Chlamydia pneumoniae* (*Cpn*), promotes the production of Aβ in the CNS, human astrocytes were infected with *Cpn* for 6-72 hrs. The protein and mRNA expression of ADAM10, BACE1, and PSEN1 proteases were localized by confocal microscopy and quantified by western blot and RT-PCR. Our results showed that relative to that of uninfected astrocytes, membrane-localized fluorescent labeling of BACE1 and cytoplasmic labeling of PSEN1 was enhanced at 24 and 48-72 hrs. post-infection (hpi), respectively, whereas ADAM10 labeling was unaltered. Immunoblot-quantified levels of BACE1 and PSEN1 followed a similar temporal increase from 24 to 72 hpi, with significance at 48 and 72 hrs. BACE1 and PSEN1 mRNA expression was significantly upregulated ($p<0.05$); BACE1 by 1.5 fold at 48 hpi and PSEN1 by 1.7 fold at 6 hpi. Interestingly, increases in intracellular $A\beta_{1-42}$ labeling corroborated increases in BACE1 and PSEN1 protein levels at 48 to 72 hpi. These findings are the first to suggest that acute *Cpn* infection promotes the formation of Aβ through upregulation of glial β-, and γ-secretases at the protein and genetic level.

Furthermore, this study provides further evidence for a direct link between a respiratory pathogen and the pathologic neurodegeneration of AD.

Disclosures: **Z. Al-Atrache:** None. **A. Cader:** None. **S. Hingley:** None. **D. Appelt:** None.

Nanosymposium

011. APP Processing and Metabolism

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NINDS grant R01NS073512.

Title: Major Aβ degrading enzymes ECE-2 and neprilysin are expressed by distinct populations of GABAergic interneurons in hippocampus and neocortex

Authors: *J. PACHECO-QUINTO, E. A. ECKMAN;
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Abstract: Impaired clearance of amyloid-β peptide (Aβ) has been postulated to significantly contribute to the amyloid accumulation typical of Alzheimer's disease. Among the enzymes known to degrade Aβ *in vivo* are endothelin-converting enzyme (ECE)-1, ECE-2, and neprilysin, and evidence suggests that they regulate independent pools of Aβ that may be functionally significant. To better understand the differential regulation of Aβ concentration by its physiological degrading enzymes, we characterized the cell and region-specific expression pattern of ECE-1, ECE-2, and neprilysin by *in situ* hybridization and immunohistochemistry in brain areas relevant to Alzheimer's disease. In contrast to the broader distribution of ECE-1, ECE-2 and neprilysin were found enriched in GABAergic neurons. ECE-2 was expressed by somatostatin-positive interneurons in hippocampus and neocortex, with transcripts detected both in soma and in regions with strong somatostatin innervation. Consistent with the possibility that ECE-2 mRNA may be transported to synapses for local translation, ECE-2 was active in isolated synaptosomes. Neprilysin was expressed mainly by parvalbumin-positive interneurons. While neprilysin mRNA was mostly confined to the soma, neprilysin protein was found enriched in regions with strong parvalbuminergic input, indicating that neprilysin catalytic activity may be concentrated at the presynaptic ends of parvalbumin interneurons. The identification of somatostatinergic and parvalbuminergic synapses as hubs for Aβ degradation is consistent with the intriguing possibility that Aβ may have a physiological function related to the regulation of inhibitory signaling. Impaired degradation leading to Aβ accumulation at inhibitory synapses

could disturb this putative function and result in synaptotoxicity, ultimately leading to cognitive impairment.

Disclosures: **J. Pacheco-Quinto:** None. **E.A. Eckman:** None.

Nanosymposium

011. APP Processing and Metabolism

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG044817

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Alzheimer Association grant ZEN-14-283969

Title: Alzheimer’s Aβ catabolism: implications for the mechanisms of brain clearance and amyloid formation

Authors: ***J. GHISO**, E. CABRERA, A. ROSTAGNO;
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Abstract: Extensive parenchymal and vascular amyloid deposits are classic pathologic hallmarks of Alzheimer’s disease (AD). Amyloid beta (Aβ), the main component of these fibrillar deposits, is also a normal soluble constituent of biological fluids and brain interstitial fluid. How this soluble-to-fibrillar conversion is regulated is a hot topic of intense scrutiny, with current data pointing to a dysregulation of brain clearance as the leading mechanism. Biochemical and proteomic analysis of brain deposits and biological fluids reveal a high degree of Aβ heterogeneity that goes far beyond the classical Aβ40/Aβ42 dichotomy, displaying numerous post-translational modifications and exhibiting multiple truncations at both N- and C-terminal ends of the molecule which likely reflect the local action of resident enzymes. In spite of innumerable studies focusing in Aβ, the relevance of N- and C-terminal truncated species in the mechanism of AD pathogenesis remains largely understudied. Using novel antibodies specifically recognizing Aβ N-terminally truncated at position 4 or C-terminally truncated at position 34 we provide a clear assessment of the differential topographical localization of these species in brain specimens from AD cases as well as from different APP transgenic models. Immunoprecipitation combined with mass spectrometry demonstrate the presence of selected C-

and N-terminal truncated fragments in brain homogenates whereas their differential fractionation characteristics in water-soluble and formic acid-soluble brain extracts as well as their selective access to the cerebrospinal fluid provide a clear indication of their dissimilar biochemical/biophysical properties. Additional studies with synthetic homologues confirmed the differences in solubility and revealed contrasting oligomerization/fibrillization characteristics of these species. *In vivo* brain removal studies following intrahippocampal inoculation of radiolabeled Aβ fragments differentially truncated at their N-and C-terminal end further established a direct correlation between degree of oligomerization and clearance propensities with oligomerization significantly increasing brain retention. Overall, the data indicate that C-terminal degradation at position 34 leads to the production of more soluble, clearance-driven fragments whereas N-terminal truncation at position 4 generates poorly soluble, aggregation prone peptides with high amyloidogenic propensity and the potential to exacerbate the fibrillar deposits self-perpetuating the amyloidogenic loop.

Disclosures: J. Ghiso: None. **E. Cabrera:** None. **A. Rostagno:** None.

Nanosymposium

011. APP Processing and Metabolism

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Presentation Number: 11.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Roy and Christine Sturgis Charitable Trust

Strategies Toward Overcoming and Preventing (STOP) Alzheimer's Fund

Title: Amyloid β-peptide modulation of glucose responses and insulin signaling requires endogenous APP

Authors: *R. D. HENDRIX[1], A. M. MOERMAN-HERZOG[2], W. WANG[2], S. W. BARGER[3,4]; [1]Dept. of Neurobio. & Developmental Sci., Univ. of Arkansas For Med. Sci., Little Rock, AR; [2]Geriatrics, [3]Geriatrics, Neurobio. and Developmental Sci., Univ. of Arkansas for Med. Sci., Little Rock, AR; [4]Geriatric Research, Educ. and Clin. Ctr., Central Arkansas Veterans Healthcare Syst., Little Rock, AR

Abstract: Alzheimer's disease (AD) is associated with Type-2 diabetes mellitus epidemiologically, and studies performed in mouse models of AD suggest a mechanistic relationship whereby amyloid β-peptide (Aβ) drives metabolic derangement in the periphery. However, many preclinical models of AD rely on expressing a familial-AD mutation of the

amyloid precursor protein (APP), and Aβ is only one cleavage product of APP. The additional fragments (β-CTF, AICD, sAPPα, sAPPβ) have been shown to interpose their own consequences on cellular metabolism. In order to determine the effects of neuronal expression of Aβ42 alone *in vivo*, we used the transgenic BRI-Aβ42 mouse model, which produces only Aβ1-42 released from the surface of neurons. Elements of metabolism in both CNS and peripheral tissues were assessed, with and without the influence of a high-fat, high-sucrose ("western") diet. We found increased markers of insulin resistance in both the CNS and periphery in the transgenic mice, including impaired glucose tolerance and diminution of cerebral insulin receptor substrate 1 (IRS1); synaptophysin levels in the hippocampus were diminished and hyperphosphorylated tau was evident. However, Aβ-transgenic mice lacking endogenous mouse APP maintained normal glycemic control. Together, our data indicate that Aβ has systemic metabolic influences on pathways involved in glycemic control and that APP is required for the peripheral effects on blood glucose. These findings add intriguing nuances to the hypothesis that Aβ may contribute to both normal metabolic control and its derangement in diabetes; they also highlight a caveat to the use of models that overexpress APP.

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Nanosymposium

011. APP Processing and Metabolism

Location: SDCC 33C

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 11.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RIKEN

Title: Identification of two somatostatin receptor subtypes regulating the major amyloid beta-degrading enzyme neprilysin

Authors: *P. NILSSON[1], K. SÖRGJERD[1], N. KAKIYA[1], M. SEKIGUCHI[1], A. PETRISH[2], S. SCHULZ[2], T. SAITO[1], T. C. SAIDO[1];
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Abstract: Abnormally altered amyloid beta metabolism leads to Alzheimer's disease (AD). Especially in sporadic AD, which lacks robust genetic components, a decrease in the degradation of amyloid beta may contribute to the increased amyloid beta levels. In previous work, we

identified neprilysin as the major amyloid beta-degrading enzyme. We subsequently found that neprilysin activity is regulated in the neurons by somatostatin, a neuropeptide decreased with aging and in AD possibly due to the death of somatostatinergic interneurons. Here we have identified the somatostatin receptor (SSTR) subtypes responsible for the regulation of neprilysin by doubly knocking out SSTR subtypes expressed in cortex and hippocampus in a combinatorial manner. Remarkably, a simultaneous knockout of two of the SSTR subtypes led to a specific downregulation of neprilysin both in vitro and in vivo. These findings indicate presence of functional redundancy among the two SSTR subtypes in the regulation of neprilysin. In addition, genetic deficiency of these two receptors led to increased amyloid beta levels and impaired memory in the mice, indicating an important role of these two receptors in cognitive function. Here we will present the identity of the SSTR subtypes involved in the regulation of neprilysin.

Disclosures: **P. Nilsson:** None. **K. Sörgjerd:** None. **N. Kakiya:** None. **M. Sekiguchi:** None. **A. Petrish:** None. **S. Schulz:** None. **T. Saito:** None. **T.C. Saido:** None.

Nanosymposium

011. APP Processing and Metabolism

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Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 11.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MR/L007533/1

Title: Investigating the role endosome-to-Golgi trafficking plays in APP biology

Authors: ***A. MUKADAM**[1], S. Y. BREUSEGEM[2], M. N. J. SEAMAN[2];
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Abstract: Retromer dysfunction is implicated in a number of nervous system disorders, including Alzheimer’s disease. Retromer is a pentameric protein complex that plays key roles in endosomal trafficking, both, in a retrograde direction to the *trans*-Golgi network, and in recycling cargoes from the endosome to the plasma membrane. Disruption of endosome-to-Golgi trafficking by malfunctioning retromer is hypothesised to increase pathogenic processing of APP. The Seaman laboratory has screened a genome-wide siRNA library to identify novel components of the endosome-to-Golgi retrieval pathway. Taking these findings further, the goal of this project is to identify components of the endosome-to-Golgi pathway that influence the localisation and processing of APP. Forty novel components of the endosome-to-Golgi retrieval pathway were individually silenced and the effect on APP processing and Aβ secretion was

analysed. PLD3 was identified as a component of retrograde trafficking and, interestingly, silencing PLD3 significantly increased levels of secreted Aβ. We have characterised the intracellular localisation of PLD3 and have explored its role in endosome-to-Golgi trafficking and APP processing. We present our findings here.

Disclosures: **A. Mukadam:** None. **S.Y. Breusegem:** None. **M.N.J. Seaman:** None.

Nanosymposium

011. APP Processing and Metabolism

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Presentation Number: 11.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG044404

Title: Cytosolic phospholipase A_2 facilitates internalization of amyloid-β in microglia

Authors: ***J. C.-M. LEE**[1], D. RIDGLEY[2], T. TENG[2], L. DONG, 65211[3], S. TOLBERT[2], G. SUN[3];
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Abstract: Microglia are important immune cells in the Central Nervous System (CNS) playing multiple physiological roles in defense against foreign pathogens, removal of cellular debris, and maintenance of tissue redox homeostasis. Microglia have been observed to surround Amyloid-β (Aβ) senile plaques in Alzheimer's disease (AD) brains. In fact, impaired microglia-mediated Aβ clearance resulting in the buildup of Aβ has been implicated in AD pathology.Cytosolic phospholipase A_2 ($cPLA_2$) is an enzyme that plays a role in modulating membrane phospholipids and production of lipid mediators. In microglial cells, cPLA2 is activated in response to stimulation by endotoxin and activation by ERK1/2. In this study, we investigate the role of cPLA2 in the processing of Aβ in microglial cells. Inhibition of $cPLA_2$ activation reduces Aβ uptake in human and mouse immortalized microglia by up to 90%. However, inhibition of $cPLA_2$ does not alter the degradation of internalized Aβ which occurs within the lysosomes of microglia. Moreover, our data fit well with a 1^{st} order kinetic model and the ratio of Aβ uptake rate constant to degradation rate constant (k_{up}/k_d) decreases linearly with an increasing dose of $cPLA_2$ inhibitor. This work demonstrates that $cPLA_2$ is a key enzyme governing the dynamics of Aβ clearance by microglial cells. Further understanding the mechanism of the cPLA2-induced Aβ uptake process by microglia will contribute to our current understanding of AD pathology.

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Nanosymposium

011. APP Processing and Metabolism

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Swedish Alzheimer's Foundation

Title: Mechanisms of neuron to neuron transfer of toxic amyloid-beta oligomers via exosomes

Authors: **M. S. SINHA**[1], A. ANSELL[1], L. CIVITELLI[1], C. HILDESJÖ[1], M. LARSSON[1], M. INGELSSON[2], *M. HALLBECK[3,1];
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Abstract: Progressive accumulation of specific protein aggregates, is a defining feature of many major neurodegenerative diseases, including Alzheimer's disease (AD). Several studies emphasize synaptic transfer of aggregation-prone proteins such as amyloid beta peptide (Aβ), from neuron to neuron as one disease mechanism. This prion like spread of oligomeric Aβ continues to other brain regions along the synaptic connections, thereby contributing to the development of the disease. However, the cellular mechanisms of this spread are not known nor weather it is possible to interfere with the transfer. In this study we investigated whether exosomes have any direct role in transferring pathogenic oligomeric Aβ (oAβ) from one cell to another. We show that oAβ co-localized with the exosome protein Flotillin1 in AD patients' brains. Interestingly, the level of oAβ was significantly higher in exosomes isolated from AD brains compared to controls. In addition, using our newly developed neuronal 3D co-culture model using induced pluripotent stem cells (iPSC), we observed that donor cells internalized intact AD brain exosomes carrying oAβ and transferred the protein to neighboring cells. Subsequently, the transfer of exosomes containing oAβ induced toxicity in the acceptor cells as demonstrated by MTT assay. To deepen the mechanistic role of exosomes in transferring oAβ, iPS cells were treated with fluorescently labeled oAβ and after 48h exosomes were isolated from the medium. The characterization and presence of oAβ in secreted exosomes isolated from conditioned media was confirmed by tunable resistive pulse sensing and size exclusion

chromatography. In addition, iPS cells fed with isolated exosomes internalized oAβ, thus demonstrating that oAβ can be transmitted from neuron-to-neuron via exosomes. Interestingly, the cellular transfer of oAβ containing exosomes could be inhibited using dynasore, an inhibitor of dynamin dependent endocytosis and using siRNA targeting TSG101 and VPS4A to down-regulate exosome biogenesis and secretion. In conclusion, our study shows that AD brain exosomes contain a high level of oAβ and exosomes can spread oAβ from cells to cells via, thus potentially contributing to neurodegeneration. Blocking exosome secretion or uptake decreases oAβ transfer significantly. These results have important implications for understanding the disease propagation in AD pathogenesis and targeting exosomes as a new therapeutic approach.

Disclosures: **M.S. Sinha:** None. **A. Ansell:** None. **L. Civitelli:** None. **C. Hildesjö:** None. **M. Larsson:** None. **M. Ingelsson:** None. **M. Hallbeck:** None.

Nanosymposium

012. Molecular and Neuroimaging Biomarkers for Alzheimer's Disease

Location: SDCC 32B

Time: Saturday, November 12, 2016, 1:00 PM - 4:30 PM

Presentation Number: 12.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Carraway Foundation Grant

UMMC MIND Center Operating Grant

NIH Grant AA020103

Title: Using a single molecule digital analyzer to measure blood based biomarkers for neurodegenerative diseases

Authors: ***J. WANG**[1], Q. ZHANG[2], B. ZHENG[2], J. LAGE[2], C. STOCKMEIER[2], T. MOSLEY[2];
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Abstract: Neurodegenerative diseases, such as Alzheimer’s, Parkinson’s diseases, are incurable and debilitating conditions that result in progressive neurodegeneration which may start 20-30 years before the symptoms are noticed. Developing specific and sensitive predictive biomarkers is one of the major challenges so far for establishing therapeutic strategies for these diseases. The major barrier is lack of highly sensitive and reliable tools to measure the gradient difference of molecules at the pre-symptomatic stage. Recently we have established such a tool for detecting multiple molecules simultaneously, in a supersensitive and reliable manner. Using Quanterix

technology, we have successfully developed an ultra-sensitive, single molecule digital analyzer platform, which can measure the molecules in plasma and tissue homogenates at serial pg/ml to fg/ml in a very reliable manner, R square values are as high as 99 in serial dilution tests. Now we have successfully established the tests for several cytokines and also for a neurosteroid, allopregnanolone, in human plasma samples. Further spike and recovery experiments in diluted samples help us accurately measure the spiked samples in calibrator into those diluted brain samples (recovery 70-93%). The high sensitivity also indicate that these molecules can be measured in a small size quantity/volume (less than 200µl) of samples. Therefore, this highly sensitive and reliable measurement platform can be used to determine the blood based, early changes of bioactive molecules for neurodegenerative disease. The results will also provide targets for developing therapeutic strategies for these neurodegenerative diseases.

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Nanosymposium

012. Molecular and Neuroimaging Biomarkers for Alzheimer's Disease

Location: SDCC 32B

Time: Saturday, November 12, 2016, 1:00 PM - 4:30 PM

Presentation Number: 12.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ASU/Mayo Partnership Program

DOD Grant

Title: Blood-based protein variant biomarkers to facilitate presymptomatic diagnosis and staging of Alzheimer’s disease

Authors: ***S. M. WILLIAMS**[1], P. SCHULZ[1], T. L. ROSENBERRY[2], R. J. CASELLI[3], M. R. SIERKS[1];
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Abstract: Oligomeric forms of beta-amyloid, tau, and TDP-43 play important roles in Alzheimer’s disease (AD), and therefore are promising biomarkers. We previously generated single chain antibody fragments (scFvs) that selectively bind disease related variants of these proteins including A4, C6T and E1 which bind different beta-amyloid oligomers, D11C which binds oligomeric tau, and AD-TDP1 and AD-TDP2 which bind TDP-43 variants. To determine

if these protein variants were useful early biomarkers for AD, we first analyzed 11 human sera samples obtained from patients ~2 years prior to an initial diagnosis of MCI, and while the biomarker profile varied from case to case, all displayed elevated reactivity relative to cognitively normal age-matched controls. To determine specific protein variant profiles indicative of different stages of AD, we next examined longitudinal human plasma from four AD (encompassing time points prior to initial MCI diagnosis to after conversion to AD) and two control cases. Levels of A4 and C6T reactive oligomeric beta-amyloid were significantly higher with all AD stages compared to the controls; levels of D11C reactive oligomeric tau increased with AD progression, and levels of AD-TDP1 and AD-TDP2 reactive TDP-43 variants decreased with AD progression. Pre-MCI samples were characterized by high TDP-43, moderate beta-amyloid and low tau variant levels; MCI samples by moderate TDP-43 and tau, and high beta-amyloid variant levels; and AD samples by low TDP-43 and high beta-amyloid and tau variant levels. Sample time points ranged from ~7 years pre-MCI to ~9 years after AD conversion. Bivariate correlations utilizing this range showed a strong positive correlation between cumulative beta-amyloid, tau and TDP-43 levels with disease progression indicating an increase in neurodegenerative processes with time in AD. Our scFv panel not only readily selects AD cases from controls, but also discriminates between AD stages, and detects the presence of blood-based AD biomarkers more than 7 years prior to an initial diagnosis of MCI.

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Nanosymposium

012. Molecular and Neuroimaging Biomarkers for Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

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P30AG10161

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Title: CREB signaling components as blood biomarkers for cognitive function in Alzheimer’s disease

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Abstract: Cognitive dysfunction and progressive memory loss characterize Alzheimer's disease (AD). The challenging lack of correlation between the pathological hallmarks of AD and cognitive performance raises the urgent need for a biomarker of cognitive dysfunction and disease progression. CREB signaling components are important factors in the formation of new memories in the brain. We have previously shown that CREB signaling is significantly decreased in young adults of a mouse model of familial AD (FAD). Here, we show that activated CREB phosphorylated on Ser133 (pCREB) is decreased in peripheral blood mononuclear cells (PBMC) of FAD mice, indicating that CREB signaling components in the PBMC could be indicative of CREB signaling in the brain, and, by extension, cognitive function. Importantly, we show that these changes in CREB signaling are mirrored in the prefrontal cortex and PBMC of human AD patients. We further show that pCREB expression in PBMC during life is positively correlated to pCREB expression in postmortem samples of prefrontal cortex of these AD patients. These results suggest that CREB signaling in PBMC reflects expression in the brain and can be used as a biomarker for cognitive function in AD.

Disclosures: N. Bartolotti: None. **D.A. Bennett:** None. **O. Lazarov:** None.

Nanosymposium

012. Molecular and Neuroimaging Biomarkers for Alzheimer's Disease

Location: SDCC 32B

Time: Saturday, November 12, 2016, 1:00 PM - 4:30 PM

Presentation Number: 12.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant U01 AG024904

NIH grant P30 AG10133

NIH grant K01 AG049050

Title: Comparison of CSF and plasma tau biomarker associations with atrophy on MRI in Alzheimer's disease and mild cognitive impairment

Authors: *K. D. DETERS[1], S. R. RISACHER[2], K. NHO[2], S. KIM[2], J. D. WEST[2], K. BLENNOW[3], H. ZETTERBERG[3], M. W. WEINER[4], A. J. SAYKIN[2];
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Abstract: Neurofibrillary tangles composed of hyperphosphorylated tau protein are one of the main pathological hallmarks of Alzheimer's disease (AD). Tau pathology can be directly measured using neuroimaging or indirectly using fluid measurements from cerebrospinal fluid (CSF) and more recently plasma. It has been well established that individuals with AD and mild cognitive impairment (MCI) have increased CSF tau levels compared to cognitively normal controls (CN). Furthermore, CSF tau has been correlated with neuroimaging phenotypes such as decreased gray matter density (GMD) in the medial temporal regions. Plasma tau is also significantly increased in AD and MCI, yet very limited information is available regarding relationships with disease markers. The goal of this study was to compare the association between atrophy indicated by GMD with central (i.e., CSF) and peripheral (i.e., plasma) measures of tau. In this study, cross-sectional data from 90 AD, 172 MCI, and 106 NC participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) were collected for the following variables: clinical, CSF total tau, plasma total tau, and structural magnetic resonance imaging (MRI) on a 1.5T scanner. Across all participants, the relationships of CSF tau and plasma tau with GMD were assessed on a voxel-by-voxel basis using regression models in SPM8. For all analyses, age, gender, *APOE* ε4 status, and total intracranial volume were used as covariates. A composite statistical map was created to illustrate the overlap between CSF and plasma tau associations with GMD. Results are displayed at a voxel threshold (k) of 50 voxels and $p<0.001$ (uncorrected). Both CSF and plasma tau were negatively correlated with GMD in the hippocampus, fusiform, and posterior cingulate cortex. Increased CSF tau was primarily correlated with decreased GMD in cortical regions including the precuneus and middle temporal gyrus. Increased plasma tau was primarily correlated with decreased GMD in subcortical regions such as the striatum, as well as the parahippocampal gyrus. Overall, CSF and plasma tau were negatively correlated with GMD with an overlapping but notably different pattern of association. Most overlap occurred in AD-specific regions. These findings suggest that central (CSF) and peripheral tau (plasma) may be reflecting related but also somewhat different pathological substrates of AD. This may in part be due to differences in tau isoforms detected by the various assays which is a topic warranting further investigation.

Disclosures: K.D. Deters: None. **S.R. Risacher:** None. **K. Nho:** None. **S. Kim:** None. **J.D. West:** None. **K. Blennow:** None. **H. Zetterberg:** None. **M.W. Weiner:** None. **A.J. Saykin:** None.

Nanosymposium

012. Molecular and Neuroimaging Biomarkers for Alzheimer's Disease

Location: SDCC 32B

Time: Saturday, November 12, 2016, 1:00 PM - 4:30 PM

Presentation Number: 12.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Basal forebrain degeneration precedes and predicts the cortical spread of Alzheimer's pathology

Authors: *T. W. SCHMITZ[1], R. N. SPRENG[2];
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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by distributed amyloid and tau pathophysiology throughout the brain. Recent breakthroughs in molecular genetics have identified a trans-synaptic mechanism by which these pathologies spread across anatomically and functionally linked cortical regions. These findings have potential for novel biomarkers and therapeutic strategies aimed at identifying the earliest signs of pathology and preventing its spread, prior to the onset of clinical AD.
However, the initial stages of AD pathophysiology remain ill-defined, preventing a clear picture of what regions to target as the earliest points of spread. The prevailing model suggests that amyloid and tau deposition first appear within the transentorhinal and entorhinal cortex (EC). This model has been called into question by histological and in vivo structural imaging evidence of early pathological change to the nucleus basalis of Meynert (NbM) in the basal forebrain. One possible explanation for these competing findings is that the early emergence of pathology in NbM and EC occurs in parallel. A second unexplored possibility points to pathological spread from one structure to the other.
In the present study, we evaluated the hypothesis of predictive pathological spread first by examining if volumetric MRI indices of gray matter degeneration in NbM and EC over time exhibits interdependence and directionality. However, by itself, such a relationship does not indicate an underlying pathology drives the interregional degenerative cascade. We therefore integrated our volumetric measures, in the same individuals, with a molecular biomarker of neuronal amyloid deposition that is extremely sensitive to AD pathophysiology at early presymptomatic stages of disease.
Longitudinal changes in NbM and EC volume were examined over 2 years in age-matched older adults ranging from cognitively normal to advanced AD. We observed a predictive spread of degeneration from NbM to EC. Competing models of parallel degeneration or entorhinal origin received negligible support. Comparison between cognitively matched normal adult subgroups, delineated according to the AD biomarker, revealed abnormal degeneration in NbM, but not EC. Abnormal degeneration in both NbM and EC was only observed at later stages of disease, among

mildly amnestic individuals. We provide unprecedented evidence that NbM pathology precedes and predicts both EC pathology and memory impairment, challenging the widely held belief that AD has a cortical origin.

Disclosures: **T.W. Schmitz:** None. **R.N. Spreng:** None.

Nanosymposium

012. Molecular and Neuroimaging Biomarkers for Alzheimer's Disease

Location: SDCC 32B

Time: Saturday, November 12, 2016, 1:00 PM - 4:30 PM

Presentation Number: 12.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG039452

AG23084

P50 AG05142

NS34467

Zilkha Senior Scholar Support

Title: Impact of APOE4 genetic risk on CSF and MRI biomarkers of the neurovascular unit in preclinical Alzheimer's disease

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Abstract: Vascular dysfunction is increasingly recognized in the pathophysiology of Alzheimer's disease (AD), and measures of vascular dysfunction can be evaluated using cerebrospinal fluid (CSF) and imaging-based biomarker approaches. A clinical need exists to identify reliable biomarkers for early AD diagnosis, and to aid in identifying novel treatment targets and evaluating the efficacy of clinical trials. Thus, we quantified novel CSF biomarkers

of responses/injury to the neurovascular unit (NVU) – comprising vascular cells, glia, and neurons – using antibody-based single/multiplex assays, and evaluated quantitative regional cerebrovascular integrity using multiple magnetic-resonance imaging (MRI) approaches including dynamic contrast-enhanced (DCE)-MRI for blood-brain barrier (BBB) K_{trans} permeability and diffusion tensor imaging (DTI)-MRI for analysis of fiber integrity. CSF and MRI scans were obtained from human subjects of the USC Alzheimer's Disease Research Center (ADRC), Huntington Medical Research Institutes, and the Knight ADRC. CSF and imaging biomarkers of the NVU were analyzed in relation to subjects' cognitive status [no cognitive impairment (NCI) vs. mild cognitive impairment (MCI)] and AD genetic risk factor apolipoprotein E-ε4 (*APOE4*) [carriers vs. non-carriers]. We found that K_{trans} and CSF-based biomarkers of BBB/vascular injury are altered in *APOE4* carriers versus non-carriers with NCI and these markers are further altered in *APOE4* carriers versus non-carriers with MCI. We also found that K_{trans} and CSF-based biomarkers of BBB/vascular injury positively correlate with the DTI metric mean diffusivity (MD) within the hippocampus. Furthermore, preclinical changes in the pericyte vascular injury marker, soluble platelet-derived growth factor receptor-β (sPDGFRβ), is related to subtle impairments in executive function and attention, independent of CSF amyloid-β $(Aβ)_{42}$ levels, and predict longitudinal decline in memory function prior to changes in established AD biomarkers (CSF Aβ and tau). Overall, these data suggest that CSF and MRI-based biomarkers of cerebrovascular dysfunction are altered in *APOE4* carriers, and that CSF sPDGFRβ may be a useful predictor of subtle deficits in neuropsychological domains in preclinical AD and across the preclinical-MCI spectrum.

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Nanosymposium

012. Molecular and Neuroimaging Biomarkers for Alzheimer's Disease

Location: SDCC 32B

Time: Saturday, November 12, 2016, 1:00 PM - 4:30 PM

Presentation Number: 12.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR MOP115011

Title: Aging amygdala sub-nuclei: a high-field magnetic resonance imaging study

Authors: *A. AGHAMOHAMMADI SERESHKI[1], Y. HUANG[2], F. OLSEN[2], R. CARTER[2], N. V. MALYKHIN[3];

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Abstract: Introduction: Dramatic growth of the elderly population is now affecting many countries in the world. Success in diagnosis and treatment of age-related brain diseases depends on understanding healthy aging. The amygdala (AG) is a group of nuclei in the medial temporal lobe involved in the neural circuits of fear and reward learning, as well as aggressive, sexual, maternal, and feeding behaviours. AG consists of five major subnuclei groups: lateral nucleus (LA), basal nucleus (BA), accessory basal nucleus (AB), cortical (Co) and Centro-medial (CeM) groups. The effects of healthy aging on AG subnuclei have not been investigated in humans due to limitations of conventional Magnetic Resonance Imaging (MRI). The goal of the present study was apply our recently developed high-field volumetric MRI method in order to investigate how AG sub-nuclei are affected in healthy aging.**Methods:** 167 healthy subjects (74 males, 93 females, 18-85) were recruited in this study. Participants were excluded if they had unstable medical illness, history of psychiatric or neurological disorders and the use of medications that might affect brain structure. Written informed consent was obtained and the research was approved by the University of Alberta Health Research Ethics Board. Images were acquired on a 4.7T Varian Inova scanner. The program DISPLAY (MNI, Montreal) was used to trace intracranial volumes (ICV) on the T1-weighted MPRAGE images and volumes of amygdala sub-nuclei on the T2-weighted FSE images.**Results:** We found that total AG volume declined with age only in males (left r=-.198 (p=.09) right r=-.317 (p=.006)) but not in females. BA AG was the most affected by age (left r=-.326, p= .005, right r=-.377, p= .001), following by AB (left r=-.229, p= .05, right r=-.285, p= .014) and LA (left r= -.192, p= .1, right r=-.274, p= .018), while Co and CeM AG were not affected by age. All AG subnuclei were preserved with age in females.**Conclusion**: We found that in females there was a preservation of all AG subnuclei, while in males most of the AG subnuclei undergo significant atrophy with age. Our results suggest that there is a gradient in AG volume loss in male participants: BA and AB AG were the most affected by age, following by LA nucleus, while Co and CeM AG were resilient to age-related volume loss.

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Nanosymposium

012. Molecular and Neuroimaging Biomarkers for Alzheimer's Disease

Location: SDCC 32B

Time: Saturday, November 12, 2016, 1:00 PM - 4:30 PM

Presentation Number: 12.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Canadian Institutes of Health Research (CIHR MOP115011)

Alberta Innovates Health Solutions Graduate Studentship

Title: Effects of ApoE and BDNF polymorphisms on hippocampal subfield volumes in a healthy cognitive aging.

Authors: *S. G. TRAVIS[1], Y. HUANG[2], F. OLSEN[2], R. CARTER[2], R. CAMICIOLI[2], N. V. MALYKHIN[2];
[1]Ctr. for Neurosci., [2]Univ. of Alberta, Edmonton, AB, Canada

Abstract: Background: One of the greatest challenges facing developed societies today is addressing the health concerns of an aging population. Recently, we've seen increased evidence that the presence of specific ApoE and BDBF polymorphisms can affect the aging brain. ApoE4 has been linked to atrophy in the volume of the hippocampus (HC), and the rate at which new neurons are generated in the HC and their survival is attenuated with reduced BDNF levels. The main goal of the present study was to investigate effects of ApoE and BDNF polymorphisms on HC subregion and subfield volumes in healthy cognitive aging.
Methods: 140 healthy volunteers (64 M, 76 F, ages 18-85) were recruited. Exclusion criteria were neuropsychiatric disorders, and a MOCA score <26. A TAGC analysis was performed from cheek swabs. Ultra-high resolution HC images were acquired using a T2-weighted, 2D FSE sequence on a 4.7T Varian scanner. HC subfields were manually segmented into the cornu amonis (CA), dentate gyrus (DG), and subiculum (Sub). Subjects were coded for presence of an e4 allele (ApoE), and the presence of the met variant of BDNF. Memory was assessed using the Wechsler Memory Scale, 4th Edition (WMS-IV). We then performed a One-way MANOVA to determine the effects of polymorphisms on total HC volume, its subregions and subfields. Written, informed consent was obtained, and the research was approved by the University of Alberta Health Research Ethics Board.
Results: Subjects with ApoE2 allele(s) had significantly larger total HC volume (p=.021), HC Head volume (p=.008), total CA (p=.009), total Sub (.008), HC Head CA (p=.011), head Sub (p=.04) and HC Body Sub (p=.04) compared to groups with e3 and e4 alleles, after controlling for age and gender. For BDNF, significantly smaller DG volume in the HC Body (p=.022) and HC Head (p=.001) were observed in subjects with BDNF met variant compared to no met variant, controlled for age/sex, with a trend towards smaller total DG volume (p=.06), total HC (p=.058) and HC Head (p=.057) volumes. Neither ApoE nor BDNF polymorphisms had a significant effect on performance in the memory indexes in the WMS-IV.
Conclusions: ApoE polymorphisms e3 and e4 were associated with reduced global HC volume, HC head, and global volumes of CA and Sub, compared to e2 carriers. This was shown in our sample of screened, cognitively healthy adults, suggesting that ApoE4-induced HC atrophy might precede any cognitive loss. In contrast, subjects with BDNF met variant had smaller DG volumes within the HC Head and Body.

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Nanosymposium

012. Molecular and Neuroimaging Biomarkers for Alzheimer's Disease

Location: SDCC 32B

Time: Saturday, November 12, 2016, 1:00 PM - 4:30 PM

Presentation Number: 12.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R03MH106922

Burroughs Wellcome Fund

ACE Autism Center of Excellence P50HD055784

Title: Hemodynamic bias in fmri studies of aging

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Abstract: Introduction:
Functional MRI (fMRI) has been used to identify individuals at-risk for dementia, yet these findings rely on the false assumption that the hemodynamic response function (HRF) to neuronal stimuli is independent of age, and is similar in both healthy and diseased patients. Given that the HRF changes with aging and disease, we hypothesized that false positives would increase when aging and disease-related hemodynamic changes were ignored.
Methods:
We test this hypothesis using an fMRI study comparing functional activations among Younger Healthy (n=14), Older Healthy (n=14) and Older Dementia patients (n=13). We first performed two standard statistical analyses comparing the activation differences seen between (Older Healthy, Younger Healthy) patients, and then (Old Healthy, Old Demented) patients using a both standard multi-level GLM analyses with a fixed HRF. We then repeated this analysis using models in which the hemodynamic effects of aging and disease are estimated within each subject using FLOBS in FSL.
Results:
Within older patients, over 50% of the statistically significant differences for dementia disappeared from significance when using proper HRFs, with the number of statistically significant “different” voxels going from 12,644 to 5,449. When comparing older healthy and younger healthy patients, 32% fewer voxel differences were observed after accounting for the changing hemodynamic response (5,903 vs. 4,030).
Conclusions:
Deviations in the HRF have a practical and measureable impact, diluting the quality of fMRI research studies since observed activations are usually attributable to a neuronal - and not a

hemodynamic - source. More generally, this confirms that hemodynamic changes which occur with aging and disease are measureable using even standard fMRI imaging techniques.

Disclosures: **A. Anderson:** None. **P.K. Douglas:** None. **S.Y. Bookheimer:** None.

Nanosymposium

012. Molecular and Neuroimaging Biomarkers for Alzheimer's Disease

Location: SDCC 32B

Time: Saturday, November 12, 2016, 1:00 PM - 4:30 PM

Presentation Number: 12.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Italian Ministry of Health 204/GR-2009-1606835

Italian Ministry of Health RC 10-11-12-13/A

Disorders and Stroke award R01 NS074980 (DWS)

Title: The superficial white matter in alzheimer's disease

Authors: ***M. DI PAOLA**[1], O. PHILLIPS, 00199[2], S. JOSHI[3], F. PIRAS[5], M. ORFEI[5], M. IORIO[5], K. NARR[4], D. SHATTUCK[4], C. CALTAGIRONE[6], G. SPALLETTA[5];
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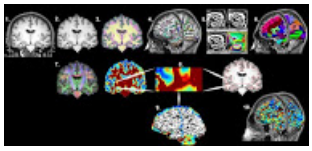
Abstract: White matter abnormalities have been shown in the large deep fibers of Alzheimer’s disease patients (Sachdev et al., 2013; Matsuda, 2013; Liu et al., 2011). However, the late myelinating superficial white matter comprised of intracortical myelin and short-range association fibers has not received much attention. In order to investigate this area, we extracted a surface corresponding to the superficial white matter beneath the cortex, and then applied acortical pattern-matching approach which allowed us to register and subsequently sample diffusivity along thousands of points at the interface between the gray matter and white matter in 44 patients with Alzheimer’s disease (Age: 71.02+5.84, 16M/28F) and 47 healthy controls (Age 69.23+4.45, 19M/28F). In patients we found an overall increase in the axial and radial diffusivity across most of the superficial white matter ($p < 0.001$) with increases in diffusivity of more than 20% in the bilateral parahippocampal regions and the temporal and frontal lobes. This pattern appears to be strongly related to the described progression of Alzheimer’s disease (Braak et al., 1999). Furthermore, diffusivity correlated with the cognitive deficits measured by the Mini-Mental State Examination scores ($p < 0.001$). The superficial white matter has a unique microstructure and is critical for the integration of multimodal information and during brain

maturation and aging. Here we show that there are major abnormalities in patients and the deterioration of these fibers relates to clinical symptoms in Alzheimer's disease.



Disclosures: **M. Di Paola:** None. **O. Phillips:** None. **S. Joshi:** None. **F. Piras:** None. **M. Orfei:** None. **M. Iorio:** None. **K. Narr:** None. **D. Shattuck:** None. **C. Caltagirone:** None. **G. Spalletta:** None.

Nanosymposium

012. Molecular and Neuroimaging Biomarkers for Alzheimer's Disease

Location: SDCC 32B

Time: Saturday, November 12, 2016, 1:00 PM - 4:30 PM

Presentation Number: 12.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Swedish Research Council (#2012-1593, #2012-2172)

Alzheimerfonden

Uppsala Berzelii Technology Centre for Neurodiagnostics

Title: PET imaging of amyloid-beta protofibrils with a bispecific antibody-based radioligand

Authors: ***X. T. FANG**[1], D. SEHLIN[1], L. CATO[1], G. HULTQVIST[1], G. ANTONI[2,3], L. LANNFELT[1], S. SYVÄNEN[1];
[1]Publ. Hlth. and Caring Sci., [2]Medicinal Chemistry, Preclinical PET platform, Uppsala Univ., Uppsala, Sweden; [3]Uppsala Univ. Hosp. PET-Center, Uppsala, Sweden

Abstract: The goal of this study was to enable antibody based PET imaging of amyloid-β (Aβ) protofibrils, which are suggested to cause neurodegeneration in Alzheimer's disease (AD). This was achieved by increasing the brain uptake of the Aβ protofibril selective antibody mAb158 by means of transferrin receptor (TfR) mediated transcytosis across the blood-brain barrier. A bispecific fusion protein was created by chemically conjugating a F(ab')2 fragment of mAb158 to the TfR antibody 8D3. This fusion protein, 8D3-F(ab')2-158 displayed a 15-fold increased brain uptake compared with F(ab')2-158 72 h post injection. Ex vivo experiments demonstrated that the brain retention of 125I radiolabelled fusion protein increased with age and correlated closely with brain levels of Aβ protofibrils in AβPP transgenic mice (tg-ArcSwe), whereas it was

low in wild type (wt) mice. PET imaging with 124I labelled fusion protein confirmed the ex vivo results, showing a high signal in >12 months old tg-ArcSwe mice, and it increased further with age while remaining low in wt mice of all ages. The PET-signal correlated with brain levels of Aβ protofibrils but not with total brain levels of Aβ40 and Aβ42, suggesting that the PET signal reflected protofibril levels rather than plaque load. By recombinantly expressing the bispecific fusion protein 8D3-rec158 we were able to further increase brain uptake another 10-fold compared with the chemically conjugated variant, which is a strong step towards enabling clinical antibody-based PET. The concept of bispecific fusion proteins with the means to cross the blood-brain barrier to enable in vivo PET imaging may be viable for other CNS targets also.

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Nanosymposium

012. Molecular and Neuroimaging Biomarkers for Alzheimer's Disease

Location: SDCC 32B

Time: Saturday, November 12, 2016, 1:00 PM - 4:30 PM

Presentation Number: 12.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: This research was supported entirely by the Intramural Research Program of the National Institute on Aging, NIH

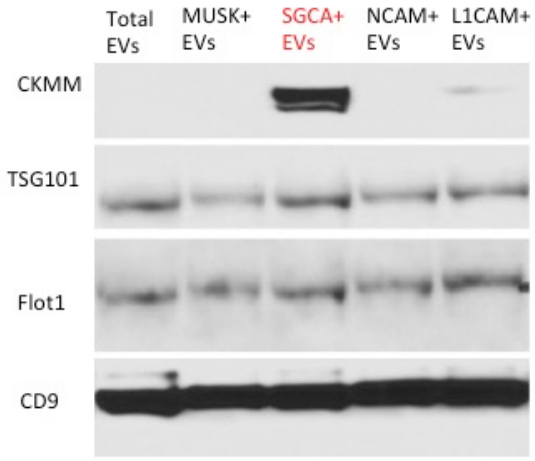
Title: A novel tool for muscle research: plasma Extracellular Vesicles enriched for myocytic origin

Authors: *D. KAPOGIANNIS[1], S. BERKOWITZ[2], E. EITAN[2], M. P. MATTSON[2], L. FERRUCCI[2];
[1]Natl. Inst. on Aging (NIA/NIH), Baltimore, MD; [2]Natl. Inst. on Aging, Baltimore, MD

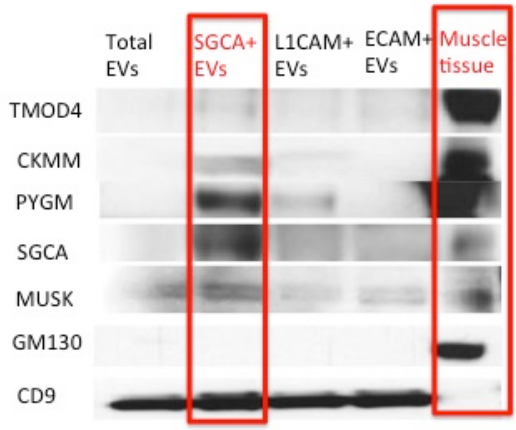
Abstract: A previous study claimed isolation of muscle-derived extracellular vesicles (EVs) from the bloodstream by demonstrating muscle-specific miRNA content. In our Lab, we have pioneered studies of neural origin-enriched EVs (isolated by immunoprecipitation towards neural antigens) as source of biomarkers for Alzheimer's disease. We modified these immunoprecipitation techniques to target muscle-specific antigens and characterized isolated EVs to demonstrate enrichment for muscle-origin. First, we isolated total EVs from plasma of normal subjects using a high-yield precipitation method (Exoquick), followed by immunoprecipitation against various dystrophin glycoprotein complex proteins, such as a-sarcoglycan (SGCA); we also performed immunoprecipitations for EpCAM and NCAM/L1CAM from the same plasma samples as controls (for epithelial and neural EVs,

respectively). Immunoprecipitation for SGCA yielded 4% of the total EV population. To characterize putative muscle-origin SGCA+ EVs against controls, we performed a series of Western blots for muscle-specific proteins CKMM, PYGM, MusK and TMOD4, loading equal numbers of each EV type, therefore, controlling for differential yields. We demonstrated that putative muscle-origin SGCA+ EVs contained equal amounts of common exosomal markers TSG101, Flot1, and CD9, but were highly enriched for CKMM, PYGM, MusK and TMOD4, relative to L1CAM+, EPCAM+ and total EV populations; human muscle tissue served as positive control. To further characterize putative muscle-origin EVs, we performed a series of protein arrays for metabolic, apoptotic, and autophagy-related factors and demonstrated unique signatures. We have provided evidence that EVs of myocytic origin can be isolated from plasma and contain a range of potential biomarker molecules. In ongoing studies, we are exploring the utility of muscle-derived EVs as a diagnostic platform for sarcopenia and other skeletal-muscle diseases.

A. N = 1 healthy subject, EVs derived from single plasma sample via alternative immunoprecipitations for MUSK, SGCA, NCAM, L1CAM



B. EVs derived from plasma samples of 4 healthy subjects via alternative immunoprecipitation for SGCA, L1CAM, EPCAM were pulled and compared to total EVs and muscle tissue homogenate.



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Nanosymposium

012. Molecular and Neuroimaging Biomarkers for Alzheimer's Disease

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Time: Saturday, November 12, 2016, 1:00 PM - 4:30 PM

Presentation Number: 12.13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Intramural research Program of the NIH, National institute on Aging.

Title: Extracellular vesicle -based biomarkers for Alzheimer's disease in the Baltimore Longitudinal Study of Aging

Authors: *M. MUSTAPIC[1], E. EITAN[1], S. T. BERKOWITZ[1], T. C. DIEHL[1], S. GULYANI[1], Y. AN[1], M. P. MATTSON[1], S. M. RESNICK[1], E. J. GOETZL[1,2], L. FERRUCCI[1], D. KAPOGIANNIS[1];
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Abstract: Neural cell adhesion molecule L1CAM may be used for enriching blood extracellular vesicles (EVs) for neuronal origin. Our previous studies generated evidence that Aβ42, pTau181, and phospho-insulin receptor substrate-1 (pSer312-IRS-1 and p-panY-IRS-1) in plasma L1CAM+ EVs may be diagnostic biomarkers for Alzheimer's disease (AD). In the present study, we are analyzing samples from the Baltimore Longitudinal Study of Aging (BLSA) to replicate previous findings and assess whether candidate biomarkers predict AD diagnosis in the clinical and preclinical stage. We studied 969 samples from 373 BLSA participants, 128 diagnosed with AD during a given visit, 128 age and sex-matched controls followed over the same time, and 117 control subjects followed prospectively. Besides samples from the visit when AD diagnosis was made, we studied samples from 2 to 8 visits before. The average times between diagnosis and the two preceding visits were 2.3 and 4.5 years respectively; the average number of visits was 2.6. We have completed isolation of total plasma EVs using Exoquick®, as well as immunoabsorption with anti-L1CAM antibody to isolate L1CAM+ EVs. Protein quantification is ongoing using electrochemiluminescence-based assays (Mesoscale Discovery) for Aβ38,40 and 42, total-tau, pTau181, pSer312-IRS-1, p-panY-IRS-1, and the EV markers Alix and TSG101. In addition, EV concentration and size are being determined by Nanoparticle tracking analysis (NTA). Biomarker concentrations will be normalized to EV concentration, Alix and TSG101. For quality control, in each Mesoscale plate, we include one sample from a single isolation batch to assess between-plate variance. A separate control sample isolated with each set of samples is being used to assess between-isolation variance. For measured analytes, the mean values and the range (mean ± SD; range) are: Aβ42 (2.5±1.6; 0.1-8.7 pg/ml), Aβ40 (7.2±6.1; 1.1-30.1 pg/ml), Aβ38 (39.1±22.9; 2.3-115.7 pg/ml), total Tau (27.8±7.9; 9.0-52.0 ng/ml), pTau181 (39011±21594; 3488-111649 arbitrary units (AU)), pSer312-IRS-1 (3462±5662;110-45115 AU),

p-panY-IRS-1 (526.81±587.73;103-4942 AU), Alix (1350.6±1138.2; 360-3612 ng/ml) and TSG101 (849±635;181-1674 pg/ml). Average concentration (mean±SEM) of L1CAM+ EVs was 1.3E11 ± 9.6E9 particles/ml with mode size (mode±SEM) of 137.8±6.8 nm. Investigators conducting EV isolation and assays will remain blinded until we complete all measurements. Statistical analysis will be performed by co-investigators from a different Laboratory with experience in statistics in BLSA cohorts. Final statistical results are still pending, but will be completed by the time of presentation.

Disclosures: **M. Mustapic:** None. **E. Eitan:** None. **S.T. Berkowitz:** None. **T.C. Diehl:** None. **S. Gulyani:** None. **Y. An:** None. **M.P. Mattson:** None. **S.M. Resnick:** None. **E.J. Goetzl:** None. **L. Ferrucci:** None. **D. Kapogiannis:** None.

Nanosymposium

012. Molecular and Neuroimaging Biomarkers for Alzheimer's Disease

Location: SDCC 32B

Time: Saturday, November 12, 2016, 1:00 PM - 4:30 PM

Presentation Number: 12.14

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Evaluation of cribriform plate morphology in Alzheimer's disease patients and controls

Authors: *D. W. ETHELL[1], R. WOLTJER[2], L. WOOD[1];
[1]Grad. Col. of Biomed. Sci., Western Univ. of Hlth. Sci., Pomona, CA; [2]Pathology, Oregon Hlth. Sci. Univ., Portland, OR

Abstract: Alzheimer’s disease (AD) pathology reflects the accumulation of toxic metabolites in extracellular compartments of the brain. Clearance of these spaces is facilitated by the flow of interstitial fluid (ISF), including cerebrospinal fluid (CSF) that originate from cerebral ventricles. Entorhinal cortex and neighboring regions of the medial temporal lobe are among the first regions to display extracellular plaques and intracellular neurofibrillary tangles in AD. To investigate ISF/CSF clearance from this area we focused on a connection with the olfactory system through to the cribriform plate. Metabolite-laden ISF/CSF for the medial temporal lobe flows along sheaths that cover the lateral olfactory, olfactory trigone, and olfactory tract, ending at the olfactory bulb. Cranial nerve (I) fibers projecting to the olfactory bulb originate in the olfactory nasal epithelium and pass through apertures in the cribriform plate as bundles. As with all of the cranial nerves, sheaths covering cranial nerve I provides for the CSF outflow. We investigated whether changes in cribriform plate morphology may reduce the clearance of CSF from the olfactory bulbs. We evaluated cribriform plate morphology from >20 post-mortem AD subjects and controls using dissection, histology, CT imaging and image analysis. The cribriform plate was excised from each subject and scanned for bone density using a high-resolution CT

scanner. CT scans were evaluated for aperture size and placement using Amira software. Each plate was then dissected and photographed to reveal the extent of aperture occlusion by Dura mater covering the cribriform plate. The brain of each sample was evaluated for a clinical diagnosis of AD using established histopathology that stained for amyloid-beta deposits, neurofibrillary tangles, and Lewy bodies. We found evidence of age-dependent cribriform plate ossification in the majority of subjects with clinically defined AD.

Disclosures: **D.W. Ethell:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Principal, Leucadia Therapeutics. **R. Woltjer:** None. **L. Wood:** None.

Nanosymposium

013. Alpha Synuclein in Parkinson's Disease: From Astrocytes to Epigenetics

Location: SDCC 5B

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 13.01

Topic: C.03. Parkinson’s Disease

Support: ALS Association

Title: Aggregation of α-synuclein and TDP-43: effects of synthetic polymers and substoichiometric concentrations of antibodies

Authors: *L. BREYDO[1], B. NEWLAND[2], D. MORGAN[3], V. UVERSKY[1];
[1]Mol. Med., Univ. of South Florida, Tampa, FL; [2]Leibniz-Institut für Polymerforschung, Dresden, Germany; [3]Byrd Alzheimer's Institute, Morsani Col. of Medicine, Univ. of South Florida, Tampa, FL

Abstract: Protein aggregation plays a central role in pathogenesis of a number of neurodegenerative diseases, and significant efforts to better understand and control this process are ongoing. Alteration of the aggregation pathway either to produce less toxic structures or to increase aggregate clearance is a promising therapeutic route. Both antibodies and small molecules have been extensively studied for this purpose. Here we present the results of our studies on the effect of both small-molecule modified polymers and antibodies on aggregation of α-synuclein as well as analysis of the mechanism of aggregation of TDP-43.
Dopamine is known to direct aggregation of α-synuclein into oligomers via both covalent and non-covalent interactions. In order to explore the importance of direct dopamine-α-synuclein interaction of this activity, we synthesized a highly branched polymer modified with dopamine and tested its effect on α-synuclein aggregation. We found that the dopamine-modified polymer was much less effective in diverting α-synuclein aggregation to oligomers than dopamine itself

indicating that direct interaction of dopamine with the protein is important for its action. Anti-α-synuclein antibodies have been used as therapeutic agents as well. However, most antibodies are too large to cross the blood-brain barrier effectively and thus their levels in the brain are significantly lower than that of α-synuclein. We have examined the effects of substoichiometric concentrations of anti-synuclein antibodies on α-synuclein aggregation. We found that the antibody against residues 121-125 of α-synuclein significantly delayed its aggregation at 1: 100 or lower antibody to protein ratios, and a few more antibodies were effective at higher ratios. Antibodies against this site have been previously shown to be effective in *vivo*.
We have also investigated the mechanism of aggregation of TDP-43, a protein playing an important role in ALS. We examined formation and interconversion of aggregates of this protein as well as their structure and morphology.

Disclosures: **L. Breydo:** None. **B. Newland:** None. **D. Morgan:** None. **V. Uversky:** None.

Nanosymposium

013. Alpha Synuclein in Parkinson's Disease: From Astrocytes to Epigenetics

Location: SDCC 5B

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 13.02

Topic: C.03. Parkinson’s Disease

Title: Functional interaction of the Parkinson´s disease risk factor RIT2 with alpha-synuclein

Authors: *M. VOLTA, J. OBERGASTEIGER, C. CORTI, A. LAVDAS, C. ASCIONE, C. ÜBERBACHER, P. P. PRAMSTALLER, A. A. HICKS;
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Abstract: Parkinson´s disease (PD) has a multifactorial etiology, hypothesized to result from combination of genetic and environmental factors. Several gene mutations cause rare familial PD. Conversely, genome wide association studies (GWAS) have identified genetic risk factors in the general population that could participate in the onset of sporadic PD. A current theory posits that this genetic variability can synergize and precipitate the effects of pathogenic gene abnormalities. Thus, we are investigating the convergence of alpha-synuclein (aSyn) and the novel GWAS hit RIT2 onto cellular mechanisms that might be altered in PD. While aSyn mutations are well established causes of familial PD, that SNCA locus variability is the most reproducible susceptibility factor and that aSyn inclusions are the pathologic hallmark of the vast majority of PD cases, little is known about RIT2 and its coded protein Rin. Importantly, RIT2 gene expression is enriched in the dopamine (DA) neurons of the substantia nigra (SN) and is reduced in PD brains. Rin belongs to the Rit subfamily of Ras-like small GTPases and mediates

neuritogenesis through p38 MAPK and ERK pathways. Notably, Rin mediates the internalization of the DA transporter through direct interaction. We used the human aSyn overexpressor *Drosophila* model and knocked down its ortholog *Ric* through RNA interference: aSyn-induced motor deficits were attenuated, indicating a functional genetic interaction. Consistently, we detected a strong trend for increased Rin protein expression in the cortex of Thy1-aSyn transgenic mice, while mRNA levels in the same area were unchanged. Thus, we studied the expression of Rin in the mouse brain. Rin is expressed in the DA neurons of the SN and their striatal DA terminals. Lastly, we are using SK-N-SH neuroblastoma cell lines overexpressing aSyn or Rin to study their physical molecular interaction and consequent modulation of intracellular signaling. Our data confirm that Rin is expressed in PD-relevant areas in the mouse brain and point towards a functional interaction with aSyn. Modulation of this interaction in cell models of increasing complexity (cell lines, primary neurons, human iPSC-derived DA neurons) will indicate a possible pathologic mechanism that could be targeted for therapy.

Disclosures: **M. Volta:** None. **J. Obergasteiger:** None. **C. Corti:** None. **A. Lavdas:** None. **C. Ascione:** None. **C. Überbacher:** None. **P.P. Pramstaller:** None. **A.A. Hicks:** None.

Nanosymposium

013. Alpha Synuclein in Parkinson's Disease: From Astrocytes to Epigenetics

Location: SDCC 5B

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 13.03

Topic: C.03. Parkinson’s Disease

Support: PICT 2013-0402

UBACyT 2014-2016

UBA-PDTS 2012

Title: α-Synunclein membrane association induces a mitochondrial fragmentation phenotype in a human Parkinson’s Disease models

Authors: V. POZO DEVOTO[1,2], N. DIMOPOULOS[3], M. ALLOATTI[1], T. SAEZ[4,1], G. OTERO[1], L. CROMBERG[1], G. SEVLEVER[3], *T. L. FALZONE[5,1,4];
[1]IBCN-CONICET, Buenos Aires, Argentina; [2]Intl. Clin. Res. Ctr., Brno, Czech Republic; [3]FLENI, Buenos Aires, Argentina; [4]IBYME-CONICET, Buenos Aires, Argentina; [5]Univ. of Buenos Aires, Buenos Aires, Argentina

Abstract: Parkinson’s Disease (PD) converges into a common pathogenic pathway of mitochondrial defects that are supported by genetic, pathological and pharmacological evidence.

Although initially α-Synuclein (α-Syn) studies associated to PD have focused on its role on aggregation and toxicity and away from the mitochondria, recently, growing interest is dedicated to the possible role that α-Syn exerts over the mitochondrial quality control. Biophysical properties of WT α-Syn or the dominant mutations A30P and A53T α-Syn suggest differential lipid binding affinities, which can give a hint on α-Syn interaction mechanism with mitochondria. To test whether α-Syn drives a mitochondrial phenotype we developed different human PD neuronal models derived from hESC or genetically modified hIPSC to analyze the effect of WT, A30P and A53T α-Syn on mitochondrial axonal transport, membrane potential and morphology. Initially, by live imaging of fluorescent mitochondria in axons we analyzed whether WT α-Syn or PD related mutants impairs the axonal transport of mitochondria. Significant defects in mitochondrial flux were observed for A53T α-Syn overexpression and milder defects for WT and A30P. Interestingly, a significant reduction in mitochondrial size plus an increase in axonal mitochondrial density was observed upon A53T α-Syn overexpression. These defects correlated with a high to low ratio of α-Syn localization within the mitochondrial fraction for A53T, WT and A30P, respectively. Therefore, we designed a mitochondrial targeting system based on FKBP-FRB dimerization that delivers α-Syn to the outer mitochondrial membrane (OMM) to test whether α-Syn interaction with the mitochondrial membrane in human neurons mediates the morphology effect. Surprisingly, we found that WT α-Syn delivery to the OMM reduced the size of the mitochondria to similar levels than A53T α-Syn, while A30P induced no effect. These results provide direct evidence of membrane interaction derived effect of α-Syn on mitochondrial size. Finally, CRISP-r/CAS9 genome edition in hIPSC were generated to test whether the N-terminal domain of α-Syn mediates fragmentation. Human neurons derived from CRISP modified IPSC revealed the opposite to fragmentation with abnormal elongated and ramified axonal mitochondria. Interestingly, the mitochondrial enlarged phenotype was lost after A53T α-Syn overexpression in this neurons. All together, our findings identify a new and relevant neuronal physiological role for α-Syn in the maintenance of mitochondrial morphology that when impaired by α-Syn overexpression or mutations can lead to abnormal fragmentation phenotypes observed as a common feature of PD.

Disclosures: **V. Pozo Devoto:** None. **N. Dimopoulos:** None. **M. Alloatti:** None. **T. Saez:** None. **G. Otero:** None. **L. Cromberg:** None. **G. Sevlever:** None. **T.L. Falzone:** None.

Nanosymposium

013. Alpha Synuclein in Parkinson's Disease: From Astrocytes to Epigenetics

Location: SDCC 5B

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 13.04

Topic: C.03. Parkinson’s Disease

Support: Fonds National de la Recherche Luxembourg

Elan Pharmaceuticals

Title: Analysis of microarray data using a PD cellular pathway map reveals early effects of alpha-synuclein on PD pathogenesis

Authors: *A. ASHRAFI[1], P. GARCIA[1], M. OSTASZEWSKI[1], P. GAWRON[1], J. JOHNSTON[2], L. MCCONLOGUE[2], W. ZAGO[3], E. GLAAB[1], R. BALLING[1], M. BUTTINI[1], S. GEBEL[1];
[1]Luxembourg Ctr. For Systems Biomedicine, Esch-Sur-Alzette, Luxembourg; [2]Elan Pharmaceuticals, South San Francisco, CA; [3]Prothena Biosci., South San Francisco, CA

Abstract: The identification of early disease markers for Parkinson's disease (PD) is essential, since the disease is already in an advanced state when motor symptoms become obvious. Large-scale omics data analysis from animal models may help to identify perturbations in molecular pathways before histological or behavioural changes appear. Thus, identifying early disease-related mechanisms can facilitate the development of human biomarkers and new therapeutic approaches. To enable a disease-specific analysis of perturbed molecular pathways from omics data, we have compiled a significant amount of literature-based knowledge (>1000 PMID) on PD into a molecular interaction map. This "PD map" is a constantly updated, freely accessible knowledge repository (http://pdmap.uni.lu), enabling the upload of omics data and interpretation in a PD-related cellular context. In this study, ventral midbrain (containing Substantia Nigra (SN)) from transgenic mice moderately overexpressing E46-mutated alpha-synuclein (about 1.5x over endogenous) was prepared from 3, 9 and 13 month old animals. This mouse line shows mild PD-like pathology (e.g. striatal TH deficits) starting at 9 months of age, but no loss of SN tyrosine-hydroxylase positive neurons. RNA was prepared and analysed on DNA microarrays. Differentially expressed genes were uploaded to the PD map and displayed within the molecular network. Data from the 3 months time point showed a distinct under-expression of genes (e.g., TH, DDC, DAT, VMAT2) related to dopamine metabolism and secretion. In addition an over-expression of genes involved in receptor mediated calcium signalling was detected. Interestingly, no motor symptoms, loss of TH positive neurons or striking alpha-synuclein aggregations were visible at this early age. Gene expression analysis using the PD map highlights early molecular changes in the ventral midbrain of alpha-synuclein over-expressing mice. Direct perturbation of dopamine and calcium signalling related cellular pathways by alpha-synuclein, e.g., through interaction with upstream transcription factors such as NR4A2, might be a key mechanism in early PD pathogenesis, independent of high-molecular, aggregated alpha-synuclein and actual degeneration of SN neurons.

Disclosures: **A. Ashrafi:** A. Employment/Salary (full or part-time): University of Luxembourg. **P. Garcia:** A. Employment/Salary (full or part-time): University of Luxembourg. **M. Ostaszewski:** A. Employment/Salary (full or part-time): University of Luxembourg- LCSB. **P. Gawron:** A. Employment/Salary (full or part-time): University of Luxembourg. **J. Johnston:** None. **L. McConlogue:** None. **W. Zago:** None. **E. Glaab:** A. Employment/Salary (full or part-time): University of Luxembourg. **R. Balling:** A. Employment/Salary (full or part-time):

University of Luxembourg. **M. Buttini:** A. Employment/Salary (full or part-time): University of Luxembourg. **S. Gebel:** A. Employment/Salary (full or part-time): University of Luxembourg.

Nanosymposium

013. Alpha Synuclein in Parkinson's Disease: From Astrocytes to Epigenetics

Location: SDCC 5B

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 13.05

Topic: C.03. Parkinson's Disease

Title: High-content siRNA screen identifies cellular modifiers of pre-formed alpha-synuclein fibril uptake

Authors: *R. KUMARAN[1], D. FERNANDEZ[2], J. W. WERNER-ALLEN[3], E. BUEHLER[2], A. BAX[3], M. LAL-NAG[2], M. R. COOKSON[1];
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Abstract: The alpha-synuclein gene is the strongest genetic risk factor for Parkinson's disease (PD), a major neurodegenerative disorder. Under pathological conditions, excess physiological alpha-synuclein may undergoes fibrillization to form toxic fibrils. Once formed, these pre-formed fibrils can be released into the extracellular microenvironment where they are internalized by neighbouring cells. Here they can act as seeds to promote further fibrilization of monomeric alpha-synuclein. This action provides a possible explanation for the progressive development of Lewy bodies in the brain of PD patients.
Currently the mechanism(s) involved in the uptake of pre-formed alpha-synuclein fibrils are poorly understood. Therefore in order to study cell-to-cell transmission of fibrils and identify genes/pathways specifically involved in their uptake, we developed a high-throughput assay. We designed fluorescently labeled pre-formed fibrils that enabled us to monitor internalization in Hela cells and performed a whole genome siRNA screen, targeting approximately 22,500 genes. Preliminary analysis has shown that loss of a number genes associated with Coatomer protein complex formation greatly inhibits the internalization of pre-formed fibrils by Hela cells. We are currently validating hits using a more focused secondary screen. Hits that survive will then be studied using primary murine cells and human iPSCs. Finally we will attempt to identify pharmacological agents that can target the proteins and pathways of most interest and hopefully this will form the basis of treatments capable of slowing down disease progression in patients.

Disclosures: R. Kumaran: None. **D. Fernandez:** None. **J.W. Werner-Allen:** None. **E. Buehler:** None. **A. Bax:** None. **M. Lal-Nag:** None. **M.R. Cookson:** None.

Nanosymposium

013. Alpha Synuclein in Parkinson's Disease: From Astrocytes to Epigenetics

Location: SDCC 5B

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 13.06

Topic: C.03. Parkinson’s Disease

Title: Structurally distinct strains of α-synuclein fibrils differentially seed Lewy Body-like inclusion formation and impair autophagy in a cell model of PD.

Authors: *A. PANDRAUD, C. KERRIDGE, P. CRAIG, S. BOSE;
Eli Lilly and Co., Windlesham, United Kingdom

Abstract: Recent studies have suggested the existence of strains of aggregated α-synuclein which may partially explain the variable phenotypes associated with synucleinopathies (1). The ubiquitin-proteasome system (UPS) and the autophagy-lysosomal pathway (ALP) are compromised in the presence of α-synuclein aggregates. The mechanism of clearance of insoluble α-synuclein aggregates remains unclear. This study characterized wild type (WT) and familial Parkinson’s disease (PD)-linked mutant A53T *in vitro* pre-formed fibrils (PFFs) generated in high and low-salt buffers and examined whether the strain of α-synuclein PFF and/or the presence of the mutation differentially affects the seeding potency and clearance pathway of α-synuclein inclusions in a PD cell model. Strains of PFFs were prepared by aggregation of WT and A53T recombinant α-synuclein at 37°C, shaking for 14 days with different buffers, and characterized by Thioflavin-T binding, dynamic light scattering, electron microscopy, and proteinase K (PK) digestion. To determine seeding efficiency and effects on clearance, the number of α -synuclein aggregates and co-localization with proteins involved in the UPS and ALP were assessed using confocal and high content imaging. PFFs generated in high-salt conditions were more resistant to PK treatment compared to low-salt PFFs. Different strains of WT and A53T PFFs were internalized, recruited endogenous α-synuclein and induced the formation of intracellular protein deposits reminiscent of Lewy body (LB) inclusions. Higher seeding potency was observed with the A53T PFFs generated in low-salt compared to high-salt buffer. This difference was less pronounced for the WT PFFs. Irrespective of the presence of the mutation or the buffer, α-synuclein aggregates were phosphorylated and co-localized with p62 but not with Lamp-1, suggesting that these inclusions are targeted for autophagy but may not be delivered to autophagolysosomes for degradation. A subset of inclusions was ubiquitinated. This study reveals that strains of PFFs display unique biophysical properties that result in differences in seeding potency, but all led to the formation of inclusions with features similar to human LBs. Inclusions were associated with the autophagy machinery but could not be degraded, suggesting that this pathway is compromised. A deeper understanding of the strains of α-synuclein aggregates and their associated neurotoxicity will be critical when targeting treatments towards

specific conformations of misfolded α-synuclein. Reference: (1) Bousset L, et al. (2013) Structural and functional characterization of two alpha-synuclein strains. Nat Commun. 4:2575.

Disclosures: **A. Pandraud:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **C. Kerridge:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **P. Craig:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **S. Bose:** A. Employment/Salary (full or part-time): Eli Lilly and Company.

Nanosymposium

013. Alpha Synuclein in Parkinson's Disease: From Astrocytes to Epigenetics

Location: SDCC 5B

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 13.07

Topic: C.03. Parkinson’s Disease

Support: Swedish Research Council

Parkinson's Foundation

Alzheimer's Foundation

Åhlen Foundation

Hedlunds Foundation

Title: Accumulation and spreading of alpha-synuclein oligomers in human astrocytes

Authors: ***J. ROSTAMI**[1], S. HOLMQVIST[2], V. LINDSTRÖM[1], J. SIGVARDSON[3], M. INGELSSON[1], J. BERGSTRÖM[1], L. ROYBON[2], A. ERLANDSSON[1];
[1]Publ. health and caring sciences, molecular geriatrics, Uppsala Univ., Uppsala, Sweden; [2]Dept. of Exptl. Med. Sci., Lund Univ., Lund, Sweden; [3]BioArctic Neurosci. AB, Stockholm, Sweden

Abstract: Parkinson’s disease (PD) is characterized by intracellular protein inclusions called Lewy bodies, composed mainly of aggregated alpha-synuclein. Although, alpha-synuclein deposits are primarily found in neurons, they also appear frequently in glial cells at advanced disease stages. Many lines of evidence suggest a prion-like propagation of aggregated alpha-synuclein during PD progression, but the cellular mechanisms behind the spreading is still elusive. The aim of this study was to clarify the role of astrocytes, the most prominent glial cell type, in alpha-synuclein propagation. For this purpose, human astrocytes, derived from embryonic stem cells were treated with Cy3-labelled monomeric or oligomeric alpha-synuclein for 24 h. Following exposure, the cells were thoroughly washed and incubated for 0, 3 or 6 days

in alpha-synuclein free medium. Using time lapse microscopy and immunocytochemistry we investigated intracellular localization, toxic effects and spreading of alpha-synuclein between the astrocytes. Our results demonstrate that human astrocytes engulfed both alpha-synuclein oligomers and monomers. Interestingly, we found that the monomers were quickly degraded, while the oligomers accumulated in the astrocytes. By immunocytochemistry, we showed that the accumulated alpha-synuclein oligomers co-localized with the lysosomal marker, LAMP-1, at the earliest time point. However, the co-localization disappeared over time, leaving large deposits of alpha-synuclein in the astrocytes, indicating an incomplete digestion. Staining with specific markers for various organelles revealed that the alpha-synuclein deposits localize to the region of the trans-golgi network. Furthermore, a clear effect of the oligomer exposure was observed on mitochondria hemostasis which was also confirmed by electron microscopy. In addition, time-lapse recordings demonstrated frequent cell-to-cell transfer of alpha-synuclein inclusions in the astrocyte culture. Interestingly, the astrocytes appeared to use different spreading mechanisms, depending on the size of the inclusions that were transferred. In summary, our results identify a possible role of astrocytes in the spreading of alpha-synuclein pathology.

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Nanosymposium

013. Alpha Synuclein in Parkinson's Disease: From Astrocytes to Epigenetics

Location: SDCC 5B

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 13.08

Topic: C.03. Parkinson’s Disease

Support: Swedish Parkinson Foundation

Swedish Alzheimer Foundation

Berzelii Technology Center for Neurodiagnostics

Swedish Research Council 2015-02671

Parkinson Research Foundation

Åhlén Foundation

Hedlund Foundation

Title: Accumulation of alpha-synuclein in cultured astrocytes can be prevented by oligomer selective antibodies

Authors: *A. ERLANDSSON[1], G. GUSTAFSSON[1], E. NORDSTRÖM[2], L. LANNFELT[1], M. INGELSSON[1], J. BERGSTRÖM[1], V. LINDSTRÖM[1];
[1]Uppsala Univ., Uppsala, Sweden; [2]BioArctic Neurosci. AB, Stockholm, Sweden

Abstract: Protein inclusions referred to as Lewy bodies and Lewy neurites are a pathological hallmark for several neurodegenerative disorders, including Parkinson's disease (PD). The inclusions consist predominantly of insoluble fibrillary alpha-synuclein, but also include soluble oligomeric forms of the protein that are known to be particularly toxic. Apart from neuronal inclusions, deposition of alpha-synuclein aggregates has been demonstrated in glial cells, including oligodendrocytes and astrocytes. To elucidate the significance of such inclusions, we have investigated engulfment, degradation and toxic effects of oligomeric alpha-synuclein in a co-culture system of primary neurons, astrocytes and oligodendrocytes. The cultures were exposed to 500 nM Cy3-labeled, HNE-induced alpha-synuclein oligomers for 24 h. The cells were then thoroughly washed and the uptake, digestion and toxicity of alpha-synuclein were studied at different time points, using immunocytochemistry, time-lapse microscopy, electron microscopy and Western blot analysis. Our results show that alpha-synuclein oligomers are engulfed by all three cell types, but that astrocytes rapidly internalize particularly large amounts of the protein. Moreover, we show that astrocytes start to degrade the ingested alpha-synuclein oligomers by the lysosomal pathway, but cannot fulfill the digestion. The overburden of the astrocytes degrading capacity results in long-term intracellular storage of alpha-synuclein and consequently detrimental processes, including mitochondrial damage. Passive immunotherapy, directed against alpha-synuclein oligomers/protofibrils has been suggested to be a promising treatment strategy for PD. However, the exact cellular mechanism behind the effect of these antibodies remains unclear. Interestingly, we here demonstrate that oligomer selective antibodies can efficiently prevent the formation of alpha-synuclein deposits in cultured astrocytes. Being the most numerous glial cell type in the central nervous system, astrocytes have a great impact on the brain environment and we suggest that processing of pathological alpha-synuclein by astrocytes should be taken into consideration when developing new treatment strategies for PD and other alpha-synucleinopathies.

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Nanosymposium

013. Alpha Synuclein in Parkinson's Disease: From Astrocytes to Epigenetics

Location: SDCC 5B

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 13.09

Topic: C.03. Parkinson’s Disease

Support: European Union's Seventh Framework Programme (FP7/2007-2013)

Innovative Medicines Initiative grant 115439

Title: Phenotypic analysis of A53T SNCA iPSC-derived dopaminergic neurons

Authors: *F. ZAMBON[1], B. RYAN[1], H. FERNANDES[1], H. BOOTH[1], O. CORDERO LLANA[1], W. HAENSELER[2], J. VOWLES[2], S. COWLEY[2], R. WADE-MARTINS[1]; [1]Physiology, Anat. and Genet., [2]Sir William Dunn Sch. of Pathology, Univ. of Oxford, Oxford, United Kingdom

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder. It is characterised by the loss of dopaminergic neurons in the midbrain and the presence of intracellular aggregates, known as Lewy bodies (LB), in the surviving neurons. The aetiology of PD is unknown but a causative role for α-synuclein (aSyn; *SNCA*) has been proposed. This protein is the main component of LBs and point mutations (A30P, E46K, A53T, H50Q and G51D) and copy number variations in the *SNCA* gene have been linked to PD. Although the function of aSyn is not well understood, a number of pathological mechanisms associated with aSyn toxicity have been proposed.

In this study, fibroblasts from healthy individuals and PD patients carrying the A53T *SNCA* mutation were reprogrammed to induced pluripotent stem cells (iPSCs) and differentiated to dopaminergic neurons (DAn) using an established protocol. DAn cultures from all iPSC lines differentiated with the same efficiency, producing overall ~50% DAn (TH^+ cells) and ~80% neurons ($TUJ1^+$ cells) indicating that they could be used for further phenotypic screening for the effect of the A53T *SNCA* mutation. Mitochondrial dysfunction has been proposed to underlie PD. Analysis of mitochondrial respiration showed decreased basal respiration (40%), maximal respiration (35%) and spare capacity (30%) in A53T *SNCA* DAn compared to controls, with no gross differences in glycolytic activity. This difference was not due to a decrease in total mitochondrial content as indicated by protein expression levels analysis of the mitochondrial marker Tom20. This mitochondrial phenotype arises only in DAn, since iPSCs from both genotypes do not show any differences in mitochondrial function or aSyn expression. Protein expression analysis of different markers showed a trend towards ER stress and increased Glucocerebrosidase (GBA) activity and protein levels, but no significant perturbation of autophagy in A53T *SNCA* DAn. Analysis of aSyn secretion in the culture media revealed that DAn secrete increasing amounts of the protein during *in vitro* maturation but no differences in

release by genotype were observed across DAn cultures. Lastly, lentiviral vectors for RNAi-mediated knockdown of aSyn were developed to study its effect on both control and A53T DAn. A ~75% reduction in protein levels was achieved compared to a scrambled control and persisted for at least 35 days post-transduction.
To conclude, iPSC-derived DAn cultures are a promising and relevant *in vitro* model for studying cellular dysfunctions in PD pathology. A53T *SNCA* DAn show deficits in mitochondrial respiration, providing an interesting insight in PD pathology.

Disclosures: F. Zambon: None. **B. Ryan:** None. **H. Fernandes:** None. **H. Booth:** None. **O. Cordero LLana:** None. **W. Haenseler:** None. **J. Vowles:** None. **S. Cowley:** None. **R. Wade-Martins:** None.

Nanosymposium

013. Alpha Synuclein in Parkinson's Disease: From Astrocytes to Epigenetics

Location: SDCC 5B

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 13.10

Topic: C.03. Parkinson’s Disease

Support: the German Center for Neurodegenerative Diseases

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the decipherPD Consortium

Title: Alpha-synuclein enhances histone H3K9 methylation

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Abstract: Background: Alpha-synuclein (αS) is a protein linked to Parkinson’s disease (PD) and related neurodegenerative disorders. It is mostly localized within synapses, but αS has also been suggested to play a role in the nucleus. The aim of this study is to explore epigenetic events through αS.
Method: Histone proteins, extracted from αS transgenic Drosophila and αS inducible SH-SY5Y neuroblastoma cells, were separated by SDS-PAGE, then histone marks were analyzed by western blotting. To determine the level of histone lysine methyltransferase (HMT) and histone lysine demethylase (KDM), mRNA levels were measured by RT-PCR. Chromatin immunoprecipitation was performed by standard protocol using H3K9me2 and REST antibodies.

Target genes were amplified by specific primer pairs.
Results: Overexpression of αS in male flies as well as in retinoic acid pre-treated neuroblastoma cells led to an elevation of histone H3K9 methylation, mostly mono- (H3K9me1) and di- (H3K9me2). The transient increase of H3K9 methylation in SY5Y cells was slightly preceded by an induction of the histone lysine methyltransferase 1C (KMT1C). Pharmacological inhibition of G9a/KMT1C reduced the H3K9 methylations. G9a and H3K9me2 can function within the REST complex. REST chromatin immunoprecipitation (ChIP) survey of REST regulated genes showed significantly increased promoter occupancy of the synaptosomal-associated protein SNAP25 gene after retinoic acid stimulation. Transcripts and protein levels of SNAP25 were decreased after αS induction.
Conclusion: αS overexpression enhances the histone modifications H3K9me1 and H3K9me2, likely involving G9a known to catalyze these histone modifications. While, probable G9a-associated transcription factor REST strongly appears at the SNAP25 promoter, possibly affecting SNARE complex assembly and hence synaptic function regulated by αS.

Disclosures: **N. Sugeno:** None. **S. Jäckel:** None. **A. Voigt:** None. **P. Kahle:** None.

Nanosymposium

013. Alpha Synuclein in Parkinson's Disease: From Astrocytes to Epigenetics

Location: SDCC 5B

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 13.11

Topic: C.03. Parkinson’s Disease

Support: CIHR Grant MOP-84501

Title: Uptake of misfolded alpha-synuclein oligomers and transmission to adjacent cells

Authors: ***A. TANDON**[1], M. M. MARANO[1], S. SRI RENGANATHAN[1], W. P. FLAVIN[2], E. M. CAMPBELL[2], P. E. FRASER[1];
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Abstract: A prion-like mechanism is thought to underlie the propagation of alpha-synuclein (asyn) pathology in Parkinson’s disease, whereby aggregated asyn is conveyed along peripheral neuronal tracts into the central nervous system. Here, we describe single and co-culture cell-based assays designed to recapitulate the uptake and transmission of misfolded asyn across cellular membranes. The accumulation of misfolded asyn into non-neuronal and neuronal cells is predominantly mediated by dynamin-dependent endocytosis. This exogenous vesicular asyn is associated with increased rupture of the endocytic vesicle membranes, thereby permitting the

misfolded lumenal asyn to interact with cytosolic asyn expressed by the host cell. When H4 neuroglioma cells expressing asyn, coupled to either luciferase or GFP, are exposed to extracellular oligomer-prone asyn mutants, we observed a reorganization of cytoplasmic asyn staining into punctate structures that contain misfolded and insoluble asyn. Moreover, co-culture of oligomeric asyn-treated cells with naïve cells expressing asyn-GFP, also induced a striking change in asyn localization in the naïve cells into small punctate structures, suggesting that the propagation of misfolded asyn from one cell population to another is induced by exposure to oligomeric asyn.

Disclosures: **A. Tandon:** None. **M.M. Marano:** None. **S. Sri Renganathan:** None. **W.P. Flavin:** None. **E.M. Campbell:** None. **P.E. Fraser:** None.

Nanosymposium

013. Alpha Synuclein in Parkinson's Disease: From Astrocytes to Epigenetics

Location: SDCC 5B

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 13.12

Topic: C.03. Parkinson’s Disease

Support: NIH Grant T32NS041234-15

Title: Defective endoplasmic reticulum dynamics and its selective autophagy in synucleinopathies.

Authors: *Y. C. WONG, D. KRAINC;
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Abstract: α-synuclein plays a central role in synucleinopathies such as Parkinson’s disease (PD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA), where it accumulates and causes degeneration. Both mutations and increased copy numbers of α-synuclein are sufficient to cause familial PD, suggesting a critical role for α-synuclein in contributing to cellular toxicity. However, how α-synuclein toxicity disrupts the real-time dynamics of key cellular pathways and organelles have not been clearly elucidated. Using live cell imaging, we examined the dynamics of multiple organelles including mitochondria, autophagosomes, lysosomes and the endoplasmic reticulum (ER) in cellular models of α-synuclein toxicity. We found that ER dynamics are disrupted by increased levels of α-synuclein, leading to decreased rates of ER tubulation and ER homotypic fusion events, ultimately disrupting ER morphology. These defects in ER dynamics consequently contribute to upregulation of selective autophagy of the ER (ERphagy) via the autophagy receptor FAM134b both under basal conditions and upon cellular stress, suggesting that the ER may be particularly vulnerable to dysfunction in

synucleinopathies. Thus, in addition to previously reported defects in ER to golgi trafficking and MAM (mitochondria-associated endoplasmic reticulum membrane) dysfunction, we now demonstrate a novel mechanism through which α-synuclein contributes to ER dysfunction via disruption of ER morphology dynamics of tubulation and fusion leading to its selective autophagy, further highlighting the ER as a critical target of α-synuclein toxicity.

Disclosures: Y.C. Wong: None. **D. Krainc:** None.

Nanosymposium

013. Alpha Synuclein in Parkinson's Disease: From Astrocytes to Epigenetics

Location: SDCC 5B

Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 13.13

Topic: C.03. Parkinson's Disease

Support: MSU Foundation Strategic Partnership Grant

Title: Alpha synuclein overexpression within the enteric nervous system impairs colonic contractility and motility

Authors: *M. J. BENSKEY[1,2,3], B. D. GULBRANSEN[4,3], X. BIAN[5], N. KUHN[2], J. J. GALLIGAN[5,3], F. P. MANFREDSSON[2,3,6];
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Abstract: Parkinson's Disease (PD) is often defined as a movement disorder, however PD is also characterized by a large non-motor component. GI dysfunction is the most common non-motor symptom associated with PD. PD-associated GI dysfunction affects virtually every level of the GI tract, from esophagus to anus, primarily presenting as gastroparesis and decreased colonic motility. Recent studies have demonstrated that the PD associated protein, α-synuclein (α-syn), forms large inclusion within neurons of the enteric nervous system (ENS), suggesting that α-syn pathology within the ENS may play a role in the etiology of GI dysfunction in PD. This hypothesis has been supported by GI dysmotility in transgenic α-syn overexpressing mice. However, α-syn overexpression within these mice occurs in cell populations throughout the entire organism, thus, it is not clear whether the GI phenotype observed in these animals is due to α-syn pathology in the central nervous system (CNS), ENS, or both. To determine if α-syn accumulation *solely* within neurons of the ENS is sufficient to produce GI dysfunction, we pioneered a novel surgical approach to overexpress human wildtype α-syn within the ENS using direct injections of adeno associated virus (AAV) to the descending colon. This approach results

in robust transduction of enteric neurons and glia, with no viral genomes detected in the CNS. To evaluate the role of α-syn in GI dysfunction adult male rats received direct injections (6 x 5µl at $6x10^{12}$vg/ml) of AAV expressing either human wildtype α-syn or a green fluorescent protein (GFP) control transgene into the descending colon. Four and eight weeks post-surgery, colonic motility was assayed by quantifying the time necessary to transit and excrete a bead through 5cm of the descending colon within ambulatory animals. Animals that received AAV-α-syn had significantly impaired colonic motility, with a mean colonic transit time of 171.5 ± 43.4 minutes compared to animals that received AAV-GFP with a mean colonic transit time of 15 ± 6.8 minutes. *Ex vivo* analyses revealed that overexpression of α-syn in the descending colon resulted in an increase in inhibitory neuromuscular junction potentials along with a corresponding decrease in neurogenic contractions of colonic circular muscle. AAV-α-syn also decreased basal levels of calcium in both neurons and glia. Importantly, a quantitative histological examination of transduced tissue revealed that α-syn overexpression did not produce any neurodegeneration. Together, these findings suggest that α-syn overexpression in the ENS results in alterations in neurotransmission that ultimately result in impaired colonic motility.

Disclosures: M.J. Benskey: None. **B.D. Gulbransen:** None. **X. Bian:** None. **N. Kuhn:** None. **J.J. Galligan:** None. **F.P. Manfredsson:** None.

Nanosymposium

014. Movement Disorders

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Time: Saturday, November 12, 2016, 1:00 PM - 3:30 PM

Presentation Number: 14.01

Topic: C.04. Movement Disorders

Title: Sustained suppression of huntingtin mRNA and protein throughout the non-human primate brain after intrathecal administration of antisense oligonucleotides

Authors: *H. B. KORDASIEWICZ[1], K. M. IKEDA-LEE[1], T. ZANARDI[1], M. STEPHAN-GUELDNER[2], D. NORRIS[1], A. SMITH[1], R. LANE[1], C. F. BENNETT[1], E. SWAYZE[1]; [1]Ionis Pharmaceuticals, Carlsbad, CA; [2]Hoffmann-La Roche AG, Basel, Switzerland

Abstract: Huntington's disease (HD) is a fatal dominantly inherited neurodegenerative disease caused by a CAG expansion in the huntingtin gene, for which there is currently no cure. Antisense oligonucleotides (ASOs) are emerging as a viable therapeutic approach and can be used to selectively target production of many of the toxic proteins involved in currently untreatable neurodegenerative diseases. ASOs are single stranded nucleotides typically 20 bases in length that bind complementary target RNA through Watson and Crick hybridization leading to selective degradation of the target RNA. Since ASOs do not cross the blood brain barrier,

CNS targets in patients can be accessed via intrathecal (IT) delivery to the CSF. ASO-mediated suppression of huntingtin mRNA in animal models of HD improves motor phenotype, anxiety, gene expression deficits and survival. The first clinical study of an ASO targeting huntingtin in HD patients is underway (NCT02519036). One key experiment in translating the rodent work to the clinic is determining the pharmacodynamics and pharmacokinetics of huntingtin-targeting ASOs in a larger brain using IT delivery, the intended clinical route of administration. To this end, we have treated non-human primates (NHP) with an ASO targeting NHP huntingtin at two dose levels and collected tissues 1, 4, or 8 weeks post-dosing. After intrathecal delivery of the huntingtin ASOs, huntingtin mRNA is suppressed throughout the NHP CNS, including cortical regions, thalamus, and caudate, all key regions implicated in HD. Remarkably, target suppression and ASO accumulation are similar in spinal cord adjacent to the injection site and in frontal cortex, the most distal region from the injection site. Target mRNA suppression is sustained and still present 8 weeks post-dosing. Using a novel assay to quantify total huntingtin protein, we also confirmed huntingtin protein levels track with RNA levels. These data support the use of intrathecal dosing to deliver huntingtin-targeting ASOs to the brain regions implicated in HD as a potential therapeutic for the treatment of HD.

Disclosures: **H.B. Kordasiewicz:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **K.M. Ikeda-Lee:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **T. Zanardi:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **M. Stephan-Gueldner:** A. Employment/Salary (full or part-time): Roche. **D. Norris:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **A. Smith:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **R. Lane:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **C.F. Bennett:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **E. Swayze:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals.

Nanosymposium

014. Movement Disorders

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Presentation Number: 14.02

Topic: C.04. Movement Disorders

Support: NIH Grant NS087986

Title: Salivary Biomarkers for Huntington's Disease

Authors: *E. A. THOMAS[1], A. AIKIN[2], S. PARK[3], A. HAQUE[3], M. GARZA[3], J. COREY-BLOOM[3];
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Abstract: Peripheral biomarkers are greatly needed in the field of neurological disorders in order to anticipate onset of disease symptoms, to monitor disease progression, and to track potential therapeutic effects. Huntington's disease (HD) is a fatal, inherited neurodegenerative disorder caused by a CAG repeat expansion in the gene encoding the huntingtin protein (Htt). Pathogenesis is associated with expression of the mutant Htt protein in the CNS; however Htt is expressed ubiquitously throughout the body. The Htt protein is the most significant molecular target for disease modifying therapies, and several therapeutic approaches that target its production, processing, and/or turnover are under development or approaching clinical trials in patients. Measurement of Htt has broad potential as a biomarker. Since non-invasive methods to quantify Htt in the CNS do not exist, measuring Htt in peripheral cells represents an essential step in biomarker discovery for HD. In the current study, we have measured Htt protein levels in saliva from symptomatic HD patients (n=31), presymptomatic HD individuals (n=10) and healthy normal controls (n=56) using an ELISA with antibodies recognizing amino acids 802-940 of human Htt protein. This assay revealed significant increases in Htt expression in saliva from HD individuals compared to normal controls. Further, salivary Htt levels were higher in symptomatic HD patients compared to pre-symptomatic or transitional patients. No correlations were detected between Htt expression and age or sex in all subjects. Further, salivary Htt levels did not show diurnal variation. We also adapted a brain-derived neurotrophic factor (BDNF) assay to measure levels of this protein in saliva using ELISA. No significant differences in salivary BDNF in HD patients compared to normal controls was detected using this method. In summary, measurements of salivary Htt offer significant promise as a relevant, non-invasive disease biomarker for HD, and its use could be immediately implemented into both translational and clinical research use.

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Nanosymposium

014. Movement Disorders

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Presentation Number: 14.03

Topic: C.04. Movement Disorders

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P30 GM103503

DE-AC05-06OR23177

Title: Whole brain imaging of a walking patient in real-time: potential and promise of a wearable PET scanner

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Abstract: The neurocorrelates of walking are understudied, especially related to balance and centering body mass over the legs. However, imaging of deep brain structures in a moving subject is not feasible with current imaging technology. Standard fMRI, PET and MEG do not tolerate head motion, and EEG and fNIRS cannot image deep brain structures. Here we discuss quantitative imaging of deep brain structures such as the striatum and eventually the whole brain, during fully upright, close to natural walking behavior using a novel wearable brain imager, Ambulatory Microdose PET (AMPET). The specially designed flexible mechanical support with an adjustable suspension point would allow upright motion such as above a treadmill or balance device, moves with a person's head and thus be capable of active image acquisition of an upright person in motion. The design is built around a safety helmet with an inner friction fit that allows it to move with the head but also suspends the weight burden off the participant. Preliminary data acquired during active wide angle head-turning motion, shows that a prototype version can obtain images during active motion. Using 18F-FDG imaging, we found activity detected in the striatum, caudate and other cortical areas to be comparable to those detected via standard PET scanner (n=2) (Bauer et al. 2016). We will discuss improvements in the head support and stabilization for walking studies, as well as design options. In addition, we will discuss options to increase temporal resolution by taking advantage of dynamic radiotracer imaging. Applications for this upright neuro-imaging technology would include understanding the mechanisms of balance, as well as normal vs. abnormal walking behaviors and gaits. Virtual reality could also be combined with an AMPET imager in order to investigate brain activity during walking and balance in environments not otherwise practical to bring to participants in the real world. Ultimately, understanding the neural correlates of walking and balance will have critical implications for rehabilitation strategies for stroke and other neurological disorders, including TBI, Parkinson’s disease and balance disorders.

Disclosures: **J.A. Brefczynski-Lewis:** None. **A. Stolin:** None. **C. Bauer:** None. **M. Mandich:** None. **P. Kinahan:** None. **J. Qi:** None. **S. Dolinsky:** A. Employment/Salary (full or part-time): General Electric. **M. Rishel:** A. Employment/Salary (full or part-time): GE. **S. Majewski:** None.

Nanosymposium

014. Movement Disorders

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Topic: C.04. Movement Disorders

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Title: Assessing the protein quality control system in the Huntington disease model R6/2 using a luciferase-based sensor

Authors: *E. SCHULZ-TRIEGLAFF, R. KLEIN, I. DUDANOVA;
Max Planck Inst. of Neurobio., Munich, Germany

Abstract: An impairment of the protein quality control system (PQCS) is believed to be a major cause of age-related neurodegenerative disorders. It has been shown that modulating the PQ machinery protects from or enhances pathological changes in cellular and animal models of neurodegenerative diseases. However, the PQCS has not sufficiently been monitored during the course of disease of mammalian models. To study the chronological sequence of impairment of the PQCS, protein aggregation, neuropathological and functional changes in the brain, we are using a protein sensor, which consists of firefly luciferase (Fluc) fused to GFP. Fluc is a metastable protein that requires chaperone assistance for proper folding and full enzymatic activity. A decrease in protein folding capacity of a cell can be detected by decreased enzymatic activity of Fluc and changes in GFP distribution in the cytoplasm, as the sensor forms inclusions when not folded correctly (Gupta R, et al. 2011. *Nature methods* 8: 879-84). We first tested the sensor's sensitivity and effectiveness in murine cortical cultures by applying different proteotoxic stress paradigms to transfected primary neurons. Either application of proteasome and chaperone inhibitors, heat stress or co-expression of aggregating proteins leads to the formation of GFP+ inclusions and decreased enzymatic activity of Fluc in transfected neurons. We next used this sensor to measure changes of the PQCS caused by the presence of a misfolded protein. We detected a reduction of specific luciferase activity in Fluc-transfected primary cortical cultures of the Huntington disease model R6/2. In order to monitor proteostasis defects in vivo, we generated transgenic mice expressing the sensor in the nervous system. Promising founder lines show expression in several brain regions. After crossing these mice to the R6/2 line we measured a decreased functionality of the PQCS in the striatum of R6/2 mice at an advanced disease stage (12 weeks). Interestingly, in presymptomatic 1 week-old mice we observed an increase in Fluc folding efficiency in the hippocampus and the cerebellum, regions that remain relatively spared at early disease stages. We are currently working on unraveling the mechanistic basis of this finding. We plan to cross these sensor mice to different disease models to assess the PQCS comparatively between neuropathological disorders. Also, we will monitor the PQCS

throughout normal aging comparing several brain regions. Our sensor mice represent a tool to monitor the PQCS during the disease, an important basis for developing new therapies based on improved functionality of the PQC machinery.

Disclosures: E. Schulz-Trieglaff: None. **R. Klein:** None. **I. Dudanova:** None.

Nanosymposium

014. Movement Disorders

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Topic: C.04. Movement Disorders

Support: NS084386

NS092024

Title: *In vivo* evidence for a moving Huntingtin Rab4 vesicle complex during axonal transport.

Authors: *S. GUNAWARDENA[1], J. WHITE, 14221[2], K. ZIMMERMAN[2], H. HOFMAR-GLENNON[2];
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Abstract: Huntingtin (HTT), the protein responsible for Huntington's disease (HD), is ubiquitously expressed and enriched in neurons. HTT associates with microtubule motors, kinesin and dynein, and is involved in the movement of vesicles within axons. However the type of vesicle or the cargo complex that HTT is present on during long distance transport within axons is unknown. We previously found that reduction of Drosophila HTT perturbed the bi directional movement of Rab4 GTPase containing vesicles in larval axons. Simultaneous dual view imaging revealed that HTT and Rab4 likely move together within larval axons. Sub pixel co localization analysis revealed that Rab4, HTT, and motor proteins co localize suggesting that a HTT motor complex likely exists in vivo during axonal transport. Intreguingly reduction of the HTT interacting protein 1 (HIP1) and a known Rab effector, Rip11, perturbed the movement of both HTT and Rab4 in vivo. However, reduction of Milton (a protein with some sequence homology to HAP1) and Nemo (a Rab8 effector) had no effect. Taken together, our observations propose a model in which HIP1 and Rip11 may aid the linking of HTT Rab4 containing complexes to motor proteins. Since expansion of polyQ repeats in the context of human HTT perturbed the motility of Rab 4, perhaps normally HTT plays a key role in facilitating Rab4 motility within axons for particular Rab4 mediated functions at the synapse. These findings have

important implications for our understanding of the complex functions of HTT, which when disrupted may initiate disease pathways.

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Nanosymposium

014. Movement Disorders

Location: SDCC 24A

Time: Saturday, November 12, 2016, 1:00 PM - 3:30 PM

Presentation Number: 14.06

Topic: C.04. Movement Disorders

Title: The MID1 protein is a regulator of several CAG repeat mRNAs

Authors: ***S. KRAUSS**, N. GRIESCHE, J. SCHILLING, V. PESCH, S. WEBER, M. ROHM; DZNE / German Ctr. of Neurodegenerative Dis., Bonn, Germany

Abstract: CAG repeat expansion disorders like Huntington's disease (HD) are monogenic diseases that are caused by expansion of CAG repeat motifs in the disease-causing genes that, if located within the coding region, translate into elongated polyglutamine (polyQ) stretches on the protein level. HD is the most common of the nine known polyQ disorders. Aggregation of Huntingtin (HTT) aminoterminal fragments with expanded polyQ stretches in the central nervous system of the affected patients is a hallmark of HD. Both loss of function of the normal protein and gain of toxic function of the mutant protein have been suggested to play a role in the pathogenesis. Recent evidence argues that another pathogenic mechanism that contributes to disease development is a gain of function at the RNA level. The CAG repeat RNA folds into hairpin structures, and several proteins can bind to these structures in a repeat-size dependent manner. The aberrant recruitment of proteins into pathological RNA structures might deplete the cell of essential factors involved in translation and could thus represent a novel mechanism contributing to HD pathogenesis. We have shown previously that one example of a protein complex that binds to mutant HTT mRNA is the MID1 protein complex. This protein complex contains, amongst other proteins, the MID1 protein, the catalytic subunit of protein phosphatase 2A (PP2Ac), and 40S ribosomal S6 kinase (S6K). Strikingly, binding of this protein complex to mutant HTT mRNA results in an increased translation and subsequently aggregation of mutant HTT. In this study we analyzed if MID1 also regulates other mRNAs with expanded CAG repeats using a panel of *in vitro* and *ex vivo* assays. Our data suggest that MID1 binds to CAG repeat mRNAs irrespective of the repeat flanking regions, suggesting that MID1 is a common regulator of CAG repeat mRNAs. This makes the MID1 complex a very interesting target to

prevent formation of both pathological RNA-protein complexes and pathological accumulations of mutant polyQ protein not only in HD but also in other polyQ disorders.

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Nanosymposium

014. Movement Disorders

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Topic: C.04. Movement Disorders

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Strategic Positioning Fund for Genetic Orphan Diseases (SPF2012/005)

Title: Cell-intrinsic effects of mutant HTT in oligodendroglial cells contribute to myelination abnormalities and behavioural deficits in a mouse model of Huntington disease

Authors: *C. FERRARI BARDILE[1], M. GARCIA-MIRALLES[1], N. CARON[2], R. T. Y. TEO[1], M. R. HAYDEN[2], M. A. POULADI[1];
[1]Translational Lab. In Genet. Med. and, Singapore, Singapore; [2]Ctr. for Mol. Med. and Therapeut., Vancouver, BC, Canada

Abstract: Clear evidence from human and animal studies indicates that white matter structures are profoundly affected in Huntington disease (HD). Although its etiology is not fully understood, white matter atrophy appears very early in the disease course suggesting that it may be a primary event preceding neuronal loss. We hypothesize that abnormalities in white matter reflect dysfunction caused by the direct effects of mutant huntingtin (mHTT) on oligodendrocytes, the myelinating cells of the central nervous system. Using the BACHD mouse model of HD, which expresses full-length human mHTT and mimics many of the behavioural and neuropathological features of the human condition, we genetically reduced mHTT expression in oligodendroglial cells by crossing BACHD mice to NG2-Cre mice. Using electron microscopy analysis of myelinated fibers of the corpus callosum we show that myelin sheaths are thinner and less compact in BACHD mice. Reduction of mHTT expression in oligodendroglial cells rescues the deficits in thickness and compactness of myelin sheaths, supporting cell intrinsic effects of mHTT on oligodendrocytes. We further show that silencing mHTT in oligodendroglia improves aspects of behavioural dysfunction in the HD mice, including motor

and psychiatric-like phenotypes. Our findings suggest that the expression of mHTT in oligodendrocytes contributes to myelin abnormalities and certain behavioural manifestations in HD. Our study provides novel insights into the etiology of white matter pathology in HD.

Disclosures: C. Ferrari Bardile: None. **M. Garcia-Miralles:** None. **N. Caron:** None. **R.T.Y. Teo:** None. **M.R. Hayden:** None. **M.A. Pouladi:** None.

Nanosymposium

014. Movement Disorders

Location: SDCC 24A

Time: Saturday, November 12, 2016, 1:00 PM - 3:30 PM

Presentation Number: 14.08

Topic: C.04. Movement Disorders

Support: CIHR Foundation FDN-143210

Title: Presynaptic alterations in corticostriatal synapses in huntington's disease model

Authors: *C. BUREN[1], A. SMITH-DIJAK[1], M. E. SCHMIDT[2], M. R. HAYDEN[2], L. A. RAYMOND[1];
[1]Psychiatry, [2]Ctr. for Mol. Med. and Therapeut., The Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Huntington's disease (HD) is an inherited neurodegenerative disorder caused by expansion of a CAG tract >35 in the huntingtin (*HTT*) gene. The disease manifests with poor movement control, cognitive decline and psychiatric disorders. Recent studies suggest that corticostriatal synaptic dysfunction precedes the motor phenotype in HD mouse models. Moreover, protein interaction studies have revealed that wildtype and mutant huntingtin interact in complexes with hundreds of different proteins, in which presynaptic proteins are prominent. Our study focused on the potential changes in presynaptic terminals of corticostriatal synapses in HD. We compared the YAC128 HD mouse model, expressing human huntingtin with 128 polyglutamine repeats on an FVB/N background, with FVB/N wild-type (WT) control mice. Using immunocytochemistry and whole-cell patch clamp in corticostraital co-cultures, we found a reduction in spontaneous release of glutamate, but an increase in Ca^{2+}-independent vesicle release, at corticostriatal synapses in YAC128 compared with FVB/N co-cultures. We also uncovered reduced expression of vGlut1 in the cortical pre-synapses, whereas in the postsynaptic striatal neurons, there was no change in GluA2 expression but miniature excitatory postsynaptic current amplitude was reduced. Taken together, these data suggest decrease of glutamate in individual vesicles. Interestingly, the number of vesicles in the presynaptic readily releasable pool remains unchanged, but vesicle replenishment is impaired in this HD model. Currently, we

are assessing vesicle replenishment at corticostriatal synapses in acute brain slices from 6-month old YAC128 mice after train stimulation by patch clamp recording as well as by imaging glutamate release using the iGluSnFr fluorescent protein. Together, our studies will enhance understanding of the corticostriatal synaptic changes contributing to early circuit dysfunction in HD.

Disclosures: C. Buren: None. **A. Smith-Dijak:** None. **M.E. Schmidt:** None. **M.R. Hayden:** None. **L.A. Raymond:** None.

Nanosymposium

014. Movement Disorders

Location: SDCC 24A

Time: Saturday, November 12, 2016, 1:00 PM - 3:30 PM

Presentation Number: 14.09

Topic: C.04. Movement Disorders

Support: NIH R01 EY014061

Title: Sirt1 neuroprotection in spinocerebellar ataxia type 7 neurodegeneration involves restoration of proper calcium homeostasis

Authors: *C. STOYAS[1], B. DAVID[3], A. SAVTCHENKO[1], C. C. NIU[1], J. W. WARD[1], T. GAASTERLAND[1], F. XIE[1], M. MERCOLA[4], V. G. SHAKKOTTAI[3], A. R. LA SPADA[2]; [2]Pediatrics, [1]UCSD, LA Jolla, CA; [3]Neurol., Univ. of Michigan, Ann Arbor, MI; [4]Stanford Univ., Palo Alto, CA

Abstract: Spinocerebellar ataxia type7 (SCA7) is an autosomal dominantly inherited neurodegenerative disorder caused by a CAG/polyglutamine (polyQ) repeat expansion in the ataxin-7 gene. It is characterized by both cerebellar disease and retinal degeneration that ultimately progresses to complete blindness. The normal function of ataxin-7 and the mechanistic basis of SCA7 have yet to be fully determined; however, ataxin-7 is known to be a core component of the transcriptional co-activator complex STAGA. As polyQ-expanded ataxin-7 integrates into the STAGA complex, transcriptional dysregulation in the presence of polyQ-ataxin-7 is a likely mechanism for SCA7 neurodegeneration. We utilized RNA-seq analysis to obtain an unbiased list of down-regulated genes in the cerebellum of our PrP-SCA7 92Q-BAC mouse model of SCA7. Pathway analysis implicated calcium homeostasis, specifically inositol (1,4,5) triphosphate receptor (IP3R) signaling, as altered in SCA7. Cerebellar electrophysiology and calcium response rates of neurons were altered in mPrP-SCA7 92Q-BAC mice. Transcription factor binding site analysis of all down-regulated genes revealed putative binding sites for peroxisome proliferator-activated receptors (PPARs), which are known Sirtuin1 (Sirt1)

targets. Eight of the identified down-regulated calcium homeostasis gene promoters had putative PPAR response element (PPRE) sites, including the IP3R gene promoter. As previous work in yeast had identified a functional and physical interaction between yeast ataxin-7 (Sgf73) and yeast Sirt1 (Sir2), we tested if this interaction is conserved in mammals, and we found compelling evidence for a physical interaction between Sirt1 and ataxin-7, which was enhanced by polyQ tract expansion. We also documented marked inhibition of Sirt1 deacetylase activity in the cerebellum of PrP-SCA7 92Q-BAC mice. Based upon these findings, we pursued a genetic rescue in two different SCA7 mouse models by crossing Sirt1 over-expressing mice with PrP-SCA7 92Q-BAC mice, and with SCA7 266Q knock-in mice. We observed an amelioration of cerebellar neurodegeneration, reduced disease progression, and significantly increased lifespan in Sirt1-SCA7 bigenic mice. In Sirt1 - SCA7 bigenic mice, we further noted significantly increased expression of calcium homeostasis genes with PPRE binding sites in their promoters. Our studies reveal a novel role for Sirt1 in positively regulating the expression of calcium homeostasis genes in the cerebellum in SCA7 neurodegeneration, and thus suggest that one key aspect of Sirt1 neuroprotection is calcium regulation.

Disclosures: **C. Stoyas:** None. **B. David:** None. **A. Savtchenko:** None. **C.C. Niu:** None. **J.W. Ward:** None. **T. Gaasterland:** None. **F. Xie:** None. **M. Mercola:** None. **V.G. Shakkottai:** None. **A.R. La Spada:** None.

Nanosymposium

014. Movement Disorders

Location: SDCC 24A

Time: Saturday, November 12, 2016, 1:00 PM - 3:30 PM

Presentation Number: 14.10

Topic: C.04. Movement Disorders

Support: Brain Research Trust Studentship

Title: Modeling spinocerebellar ataxia 15 with iPS cell derived neurons

Authors: ***S. WIETHOFF**[1], C. ARBER[2], S. WRAY[2], Y. ZHI[2], R. PATANI[1], H. HOULDEN[1]; [1]Dept. for Mol. Neurosci., Univ. Col. London, London, United Kingdom; [2]Dept. of Mol. Neurosci., UCL Inst. of Neurol., London, United Kingdom

Abstract: Introduction
Spinocerebellar Ataxia 15 (SCA15-) patients present with progressive cerebellar ataxia and possible pyramidal, extrapyramidal and cortical features (1, 2). Heterozygous deletions in the inositol-1,4,5-triphosphate-receptor type 1-gene (*ITPR1*) resulting in haploinsufficiency of the ligand-gated calcium-channel on the membrane of the endoplasmatic reticulum (ER) are the

underlying genetic cause (3). *ITPR1* is expressed in the cerebellum and cortex with an assumed role in calcium homeostasis & neurotransmitter exocytosis (4).
Methods
Fibroblasts were collected from 3 SCA15-patients via a skin biopsy, reprogramming was performed via episomal gene delivery (5), iPSC-colonies picked and expanded. Two clones per patient were validated and neurally differentiated (7).
Preliminary Results
Patient-derived iPSCs were confirmed as pluripotent via ICC and qPCR for marker pluripotency genes, karyotypically normal apart from the heterozygous ITPR1-deletion and 5 out of 6 were integration-free. Neural differentiation yields a high proportion of cortical neurons in patient and control lines. Preliminary Ca-imaging trial data in fibroblasts, iPS-cells and neurons shows disturbance of store-operated calcium entry in patient-neurons only.
Future Work
We will use ICC, live-imaging, cytotoxicity assays and electrophysiology to investigate the interplay between ER-calcium-stores, mitochondria and cytosol impacting on cell-integrity and transmitter exocytosis to conclude on the pathomechanism leading to neurodegeneration in SCA15. A phenotypic rescue will be investigated via treatment with different calcium-stabilisators (e.g. dantrolene). In a parallel string of experiments we are developing a shorter protocol to derive cerebellar-like cells from monolayer iPSCs even though challenging (8) in order to study this condition in a more region-specific neuronal subtype.

Disclosures: S. Wiethoff: None. **C. Arber:** None. **S. Wray:** None. **Y. Zhi:** None. **R. Patani:** None. **H. Houlden:** None.

Nanosymposium

015. Olfaction: Sensation and Second-Order Representation

Location: SDCC 30B

Time: Saturday, November 12, 2016, 1:00 PM - 4:00 PM

Presentation Number: 15.01

Topic: D.04. Olfaction and Taste

Support: Marie Curie CIG 334341

Israel Science Foundation 816/14

Title: Concentration change detection in the olfactory bulb

Authors: A. PARABUCKI[1], A. BIZER[1], G. MORRIS[1], M. SMEAR[2], *R. SHUSTERMAN[2,1]; [1]Univ. of Haifa, Haifa, Israel; [2]Univ. of Oregon, Eugene, OR

Abstract: Olfactory navigation requires comparing of, odor concentration across samples distributed in space and time. One strategy of localizing an odor source is to compute the difference in odor concentrations between nostrils - spatial (stereo) sampling. Another mechanism of odor source localization is sequential (temporal) sampling: the animal chooses direction in the olfactory surroundings by detecting concentration changes across consecutive inhalations (hereafter referred to as ΔC_t). While the role of the spatial strategy in olfactory navigation has been studied in various species, mechanisms of ΔC_t perception remain obscure. To study the neural substrate of ΔC_t perception, we developed an odor delivery system that allows rapid switching and stabilization of different concentrations of an odor, such that concentration can be changed on each sniff. We monitored activity of mitral/tufted (M/T) cells in the olfactory bulb of mice in response to prolonged concentration presentations and to stimuli that flicker between concentrations from sniff to sniff (ΔC_t stimuli). We find that a subset of M/T responses are tuned for ΔC_t, giving large modulations of firing rate when the concentration changes.
Our results indicate that M/T cells explicitly compute ΔC_t, providing a signal that may guide navigational decisions in downstream olfactory circuits.

Disclosures: **A. Parabucki:** None. **A. Bizer:** None. **G. Morris:** None. **M. Smear:** None. **R. Shusterman:** None.

Nanosymposium

015. Olfaction: Sensation and Second-Order Representation

Location: SDCC 30B

Time: Saturday, November 12, 2016, 1:00 PM - 4:00 PM

Presentation Number: 15.02

Topic: D.04. Olfaction and Taste

Support: MS: DFG SCHM2474/1-1 (SPP 1392)

MS: DFG SCHM2474/1-2 (SPP 1392)

MS: EU FP7 PEOPLE - Marie Curie IEF (no. 331892)

HS: SP1134/1-1

HS: SP1134/2-1 (SPP 1392)

HS: FOR 643

Title: Glomerulus-centric exploration of the chemical map in the olfactory bulb

Authors: *M. SCHMUKER[1,2], J. SOELTER[2], J. SCHUMACHER[3], H. SPORS[4,3];
[1]Univ. of Sussex, Brighton, United Kingdom; [2]Dept. of Biology, Chemistry, Pharm., Freie Univ. Berlin, Berlin, Germany; [3]Max-Planck Inst. for Biophysics, Frankfurt am Main, Germany; [4]Dept. of Neuropediatrics, Justus-Liebig-University, Giessen, Germany

Abstract: In spite its essential role in everyday life, Olfaction still is probably the most obscure of our senses. While we have largely understood how primary sensory neurons in e.g. vision encode their relevant input space, the chemical receptive range of many olfactory receptors still remains elusive. We profiled the receptive range of a genetically labelled olfactory receptor (MOR18-2) using intrinsic signal imaging in anaesthesized mice. We measured its response to 214 odorants in a total of 41 animals. Using advanced image processing methods [1] we extracted the responses of neighboring glomeruli. Based on their responses to a set of 45 diagnostic odors we identified glomeruli across individuals, enabling to relate their chemical receptive ranges with their spatial position relative to MOR18-2 (Figure 1). We found that MOR18-2 is embedded in a local tunotopic response domain that shares several ligands with spatially proximal glomeruli. Furthermore, we derived a description of MOR18-2's chemical receptive range in terms of physico-chemical properties. With regard to those properties we found a weak chemotopic embedding of MOR18-2 in a lateral-posterior domain of the dorsal olfactory bulb. Our findings provide insight how the arrangement of glomeruli in the olfactory bulb reflects the structure of chemical space.

References

[1] Soelter, J., Schumacher, J., Spors, H., and Schmuker, M. (2014). Automatic segmentation of odor maps in the mouse olfactory bulb using regularized non-negative matrix factorization. *Neuroimage* 98, 279-288. doi:10.1016/j.neuroimage.2014.04.041.

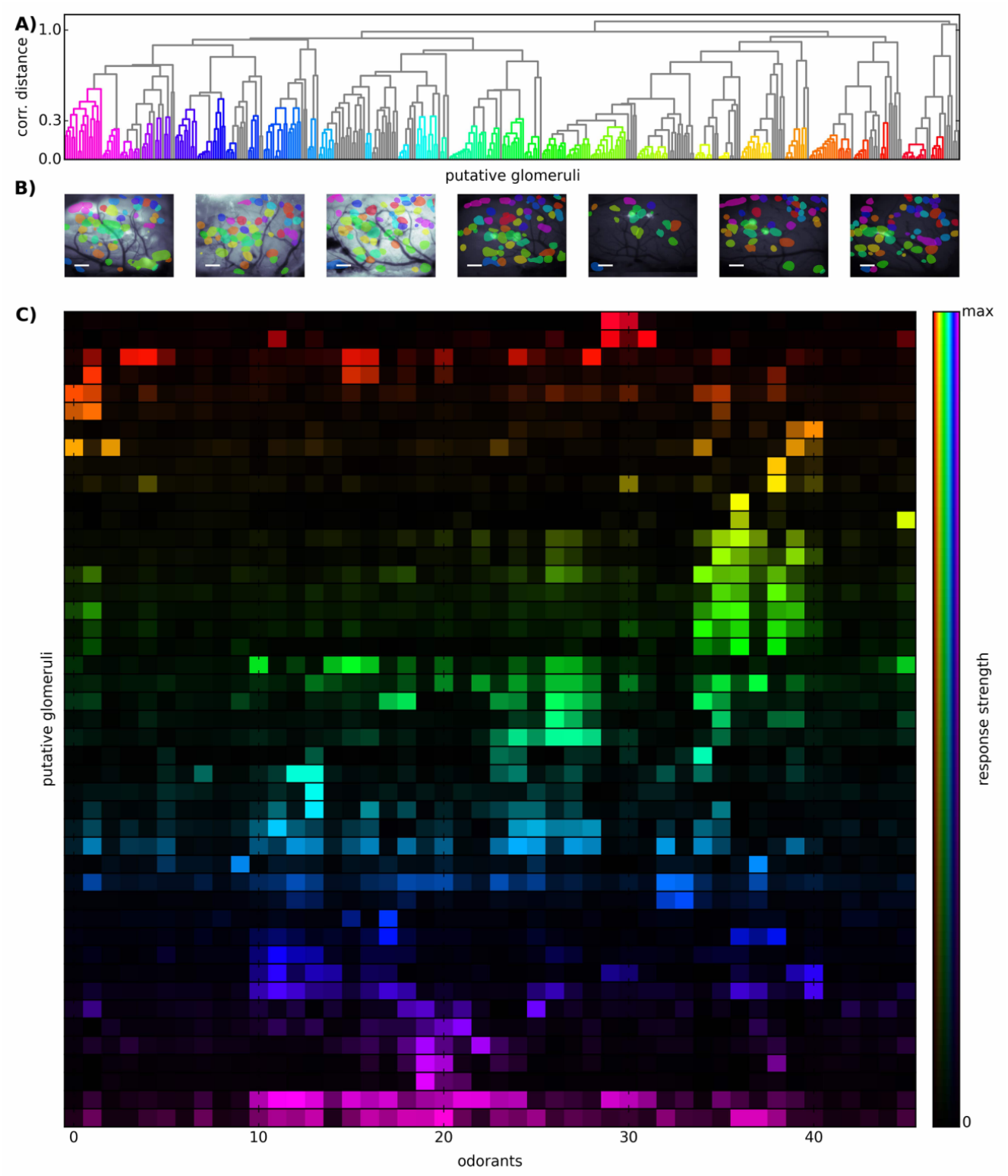

Figure 1: Response Clustering. A) Hierarchical clustering of putative glomeruli of seven mice based on the correlation between their odorant response spectra. B) Spatial location of cluster

members in the olfactory bulbs of all seven mice. Colours according to cluster colouring in A). Scale bar 100 μm. C) Median odorant spectra of all putative glomeruli in each cluster.

Disclosures: **M. Schmuker:** None. **J. Soelter:** None. **J. Schumacher:** None. **H. Spors:** None.

Nanosymposium

015. Olfaction: Sensation and Second-Order Representation

Location: SDCC 30B

Time: Saturday, November 12, 2016, 1:00 PM - 4:00 PM

Presentation Number: 15.03

Topic: D.04. Olfaction and Taste

Support: NIH R00 DC011780

NIH T32-DA07290

University of Texas Southwestern Medical Center

Title: Arc-transcribing accessory olfactory bulb internal granule cells increase their excitability through intrinsic mechanisms following intermale aggression

Authors: *H. L. CANSLER, M. A. MAKSIMOVA, J. P. MEEKS;
Dept. of Neurosci., Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Rodents rely on their olfactory systems for survival and reproduction. The accessory olfactory system (AOS) is particularly important for social behaviors, including territorial aggression. The accessory olfactory bulb (AOB) is the first and only dedicated circuit for processing AOS sensory information, and acts as a gateway for AOS-mediated behaviors. Though physiological and behavioral evidence suggests that plastic changes occur in the AOB following salient behavioral events, these changes are not well understood on a cellular level. We investigated AOB plasticity following intermale territorial aggression by examining expression of the immediate early gene *Arc*, which has been implicated in diverse forms of plasticity throughout the brain. We allowed adult male transgenic mice that express GFP in *Arc*-transcribing cells (*Arc*-d4EGFP-BAC mice) to interact for 10 minutes in a "resident-intruder" assay, and found significant GFP labeling in a subset of AOB neurons following behavior. The vast majority of these *Arc*-transcribing cells were internal granule cells (IGCs), interneurons that inhibit projecting mitral cells (MCs) via reciprocal dendro-dendritic synapses. In acute AOB slices from *Arc*-d4EGFP-BAC mice, we found that *Arc*-transcribing IGCs demonstrated an increased response to simulated sensory input, suggesting that *Arc*-transcribing cells are strongly connected to MCs and well-situated to perform MC inhibition. Using optogenetics, we confirmed that *Arc*-transcribing IGCs synaptically inhibit MCs, and suppress MC firing.

Together, these results suggest that *Arc*-transcribing IGCs have an increased ability to inhibit MCs. In order to determine whether increased IGC activation was the result of strengthened excitatory synaptic drive, we measured IGC synaptic function and dendritic spine density. We found no differences in either spontaneous EPSC or miniature EPSC amplitude/frequency, nor did we find a difference in spine density, suggesting that the increased excitability of IGCs was not due to increases in excitatory synapse strength or number. To assess IGC intrinsic properties, we used an unbiased electrophysiological assay involving 26 distinct parameters. We found that *Arc*-transcribing IGCs exhibit a fast-spiking phenotype when depolarized by a current injection and showed significantly reduced I_h currents compared to controls. These results suggest that following salient social encounters, *Arc*-transcribing IGCs increase their excitability through intrinsic but not synaptic mechanisms, resulting in an increased capacity to inhibit MCs.

Disclosures: H.L. Cansler: None. **M.A. Maksimova:** None. **J.P. Meeks:** None.

Nanosymposium

015. Olfaction: Sensation and Second-Order Representation

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Presentation Number: 15.04

Topic: D.04. Olfaction and Taste

Support: McDonnell Foundation

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NEI Grants P30 EY019005

T32 EY020503

Title: A robust feedforward model of the olfactory system

Authors: *Y. ZHANG, T. SHARPEE;
Physics, UCSD & Salk, La Jolla, CA

Abstract: Most natural odors have sparse molecular composition. This makes the principles of compressed sensing potentially relevant to the structure of the olfactory code. Yet, the largely feedforward organization of the olfactory system precludes reconstruction using standard compressed sensing algorithms. To resolve this problem, recent theoretical work has shown that signal reconstruction could take place as a result of a low dimensional dynamical system

converging to one of its attractor states. However, the dynamical aspects of optimization slowed down odor recognition and were also found to be susceptible to noise. Here we describe a feedforward model of the olfactory system that achieves both strong compression and fast reconstruction that is also robust to noise. A key feature of the proposed model is a specific relationship between how odors are represented at the glomeruli stage, which corresponds to a compression, and the connections from glomeruli to third-order neurons (neurons in the olfactory cortex of vertebrates or Kenyon cells in the mushroom body of insects), which in the model corresponds to reconstruction. We show that should this specific relationship hold true, the reconstruction will be both fast and robust to noise, and in particular to the false activation of glomeruli. We also show that the predicted connectivity rate is optimal without the reconstruction assumption.

Disclosures: **Y. Zhang:** None. **T. Sharpee:** None.

Nanosymposium

015. Olfaction: Sensation and Second-Order Representation

Location: SDCC 30B

Time: Saturday, November 12, 2016, 1:00 PM - 4:00 PM

Presentation Number: 15.05

Topic: D.04. Olfaction and Taste

Support: JST PRESTO

JSPS KAKENHI

Title: Sampling mode- and concentration-invariant temporal odor coding by airflow-driven neuronal oscillations

Authors: ***T. IMAI**, R. IWATA;
RIKEN CDB, Kobe, Hyogo, Japan

Abstract: Sensory information is represented not only as firing rate (rate coding), but also as temporal patterns (temporal coding) of activity in neurons. In the rodent olfactory bulb, odors produce rich temporal patterns of activity in mitral/tufted cells; however, roles of the temporal patterns remain enigmatic. Here we show that the temporal coding distinguishes two sensory modalities detected by input sensory neurons: odor and airflow-driven mechanical signals. Odor stimuli, but not changes in airflow speed, produced glomerulus-specific phase shifts in sniff-coupled oscillatory activities. We also found that the odor-evoked phase shifts are invariant across a wide range of odor concentrations and sniff cycles, contrary to the labile nature of the rate coding. The loss of airflow-driven oscillations impaired stability of the temporal coding,

demonstrating a role of mechanosensation in olfaction. We propose that the phase coding is a robust encoding strategy for an odor identity under fluctuating sampling and environmental conditions.

Disclosures: **T. Imai:** None. **R. Iwata:** None.

Nanosymposium

015. Olfaction: Sensation and Second-Order Representation

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Support: NIH Grant R00DC011780

NIH Grant T32NS069562

University of Texas System Neuroscience and Neurotechnology Institute

University of Texas Southwestern Medical Center

Title: Fecal bile acids are potent activators of the accessory olfactory system

Authors: *W. I. DOYLE[1], J. A. DINSER[2], H. L. CANSLER[1], X. ZHANG[1], D. D. DINH[1], N. S. BROWDER[1], I. M. RIDDINGTON[2], J. P. MEEKS[1];
[1]Dept. of Neurosci., Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [2]Dept. of Chem., Univ. of Texas at Austin, Austin, TX

Abstract: Chemosensory processing by the accessory olfactory system (AOS) is crucial for the survival and reproductive success of rodents. Despite decades of research there are only a few known ligands for this system. The major known sources of AOS chemosignals are urine and tears, but we hypothesized that other animal excretions, such as feces, may also contain AOS ligands. We investigated whether feces are a source of AOS ligands using electrophysiological recordings of accessory olfactory bulb mitral cells (AOB MCs). We performed these experiments in AOS *ex vivo* preparations that allow one to record from the AOB while candidate ligands are delivered to peripheral sensory neurons in the vomeronasal organ (VNO). We found that aqueous extracts of BALB/cJ female mouse feces produce widespread activation of MCs in the anterior AOB (aAOB), which is selectively innervated by peripheral neurons expressing members of the V1R subfamily of vomeronasal receptors. The activity elicited in the aAOB by mouse feces was nearly equivalent to the activity elicited by female mouse urine, currently the best-studied source of AOS chemosignals. We used liquid chromatography-mass spectrometry

(LC-MS) to identify candidate chemosignals in BALB/cJ female mouse feces extracts, and found that these extracts were rich in bile acids. Bile acids are produced in the liver and secreted into the gut, where they are critical for fat absorption and play additional roles in energy metabolism. Electrophysiological recordings confirmed that many AOB MCs activated by VNO stimulation with BALB/cJ fecal extracts also responded to pure bile acids, including cholic acid and deoxycholic acid, at 10 μM. AOB MCs demonstrated sex selectivity for feces, and LC-MS revealed that this difference was at least partially explained by the presence of chenodeoxycholic acid in male but not female mouse feces. We also recorded from other AOB MCs that responded selectively to lithocholic acid, a bile acid absent from rodent feces but present in heterospecific feces, including mouse predators. These results reveal that feces are a potent source of AOS cues and that bile acids are a novel class of AOS ligands. Bile acids are thus capable of providing mice with chemosensory information about important biological variables, including sex, species and gut flora.

Disclosures: **W.I. Doyle:** None. **J.A. Dinser:** None. **H.L. Cansler:** None. **X. Zhang:** None. **D.D. Dinh:** None. **N.S. Browder:** None. **I.M. Riddington:** None. **J.P. Meeks:** None.

Nanosymposium

015. Olfaction: Sensation and Second-Order Representation

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Time: Saturday, November 12, 2016, 1:00 PM - 4:00 PM

Presentation Number: 15.07

Topic: D.04. Olfaction and Taste

Support: NIH Grant DC014788

Title: Odor detection by newly generated mouse olfactory sensory neurons *In vivo*

Authors: *C. E. CHEETHAM;
Dept. of Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Olfactory sensory neurons (OSNs), the sole source of sensory input to the mammalian main olfactory bulb (OB), are generated throughout life from stem cells in the olfactory epithelium. Each mature OSN expresses a single odorant receptor, and axons expressing the same receptor converge to form a pair of glomeruli on the surface of the OB, generating a highly organized odor map. As it matures, each newly generated OSN therefore faces the problem of how to integrate into a highly organized neural circuit without disrupting existing function. We have shown recently that OSNs form synapses and can evoke robust stimulus-locked firing of OB neurons while still expressing immature markers. Furthermore, recent studies have shown that immature OSNs already express odorant receptors. This raises the question of when newly

generated OSNs begin to play a functional role in olfaction.
Here, I investigated the ability of immature OSNs to detect odors *in vivo*. I implanted cranial windows over the OB of 3 week-old mice expressing GCaMP6s in either immature (Gγ8-GCaMP6s) or mature (OMP-GCaMP6s) OSNs, and monitored odor-evoked responses in OSN axons *in vivo*. 84% of glomeruli innervated by immature OSN axons responded to at least one odor in a 7-odor panel (n = 133 glomeruli, 7 mice), demonstrating that immature OSNs can detect odors *in vivo*. I then compared the responses of immature OSN axons to those of mature OSN axons (n = 140 glomeruli, 7 mice). Fewer glomeruli in Gγ8-GCaMP6s mice than in OMP-GCaMP6s mice responded to each individual odor ($P < 0.001$, paired t-test). Furthermore, across the odor panel, odor responses were smaller in magnitude in Gγ8-GCaMP6s mice ($\Delta F/F$ [mean ± SD] 48 ± 16 %) than in OMP-GCaMP6s mice ($\Delta F/F$ 81 ± 23 %; $P < 0.001$, 2-way ANOVA).
If immature OSNs providing input to the OB have not yet completed odorant receptor selection, they could disrupt odor coding. To investigate this, I performed three additional analyses of odor selectivity. First, glomeruli in Gγ8-GCaMP6s mice responded to fewer odors (3.8 ± 0.2) than glomeruli in OMP-GCaMP6s mice (4.9 ± 0.2; $P = 0.003$, t-test). Second, a smaller proportion of glomeruli responded to all 7 odors in Gγ8-GCaMP6s mice (38 %) than in OMP-GCaMP6s mice (62 %, $P < 0.001$, χ^2 test). Finally, odor preference, defined as the quotient of the responses evoked by the first- and second-most preferred odors for each glomerulus, was very similar in Gγ8-GCaMP6s mice (1.30 ± 0.05) and OMP-GCaMP6s mice (1.32 ± 0.04; $P = 0.70$, t-test). Therefore, I found no evidence that sensory input from immature OSNs disrupts odor coding. Together with other recent studies, these findings suggest that immature OSNs may play a previously unappreciated role in olfaction.

Disclosures: C.E. Cheetham: None.

Nanosymposium

015. Olfaction: Sensation and Second-Order Representation

Location: SDCC 30B

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Presentation Number: 15.08

Topic: D.04. Olfaction and Taste

Support: FAPESP 09/00473-0

VRERI - UNICAMP 26/2016

Title: Hormonal modulation of pup odor detection by the vomeronasal system in mice.

Authors: *F. PAPES, T. S. NAKAHARA, P. H. M. NETTO;
Dept. Genet. and Evolution, Univ. of Campinas (UNICAMP), Campinas, Brazil

Abstract: Olfaction is a fundamental sense through which most animals respond to the environment. Olfactory organs, such as the main olfactory epithelium and the vomeronasal organ, possess sensory neurons that express specialized receptors tuned to detect odorous stimuli. We recently described a novel subpopulation of olfactory sensory neurons in the vomeronasal organ that non-canonically expresses receptors in the odorant receptor (OR) family, and showed that these cells are able to detect pheromones emanating from pups. Moreover, we obtained evidence that activation of such cells is dependent on the animal's socio-sexual status. In this study, we investigated how the organism's internal hormonal state modulates activity in these neurons, thereby changing how the animal detects pup odors and consequentially the display of pup-oriented behaviors.

Disclosures: **F. Papes:** None. **T.S. Nakahara:** None. **P.H.M. Netto:** None.

Nanosymposium

015. Olfaction: Sensation and Second-Order Representation

Location: SDCC 30B

Time: Saturday, November 12, 2016, 1:00 PM - 4:00 PM

Presentation Number: 15.09

Topic: D.04. Olfaction and Taste

Support: Pew Biomedical Science Scholars Program

NIH Grant DC013779

Title: Acetylcholine rapidly enhances habituated olfactory bulb odor responses and modulates odor salience

Authors: ***M. C. OGG**, M. BENDAHMANE, M. L. FLETCHER;
Anat. and Neurobio., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: The olfactory bulb (OB) receives cholinergic input from the basal forebrain (BF) and expresses a variety of cholinergic receptors. Recent work from our lab has shown that OB acetylcholine (ACh) increases individual glomerular sensitivity to odors and decreases glomerular activation thresholds. These ACh effects could enhance OB responsivity to weak or degraded input under certain conditions. We are investigating the role of cholinergic-mediated gain control in olfactory habituation at both the physiological and behavioral level.
To measure the physiological effects of cholinergic modulation, we imaged in anesthetized transgenic mice expressing calcium indicators in input (olfactory sensory neurons; OSNs) and output (mitral/tufted cells) cells. We hypothesized that OB ACh release could reinstate reduced OB odor responses after OSN adaptation, a process known as dishabituation. We find that BF

electrical stimulation following prolonged odor presentations dishabituates OB odor responses to near baseline levels and are currently investigating whether BFS *during* prolonged odor presentations has the same effect.
To measure the behavioral effects of cholinergic modulation, we recorded odor investigation behavior in an open field chamber during prolonged odor exposure in mice expressing channel rhodopsin (ChR2) in cholinergic neurons and their wildtype littermates. We find that optogenetic OB ACh release leads to increased investigation of habituated odorants, an effect not seen in wildtype littermates. Using implanted thermistors to detect respiration, we are currently investigating whether optogenetic OB ACh release leads to changes in sniff frequency associated with detection of salient odorants. Preliminary data suggest that changing the visual context of the open field also leads to increased investigation of habituated odorants. Future experiments aim to block OB ACh release during the visual stimulation to see if the effect on odor investigation is cholinergically mediated.
Overall, these studies establish a novel functional role for olfactory ACh release, whereby odor representation and salience can be rapidly and dynamically modulated.

Disclosures: **M.C. Ogg:** None. **M. Bendahmane:** None. **M.L. Fletcher:** None.

Nanosymposium

015. Olfaction: Sensation and Second-Order Representation

Location: SDCC 30B

Time: Saturday, November 12, 2016, 1:00 PM - 4:00 PM

Presentation Number: 15.10

Topic: D.04. Olfaction and Taste

Support: NIH Grant DC009413

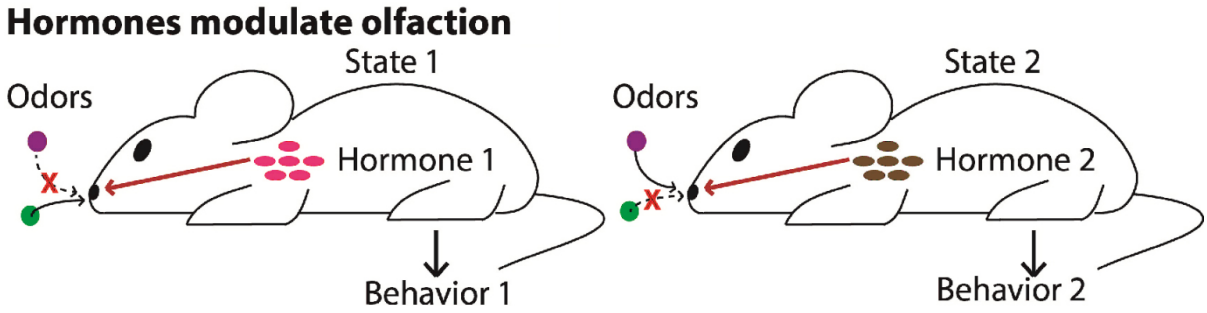
NIH Grant DC006885

Title: Hormonal control of olfactory sensitivity alters behavior

Authors: ***S. DEY**[1], L. STOWERS[2];
[2]Mol. and Cell. Neurosci., [1]The Scripps Res. Inst., La Jolla, CA

Abstract: An individual's response to a sensory cue can vary widely. Differences in the internal state often account for such behavioral fluctuations. Hormones are thought to be causative but the neural targets of endocrine action and the mechanisms that alter their activities are not fully understood. In order to understand how internal state affects behavior, it is essential to identify these neurons and how they are affected. We have shown for the first time, female reproductive hormones in diestrus (non ovulatory state of the ovulation cycle) directly silence peripheral

sensory neurons (vomeronasal neurons, VSNs). This attenuates detection of attraction pheromones in male urine and prevents the female from eliciting social behavior (Dey et al, 2016). We now show diestrus also increases sensitivity of some VSNs to a different group of male urinary odors compared to the estrus (ovulatory) phase. Behaviorally, all estrus females robustly investigate these odors whereas diestrus females show variable degrees of aversion. Calcium imaging reveals progesterone (the major diestrus hormone), acts rapidly through a non classical receptor to sensitize the VSNs. We are using transcriptome profiling, genetics and biochemistry to identify key signaling molecules in this subset of VSNs, activated by progesterone. These instances of synchronized suppression and sensitization suggest that VSNs are capable of extreme flexibility. Using this simple strategy, the olfactory system can generate practically unlimited and distinct neural codes to represent odor mixes (like male urine) uniquely in different internal states. This places the olfactory system to control state specific behaviors in a way that no other peripheral system is able to. The results of this study will reveal how synchronized modification of peripheral sensitivity affects overall perception of odor mixtures in different states.



Disclosures: **S. Dey:** None. **L. Stowers:** None.

Nanosymposium

015. Olfaction: Sensation and Second-Order Representation

Location: SDCC 30B

Time: Saturday, November 12, 2016, 1:00 PM - 4:00 PM

Presentation Number: 15.11

Topic: D.04. Olfaction and Taste

Support: NIH Grant R011DC011558

Title: A family of non-GPCR chemosensors defines an alternative logic for mammalian olfaction

Authors: *P. L. GREER[1], D. BEAR[1], J.-M. LASSANCE[3], M. BLOOM[4], T. TSUKAHARA[1], S. PASHKOVSKI[1], F. MASUDA[1], A. NOWLAN[1], R. KIRCHNER[5], H. HOKSTRA[2], S. R. DATTA[1];
[1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Dept. of Mol. and Cell. Biol., Harvard Med. Sch., Cambridge, MA; [3]Mol. and Cell. Biol., Harvard Univ., Cambridge, MA; [4]HMS, Boston, MA; [5]Biostatistics, Harvard Sch. of Publ. Hlth., Boston, MA

Abstract: Odor perception in mammals is mediated by parallel sensory pathways that convey distinct information about the olfactory world. Multiple olfactory subsystems express characteristic seven-transmembrane G-protein coupled receptors (GPCRs) in a one-receptor-per-neuron pattern that facilitates odor discrimination. Sensory neurons of the “necklace” subsystem are nestled within the recesses of the olfactory epithelium and detect diverse odorants; however, they do not express known GPCR odor receptors. Here we report that members of the four-pass transmembrane MS4A protein family are chemosensors expressed within necklace sensory neurons. These receptors localize to sensory endings and confer responses to ethologically-relevant ligands including pheromones and fatty acids in vitro and in vivo. Individual necklace neurons co-express many MS4A proteins and are activated by multiple MS4A ligands; this pooling of information suggests that the necklace is organized more like subsystems for taste than for smell. The MS4As therefore define a distinct mechanism and functional logic for mammalian olfaction.

Disclosures: **P.L. Greer:** None. **D. Bear:** None. **J. Lassance:** None. **M. Bloom:** None. **T. Tsukahara:** None. **S. Pashkovski:** None. **F. Masuda:** None. **A. Nowlan:** None. **R. Kirchner:** None. **H. Hokstra:** None. **S.R. Datta:** None.

Nanosymposium

015. Olfaction: Sensation and Second-Order Representation

Location: SDCC 30B

Time: Saturday, November 12, 2016, 1:00 PM - 4:00 PM

Presentation Number: 15.12

Topic: D.04. Olfaction and Taste

Support: Prestamo BID PICT 2013-2474

Title: Role of inhibition in the antennal lobe: gain control and odor invariance across concentrations

Authors: *E. MARACHLIAN[1,2,3], A. NALLY[2], R. HUERTA[4], F. LOCATELLI[1,2];
[1]IFIByNE CONICET-University of Buenos Aires, Buenos Aires, Argentina; [2]Dept. de

Fisiología, Biología Mol. y Celular, FCEyN , UBA, Buenos Aires, Argentina; [3]Dept. de Física, FCEyN , UBA, Buenos Aires, Argentina; [4]Biocircuits Inst., UCSD, San Diego, CA

Abstract: Animals need to extract information from complex and noisy environments. Even though the animals form their internal image of the surroundings through a multisensory system, each sense needs to be able to recognize (in some range) the identity of one stimulus regardless of its intensity and independently of complex or noisy background (if the stimulus is biologically relevant). Therefore the first processing level, where the sensory signal is adapted and organized, plays a fundamental role in sensory systems. In the olfactory system the chemical cues are detected and primarily encoded by the olfactory sensory neurons. Each specific odor recruits a particular combination of receptors that provides the input for its internal representation. The great neuronal convergence in the Antennal Lobe (approximately 60,000 olfactory sensory neurons converge into around 800 projection neurons organized in 160 glomeruli in the honey bee) requires a signal adaptation. In insects the first processing is done by the local inhibitory neurons located in the Antennal Lobe. Nevertheless the specific form in which the signal is adapted is not fully known. In this work we investigate the action of the different GABA components and their role in gain control and stabilization of neural activity patterns elicited by a range of odor concentrations. We use honey bees as model animal and perform calcium imaging to measure the neural representations from low to high odor concentrations in the projection neurons of the Antennal Lobe. With different GABA blockers we isolate and identify the specific contributions of GABA-A and GABA-B components. We also introduce math and computational models with which we confirm experiment interpretations, study the network contribution and evaluate the robustness of this mechanism for different input patterns. We show that the GABAergic neurons play an important role, stabilizing the pattern representation and regulating levels of activity, allowing animals to generalize the odor identity across concentrations. The simplicity of the system under study suggests that analogous versions could be involved in other sensory systems and even in higher levels of processing.

Disclosures: **E. Marachlian:** None. **A. Nally:** None. **R. Huerta:** None. **F. Locatelli:** None.

Nanosymposium

016. Distinct Approaches in the Study of Emotion: Integrative Informatics, Real Time fMRI, and Functional Connectivity

Location: SDCC 2

Time: Saturday, November 12, 2016, 1:00 PM - 3:30 PM

Presentation Number: 16.01

Topic: G.03. Emotion

Support: NIH R01DA035484-02S1

Neukom Institute for Computational Science

Title: Crowd sourced development and validation of neurocomputational models of social and affective processes

Authors: *L. CHANG[1], A. BURNASHEV[1], T. WAGER[2];
[1]Dartmouth Col., Hanover, NH; [2]Univ. of Colorado Boulder, Boulder, CO

Abstract: Objective biomarkers of pathology exist for a number of diseases, and play a critical role in their diagnosis and treatment. However, assessment of affective processes related to mental health disorders lags far behind and currently relies on self-report. Direct measures of brain function provide a promising area for developing biomarkers of emotion pathophysiology and recent advances in combining neuroimaging with machine learning techniques have brought the goal of brain-based assessment of affect within reach. For example, we have demonstrated that fMRI activity can predict whether an individual person is experiencing high or low emotional responses to arousing pictures with over 90% accuracy. Critically, this biomarker is sensitive and specific to emotional responses, when compared with other salient and arousing affective events such as thermal pain. This preliminary success raises a number of issues that must be addressed before fMRI-based biomarkers can be used clinically, such as demonstrating: a) robustness across laboratories and procedures, b) specificity to type of emotion and elicitation method, and c) applicability to clinical populations. However, data sharing is currently in its infancy and there are no easy-to-use software packages publically available to facilitate this process. Here we introduce http://neuro-learn.org - an open-source web application that provides machine-learning analysis tools that integrate with online data repositories. Neuro-learn accesses data stored in Neurovault.org and allows users to enter metadata and train predictive brain models using linear machine-learning techniques and cross-validation. These models can be shared with the research community and validated on any data stored in Neurovault.org. A critical innovation is that neuro-learn.org relies on the collective intelligence of users to train and test any model of their choosing. We believe that this crowd sourcing technique will accelerate the development of brain based models and ultimately contribute to a more open, collaborative, and replicable brain science of affect.

Disclosures: L. Chang: None. **A. Burnashev:** None. **T. Wager:** None.

Nanosymposium

016. Distinct Approaches in the Study of Emotion: Integrative Informatics, Real Time fMRI, and Functional Connectivity

Location: SDCC 2

Time: Saturday, November 12, 2016, 1:00 PM - 3:30 PM

Presentation Number: 16.02

Topic: G.03. Emotion

Title: Evaluation of full brain parcellation schemes using the NeuroVault database of statistical maps

Authors: *K. J. GORGOLEWSKI[1], A. TAMBINI[2], J. DURNEZ[1], V. V. SOCHAT[1], J. WEXLER[1], R. A. POLDRACK[1];
[1]Dept. of Psychology, Stanford Univ., Stanford, CA; [2]UC Berkeley, Berkeley, CA

Abstract: The task of dividing the human brain into regions has been captivating scientists for many years. In the following work we revisit this challenge and introduce a new evaluation technique that works for both cortical and subcortical parcellations. Our approach is based on data from a diverse set of cognitive experiments that employs nonparametric methods to account for smoothness and parcel size biases.
As reported before parcel variance was a function of parcel size in that smaller parcels were more likely to be homogenous (even in random data). However, when we used map-specific null distributions to account for both smoothness of statistical maps as well as number of parcels in atlases, unbiased estimates become apparent. Both Yeo et al. and Collins et al. parcellations produce scores for random data similar to those derived from real data. In contrast, Shen et al., AAL, and Gordon et al. show lower within parcel variance when applied to real data than when applied to random data (but no distinction can be made between them).
In addition to looking at within parcel variance we also applied a novel metric based on the intuition that different parts of the brain should not only be homogenous, but also different from each other. To quantify this we calculated a ratio of between and within parcel variances (standardized using individual null models). This approach indirectly penalizes parcellations with too many unnecessary parcels. Using this measure we show that Yeo et al. parcellation fits data better (Figure 1) than Collins et al. atlas despite having fewer parcels (7 vs 10).
We present a novel approach to evaluating atlases and parcellations of the human brain that captures diverse patterns observed across many cognitive studies. Our testing methodology overcomes biases introduced by the size of the parcels and smoothness of input data, but also, in contrast to previous methods, can be applied to whole brain volumetric data. We have found that in contrast to previous reports based on resting state cortico cortical conn

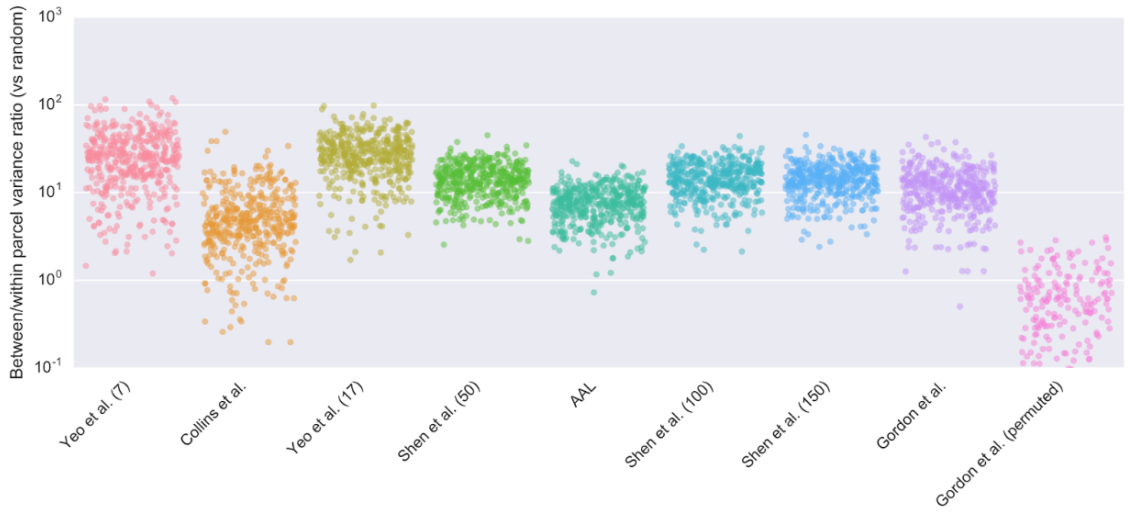


Figure 1. Comparison of parcellation schemes in terms of the ratio of within and between parcel variance - normalized by corresponding null models. Parcellations are ordered by increasing number of parcels. Each dot represents a measurement from one statistical map standardized using mean and standard deviation obtained from a null distribution of measurements based on random statistical maps matched in terms of smoothness. A between/within parcel variance ratio of zero corresponds to measurement level no different than random.

Disclosures: K.J. Gorgolewski: None. **A. Tambini:** None. **J. Durnez:** None. **V.V. Sochat:** None. **J. Wexler:** None. **R.A. Poldrack:** None.

Nanosymposium

016. Distinct Approaches in the Study of Emotion: Integrative Informatics, Real Time fMRI, and Functional Connectivity

Location: SDCC 2

Time: Saturday, November 12, 2016, 1:00 PM - 3:30 PM

Presentation Number: 16.03

Topic: G.03. Emotion

Support: R01MH096906

Title: Large-scale spatiosemantic topic modeling of the human brain

Authors: *T. YARKONI[1], T. RUBIN[2], S. KOYEJO[3], M. N. JONES[2], R. POLDRACK[3]; [1]Dept. of Psychology, Univ. of Texas at Austin, Austin, TX; [2]Indiana Univ., Bloomington, IN; [3]Stanford Univ., Stanford, CA

Abstract: Cognitive neuroscientists are in the business of understanding how neural function and mental function interrelate. One can conceptualize this enterprise partly as a search for meaningful functional-anatomical atoms, where the goal is to find a set of theoretical constructs that have relatively simple, minimally overlapping mappings onto both the biological and the psychological levels of description, thereby maximizing interpretative simplicity and minimizing model complexity. Here we introduce a novel Bayesian topic model that learns latent topics from the meta-analytic Neurosynth database of over 11,000 published fMRI studies. In contrast to previous meta-analytic approaches, this model generates topics that are simultaneously constrained by both anatomical and psychological considerations: each topic defines a single brain region that is associated with a highly interpretable, coherent set of cognitive terms. In a series of large-scale analyses that draw on data from the Human Connectome Project and the NeuroVault data repository, I demonstrate that this topic set (i) successfully captures known anatomical and functional distinctions; (ii) provides a novel data-driven metric of hemispheric specialization; (iii) enables accurate reconstruction and regularization of arbitrary whole-brain activation maps, facilitating the interpretability and stability of obtained results; and (iv) can be used to inform and even correct human experts' judgments about the psychological processes involved in performing cognitive tasks. Importantly, much of this topic modeling functionality is integrated to the NeuroVault image repository, enabling researchers to upload and topically reconstruct and interpret their own whole-brain activation maps within seconds.

Disclosures: T. Yarkoni: None. **T. Rubin:** None. **S. Koyejo:** None. **M.N. Jones:** None. **R. Poldrack:** None.

Nanosymposium

016. Distinct Approaches in the Study of Emotion: Integrative Informatics, Real Time fMRI, and Functional Connectivity

Location: SDCC 2

Time: Saturday, November 12, 2016, 1:00 PM - 3:30 PM

Presentation Number: 16.04

Topic: G.03. Emotion

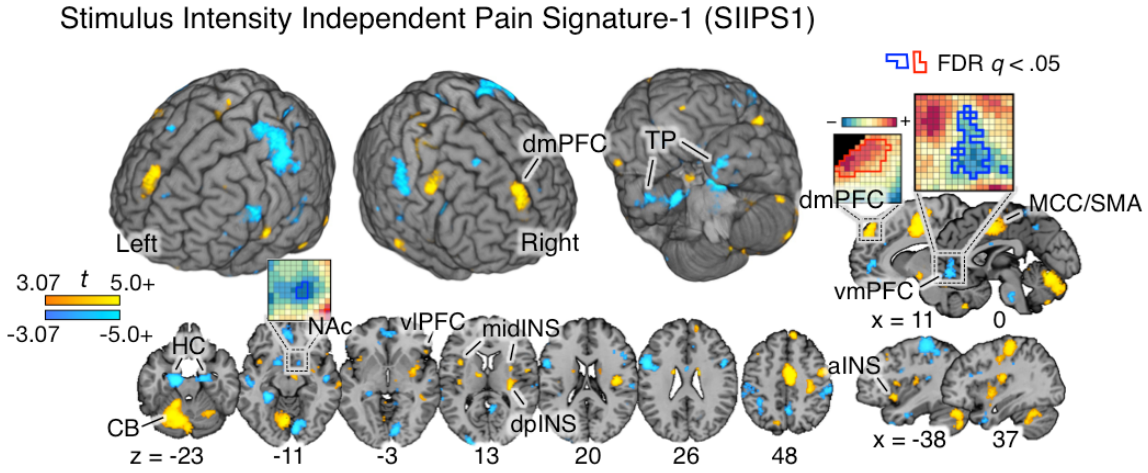
Support: NIDA R01 DA027794

NIMH R01 MH076136

Title: Quantifying cerebral contributions to pain beyond nociception: A mega-analytic approach

Authors: *C.-W. WOO[1], L. SCHMIDT[2], A. KRISHNAN[3], M. JEPMA[4], M. ROY[5], M. LINDQUIST[6], L. ATLAS[7], T. WAGER[1];
[1]Univ. of Colorado Boulder, Boulder, CO; [2]Ecole Normale Supérieure, Paris, France; [3]Brooklyn Col. of the City Univ. of New York, Brooklyn, NY; [4]Leiden Univ., Leiden, Netherlands; [5]Concordia Univ., Montreal, QC, Canada; [6]Johns Hopkins Univ., Baltimore, MD; [7]NIH, Bethesda, MD

Abstract: Cerebral processes contribute to pain beyond the level of nociceptive input and mediate psychological and behavioral influences. However, cerebral effects on pain are not yet well characterized, leading to a predominant focus on brain activity related to nociceptive input when studying pain and developing interventions. Here, we analyzed functional Magnetic Resonance Imaging (fMRI) data from 6 independent studies that involved thermal pain stimulation ($N = 183$) with machine learning techniques. Based on the training datasets (Studies 1-4, $N = 137$), we first developed a multivariate pattern signature—termed the Stimulus Intensity Independent Pain Signature-1 (SIIPS1)—that predicted pain above and beyond nociceptive input. The SIIPS1 included patterns of activity in nucleus accumbens, lateral prefrontal, parahippocampal, and other regions. We then prospectively tested the new signature on 2 independent test datasets (Studies 5-6, $N = 46$), and showed that SIIPS1 responses explained variation in trial-by-trial pain ratings not reflected in a previous fMRI-based marker for nociceptive pain. In addition, SIIPS1 responses mediated the pain-modulating effects of three psychological manipulations of expectation and perceived control. Overall, the SIIPS1 provides a generalizable and extensible fMRI signature for cerebral contributions to pain beyond nociception, and has implications for a new large-scale approach to modeling neural representations of pain and emotion.



Disclosures: **C. Woo:** None. **L. Schmidt:** None. **A. Krishnan:** None. **M. Jepma:** None. **M. Roy:** None. **M. Lindquist:** None. **L. Atlas:** None. **T. Wager:** None.

Nanosymposium

016. Distinct Approaches in the Study of Emotion: Integrative Informatics, Real Time fMRI, and Functional Connectivity

Location: SDCC 2

Time: Saturday, November 12, 2016, 1:00 PM - 3:30 PM

Presentation Number: 16.05

Topic: G.03. Emotion

Support: NIH R01MH096906

Title: Bridging psychology and genetics using large-scale spatial analysis of neuroimaging and neurogenetic data

Authors: *A. S. FOX[1], L. J. CHANG[2], K. J. GORGOLEWSKI[3], T. YARKONI[4];
[1]Psychology, Univ. of California - Davis, Madison, WI; [2]Dartmouth Col., Hanover, NH;
[3]Psychology, Stanford, Palo Alto, CA; [4]Univ. of Texas - Austin, Austin, TX

Abstract: Despite the great advances in genomics and cognitive science, there is a large gap between our molecular knowledge of the brain and the affective and cognitive processes that result. The countless hypothetical molecule-cognition relationships necessitate discovery-based techniques to guide scientists toward the most productive lines of investigation. Here, I present a novel discovery tool combining spatial patterns of neural gene expression from the Allen Institute for Brain Science (AIBS) and large-scale functional neuroimaging meta-analyses from the Neurosynth framework. The spatial similarity between over 20,000 genes from the AIBS and 48 psychological topics derived from lexical analysis of neuroimaging articles, was quantified to produce a comprehensive set of gene/cognition mappings termed the Neurosynth-gene atlas. To validate this approach, analyses tested the extent to which this approach effectively "re-discovered" well-known neurotransmitter-cognition associations, such as dopamine and reward. Permutation tests revealed support for 12/16 known associations (p's<.05). For example, the DRD gene family was associated with the Reward compared to all other topics (p=0.0004). The atlas was then used to identify a range of novel associations between individual molecules or genes and complex psychological phenomena. To demonstrate the utility of this approach, the genes associated with the Anxious Traits topic were compared to a large-scale neuroticism GWAS (n>60,000). Results demonstrated that the top 100 Anxious Traits associations were more likely to be significantly associated with neuroticism (p<.032). Based on these results we predict the genes that are implicated in the neuroticism GWAS and expressed in brain areas associated with the Anxious Traits topic (e.g. HRH1, and PTGER3) to be most likely to underlie anxiety. To test this hypothesis, used a well-validated nonhuman primate model of early-life anxiety to test the extent to which the expression of target genes within the anxiety-related dorsal amygdala region was associated with individual differences in threat-induced freezing behavior (n=47). Permutation tests demonstrated that being expressed in Anxious Traits-associated

regions increased the likelihood of observing a relationship with anxiety in the nonhuman primate above and beyond what could be predicted with the neuroticism GWAS alone (p=.0001). These results demonstrate that the Neurosynth-gene approach complements existing discovery-based methods such as GWAS, and provide a novel means of generating hypotheses about the neurogenetic substrates of complex cognitive functions.

Disclosures: **A.S. Fox:** None. **L.J. Chang:** None. **K.J. Gorgolewski:** None. **T. Yarkoni:** None.

Nanosymposium

016. Distinct Approaches in the Study of Emotion: Integrative Informatics, Real Time fMRI, and Functional Connectivity

Location: SDCC 2

Time: Saturday, November 12, 2016, 1:00 PM - 3:30 PM

Presentation Number: 16.06

Topic: H.02. Human Cognition and Behavior

Support: Williams Syndrome Association

Title: Atomic neuroscience, information processing and a candidate nexus for consciousness

Authors: *E. L. OHAYON, A. LAM;
The Green Neurosci. Lab., Neurolinx Res. Inst., San Diego, CA

Abstract: One of the most intractable problems in neuroscience is explaining the connection between conscious experience and the underlying neural correlates. Much of the focus has been at the molecular and structural level, and although these are clearly critical they are also contingent and so fail to address the most foundational aspects of the question. Other approaches ranging from quantum-level explanations and a range of schools in philosophy of mind have also attempted to identify mechanisms but fail to address the challenge of offering specific candidates that are both theoretically sound and empirically testable. Here we focus on an often overlooked level of investigation, namely the neuro-atomic level. In particular, we survey extensive evidence showing how metals play a fundamental and inexorable role in all known neural systems. In particular, we illustrate examples from our own laboratory's use of synchrotron imaging (i.e., using a particle accelerator) that demonstrates the ubiquity of multiple co-localized metals in the brain and their distribution at scales ranging from the sub-micron to whole brain in a variety of cognitive conditions (e.g., typical, Williams syndrome, epilepsy, Alzheimer's, etc). In addition, with the help of computational simulations, we describe why these properties of metals are instrumental and necessary for cognitive information flow and so may be prime candidates for the nexus of consciousness.

Disclosures: **E.L. Ohayon:** None. **A. Lam:** None.

Nanosymposium

016. Distinct Approaches in the Study of Emotion: Integrative Informatics, Real Time fMRI, and Functional Connectivity

Location: SDCC 2

Time: Saturday, November 12, 2016, 1:00 PM - 3:30 PM

Presentation Number: 16.07

Topic: G.03. Emotion

Support: NIH R21 MH098149

NIH R01 DA033369

Title: Modeling spontaneous emotion dynamics during resting-state fMRI

Authors: K. S. LABAR[1], *P. A. KRAGEL[2], A. R. KNODT[1], A. R. HARIRI[1];
[1]Duke Univ., Durham, NC; [2]Univ. Of Colorado At Boulder, Boulder, CO

Abstract: Recent work in affective neuroscience has revealed the importance of temporal dynamics in affective responding and healthy emotion regulation. For instance, limbic regions such as the amygdala often exhibit delayed recovery from emotion inductions in depression. However, it is not known how the brain transitions among discrete emotional states and whether alterations in these dynamics are predictive of psychopathology. Here we investigate this problem by modeling spontaneous dynamics of emotional brain activity using a combination of multivariate pattern analysis and stochastic process modeling in a large sample of young adults from the Duke Neurogenetics Study (N=499). Using previously developed brain-based markers of discrete emotional states, we classified time series of resting-state fMRI data into emotion categories of contentment, amusement, surprise, fear, anger, sadness, and a neutral state. This procedure yielded a sequence of classifications for each subject, which we modeled as a discrete-time Markov process with seven states. Results indicate that emotion dynamics are reliable across subjects and do not merely reflect the temporal autocorrelation of the hemodynamic response. Data were divided into self-transitions (consecutive evidence for the same affective state from one TR to the next) and transitions from one affective state to another. The proportion of self-transitions for all emotions, with the exception of neutral states, was greater than would be expected based on autocorrelation alone. Self-transitions were largest for the emotions fear, anger, and contentment. Conversely, transitions to different emotions were most often to neutral states, as opposed to fluctuating among multiple emotions. An exploratory analysis revealed that fewer transitions to neutral states predicted the clinical status of individuals in the sample. These findings suggest that human brain activity dynamically fluctuates among emotional states which

are sparsely organized about a neutral hub, the centrality of which may be disrupted in psychopathological conditions.

Disclosures: **K.S. LaBar:** None. **P.A. Kragel:** None. **A.R. Knodt:** None. **A.R. Hariri:** None.

Nanosymposium

016. Distinct Approaches in the Study of Emotion: Integrative Informatics, Real Time fMRI, and Functional Connectivity

Location: SDCC 2

Time: Saturday, November 12, 2016, 1:00 PM - 3:30 PM

Presentation Number: 16.08

Topic: G.03. Emotion

Support: Academy of Finland Grant #265917

Academy of Finland Grant #138145

ERC Starting Grant #313000

Title: Classification of emotions from brain connectivity patterns

Authors: ***H. P. SAARIMÄKI**, E. GLEREAN, D. SMIRNOV, H. MYNTTINEN, I. P. JÄÄSKELÄINEN, M. SAMS, L. NUMMENMAA;
Aalto Univ., Espoo, Finland

Abstract: Emotions can be classified from brain activity patterns measured with BOLD-fMRI (Kragel and LaBar 2014 *Emotion Review 6:*160-174), suggesting that at least some emotions have a discrete neural basis. Different emotions are represented in mostly overlapping brain areas and the current emotional state can be decoded from the distribution of voxel activations in different areas (Saarimäki et al. 2015 *Cereb Cortex*). However, little is known about whether the connectivity patterns between different emotion-related brain areas also convey information of the current emotional state.
During fMRI scanning, we induced six emotions (anger, fear, disgust, happiness, sadness, and surprise) and a neutral state in participants using 60-s-long emotional narratives. We calculated the connectivity of the whole brain and within 10 separate functional systems (Power et al. 2011 *Neuron 72:*665-678; motor and somatosensory system, cingulo-opercular task control system, auditory system, default mode system, visual system, fronto-parietal system, salience system, subcortical system, ventral attention system, and dorsal attention system). An across-participant classifier was trained to categorize the emotional states based on the brain connectivity matrix of nodes either in whole brain or within different ROIs, and the results were validated using a leave-one-participant-out crossvalidation.

Whole-brain classification accuracy was 21% (against chance level 14%) and the classification was successful for all emotions. Classification of connections within the same functional system was above chance level in default mode system and dorsal attention system.
We conclude that in addition to the differences in underlying brain activity patterns, emotions also differ in the connectivity patterns both across the brain and within functional systems including default mode system and dorsal attention system. These connectivity patterns also generalize across individuals.

Disclosures: H.P. Saarimäki: None. **E. Glerean:** None. **D. Smirnov:** None. **H. Mynttinen:** None. **I.P. Jääskeläinen:** None. **M. Sams:** None. **L. Nummenmaa:** None.

Nanosymposium

016. Distinct Approaches in the Study of Emotion: Integrative Informatics, Real Time fMRI, and Functional Connectivity

Location: SDCC 2

Time: Saturday, November 12, 2016, 1:00 PM - 3:30 PM

Presentation Number: 16.09

Topic: G.03. Emotion

Support: Studienstiftung des Deutschen Volkes (to F.B.)

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Kommission für Klinische Forschung, Technische Universität München (KKF 8762754, to V.R., KKF 8765162 to C.S)

Title: Increased global interaction across the brain's functional modules during cognitive emotion regulation

Authors: *F. BRANDL, S. MULEJ BRATEC, X. XIE, A. M. WOHLSCHLÄGER, V. RIEDL, C. MENG, C. SORG;
Technische Univ. München, München, Germany

Abstract: Cognitive emotion regulation (CER) enables humans to flexibly modulate their emotional responses. Neurobiological theories of CER differ with regard to the spatial extent of underlying brain activity. While local theories suggest that interactions between specialized local brain circuits, such as amygdala and medial prefrontal cortex (mPFC), underlie CER, global theories hypothesize global interaction increases among larger functional brain modules that

comprise local circuits. We tested the global CER hypothesis using graph-based whole-brain network analysis of functional MRI data during aversive emotional processing with and without CER. During CER, global between-module interaction across stable functional network modules increased; this was particularly driven by amygdala and cuneus - nodes of highest nodal participation -, and mPFC and posterior cingulate as relevant connector hubs. Nodes of increased nodal participation overlapped with CER-specific local activations. Results provide first evidence for the global nature of human CER that complements functional specialization of local brain circuits.

Disclosures: **F. Brandl:** A. Employment/Salary (full or part-time): Department of Neuroradiology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany, TUM-NIC Neuroimaging Center, Klinikum rechts der Isar, Technische Universität München, Munich, Germany. **S. Mulej Bratec:** A. Employment/Salary (full or part-time): Department of Neuroradiology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany, TUM-NIC Neuroimaging Center, Klinikum rechts der Isar, Technische Universität München, Munich, Germany, Ludwig-Maximilians-Universität München, Graduate School of Systemic Neurosciences, Planegg-Martinsried, Germany. **X. Xie:** A. Employment/Salary (full or part-time): Ludwig-Maximilians-Universität München, Graduate School of Systemic Neurosciences, Planegg-Martinsried, Germany, Ludwig-Maximilians-Universität München, Department of Psychology, Munich, Germany. **A.M. Wohlschläger:** A. Employment/Salary (full or part-time): Department of Neuroradiology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany, TUM-NIC Neuroimaging Center, Klinikum rechts der Isar, Technische Universität München, Munich, Germany. **V. Riedl:** A. Employment/Salary (full or part-time): Department of Neuroradiology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany, TUM-NIC Neuroimaging Center, Klinikum rechts der Isar, Technische Universität München, Munich, Germany. **C. Meng:** A. Employment/Salary (full or part-time): Department of Neuroradiology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany, TUM-NIC Neuroimaging Center, Klinikum rechts der Isar, Technische Universität München, Munich, Germany, University of Cambridge, Behavioural and Clinical Neuroscience Institute, Cambridge, United Kingdom. **C. Sorg:** A. Employment/Salary (full or part-time): Department of Neuroradiology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany, TUM-NIC Neuroimaging Center, Klinikum rechts der Isar, Technische Universität München, Munich, Germany, Department of Psychiatry, Klinikum rechts der Isar, Technische Universität München, Munich, Germany.

Nanosymposium

016. Distinct Approaches in the Study of Emotion: Integrative Informatics, Real Time fMRI, and Functional Connectivity

Location: SDCC 2

Time: Saturday, November 12, 2016, 1:00 PM - 3:30 PM

Presentation Number: 16.10

Topic: H.02. Human Cognition and Behavior

Support: This work was supported by the intramural program of the NIMH; 1 ZIA MH002920 07

Title: Direct modulation of aberrant social brain network connectivity in Autistic Spectrum Disorder through NeuroFeedback

Authors: *M. RAMOT, S. KIMMICH, J. GONZALEZ-CASTILLO, H. POPAL, E. WHITE, A. MARTIN;
NIH/NIMH, Bethesda, MD

Abstract: Patients with Autistic Spectrum Disorders (ASD) exhibit severe deficits in social functioning and a variety of changes in typical patterns of resting state functional connectivity. Specifically, some cortical areas have been shown to be significantly under-connected in patients with ASD compared to typically developing (TD) control subjects, in a manner correlated with social symptom severity. Traditional training techniques in ASD are limited and often do not generalize well beyond the training paradigm, and do not address the aberrant network structure. Recent studies have shown that real time neurofeedback can be used to train participants to modulate network activations, making neurofeedback a promising candidate for a variety of clinical applications. In this study, we developed a novel feedback mechanism, which provided feedback through the unmasking of a complex visual scene. Real time fMRI neurofeedback was used to train participants with ASD to increase the correlation between two target regions involved in social processing (STS and somatosensory cortex), while simultaneously decoupling the targets from a control region (IPL), over a period of four days. The complex nature of this feedback, which was driven by the differential connectivity of these social brain regions and IPL, precluded the formulation of an explicit strategy. Nevertheless, participants showed a large and significant change between the first and last day in the correlations between these three regions, in the direction of training. Whole brain analysis reveals that these changes were specific to the areas being trained, suggesting that neurofeedback can be used to alter clinically relevant, complex network connectivity patterns.

Disclosures: **M. Ramot:** None. **S. Kimmich:** None. **J. Gonzalez-Castillo:** None. **H. Popal:** None. **E. White:** None. **A. Martin:** None.

Nanosymposium

017. Medial Temporal Lobe Subregion Imaging in Normal and Pathological Memory

Location: SDCC 7B

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Presentation Number: 17.01

Topic: H.02. Human Cognition and Behavior

Support: NSERC CGS - master's

Ontario Graduate Scholarship - master's

Title: Unravelling subfields of the hippocampal head with 7T MRI: leveraging the dark band

Authors: *J. DEKRAKER[1,2], K. FERKO[3,2], A. KHAN[4,2], S. KOHLER[3,2];
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Abstract: There is growing interest in segmenting the hippocampus into subfields to determine their specific computational functions, and to assess their integrity as a biomarker for various diseases. However, limitations in MRI resolution, knowledge about subfield morphology, and suboptimal techniques for subfield segmentation still hinder progress. In particular the complex folding of the hippocampal head, which was recently elucidated in new histological research (Ding *et al.*, 2015), is often not addressed. This folding means that the geodesic anterior terminus of each subfield rests in the more medial and posterior uncal portion of the hippocampus rather than in the anterior tip. In the current study we aimed to improve hippocampal segmentation by using i) ultra-high resolution structural 7T MRI; ii) translation of recently published histological evidence; and iii) a hybrid semi-automated technique with manual tracing steps that guide and constrain segmentation. We collected four T2 scans with 0.6mm isotropic voxel size from 12 healthy participants. We then upsampled to 0.3mm, coregistered, and averaged the four scans from each participant. The segmentation protocol was developed based on data from a highly digitated (i.e. folded) hippocampus and a hippocampus with few digitations. Two raters then performed segmentation on the remaining data. With our acquisition protocol the hippocampal 'dark band' - a visible feature made up of high myelin strata along the vestigial hippocampal sulcus - is present throughout most of the curvature of the uncus and the digitations in the hippocampal head. Our segmentation protocol treats the subiculum, CA1, CA2, and CA3 as single folded and continuous segments, adjacent to the hippocampal dark band; the latter, in turn, wraps around the remaining inner subfields - dentate gyrus and CA4. This single, continuous tissue can be 'unfolded' to allow for histologically-informed characterization of tissue properties along the geodesic longitudinal axis, and subfield boundaries can be applied perpendicular to this axis according to predefined, anatomically precise rules. This approach builds on knowledge about hippocampal ontogeny, allows for fast segmentation, and respects the complex

morphology of the hippocampal head. Inter- and intra-rater reliability of resulting segmentations is anticipated to be comparable to that of other prominent protocols. Future work will include the validation of this protocol by direct comparison with *ex-vivo* histology, and the further integration of automation to reduce the need for manual input and reduce any influence of subjective bias.

Disclosures: **J. Dekraker:** None. **K. Ferko:** None. **A. Khan:** None. **S. Kohler:** None.

Nanosymposium

017. Medial Temporal Lobe Subregion Imaging in Normal and Pathological Memory

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Presentation Number: 17.02

Topic: H.02. Human Cognition and Behavior

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NIA grant P50 AG05146 (MAY)

NIA grant R21 AG049220 (MAY)

Title: A harmonized protocol for *In vivo* human medial temporal lobe subfield segmentation: initial results of the 3 tesla protocol for the hippocampal body

Authors: *L. WISSE[1], A. M. DAUGHERTY[2], R. K. OLSEN[3], R. S. C. AMARAL[4], D. BERRON[5], V. A. CARR[6], A. EKSTROM[7], P. KANEL[8], G. A. KERCHNER[9], S. G. MUELLER[10], J. B. PLUTA[1], C. E. STARK[11], T. A. STEVE[12], L. WANG[13], M. A. YASSA[11], P. A. YUSHKEVICH[1], R. LA JOIE[10];
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Irvine, CA; [12]Univ. of Alberta, Edmonton, AB, Canada; [13]Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Introduction: Alzheimer's disease (AD), aging and vascular pathology are proposed to differentially affect subregions of the medial temporal lobe (MTL). Characterizing these differences may provide more insight into disease processes and better biomarkers. However, comparison across studies is hampered by differences in how subfields are segmented on in vivo MRIs by various research groups. As a result, the Hippocampal Subfields Group (HSG) was formed to create a harmonized protocol for MTL subregion segmentation. Over the past year we began developing a subfield segmentation protocol for the hippocampal body using high-resolution T2-weighted 3 tesla MRI. Here we present the initial results of this effort. **Methods:** Stage 1. Establish the anterior and posterior borders of the hippocampal body as a whole and the boundaries with surrounding structures, 2. Determine subfield boundaries on histological sections throughout the hippocampal body in three specimens, 3. Identify the corresponding boundaries between adjacent subfields on in vivo MRI, 4. Solicit feedback on the initial protocol from the larger HSG, and 5. Perform formal reliability analysis by six experts. **Results:** Our initial results are from stage 1. The anterior border of the body was defined as one slice posterior to the last slice containing the uncal apex and the posterior border as the most posterior slice containing the colliculi. A reliability test yielded a Fleiss $\kappa \geq 0.75$. An initial protocol for the boundaries with surrounding structures is also developed, with the dorsal and lateral border at the interface between the gray matter of the hippocampus and the white matter of the alveus/fimbria, the ventral border placed at the parahippocampal white matter and the medial border at the most medial point of the hippocampus (coinciding with the subiculum). Finally, the histological annotations are finalized and an MRI protocol for the subfield boundaries is being prepared. **Conclusion:** The harmonized protocol, once completed, is expected to impact the field significantly by producing measurements that are comparable between labs and by making it easier to relate and pool results from different studies. Given the heterogeneity of AD, vascular and aging-related changes in the MTL, a valid and reliable measure of subfield-specific effects may lead to more powerful imaging biomarkers.

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Nanosymposium

017. Medial Temporal Lobe Subregion Imaging in Normal and Pathological Memory

Location: SDCC 7B

Time: Saturday, November 12, 2016, 1:00 PM - 3:45 PM

Presentation Number: 17.03

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant T32EB009384

1 R03 EB16923-01A1

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CBICA seed grant

Title: Mapping the structural and functional network architecture of the medial temporal lobe using 7T MRI

Authors: *P. SHAH[1], D. S. BASSETT[1], J. A. DETRE[2], J. M. STEIN[3], M. A. ELLIOTT[3], J. PLUTA[3], E. VALENCIANO[3], M. DAFFNER[2], L. E. MANCUSO[2], C. COTO[2], L. E. M. WISSE[3], B. LITT[4], K. A. DAVIS[2], S. R. DAS[3];
[1]Bioengineering, [2]Neurol., [3]Radiology, [4]Bioengineering, Neurol., Univ. of Pennsylvania, Philadelphia, PA

Abstract: The medial temporal lobe (MTL) consists of the hippocampus and extra-hippocampal cortical structures, and plays an essential role in memory function. Specific MTL subregions are differentially involved in both normal memory function and various neurological disorders such as Alzheimer's disease and temporal lobe epilepsy. However, due to lack of high-resolution MRI images and accurate subfield segmentations, there is limited prior work investigating MTL structural and functional network connectivity at the subregion level.
In this study, we aim to rigorously characterize the functional and structural network architecture of the MTL using 7T resting BOLD-fMRI and MTL-focused T2-weighted sMRI in 20 healthy subjects. We utilize a multi-atlas segmentation algorithm to identify ten MTL subregions per hemisphere (including hippocampal subfields and discrete regions of the parahippocampal gyrus). Structural connectivity matrices are generated from covariance of subregion volumes across subjects, and functional connectivity matrices are generated from linear correlations between fMRI time series in each subregion. We find a strong correlation between structural and functions networks ($r=0.57$, $p<1x10^{-14}$). Next, we calculate several key network properties (degree, clustering coefficient, efficiency) on a global and local scale, and compare those properties with those expected in appropriate null models. Furthermore, we characterize the modular architecture of the healthy MTL in both structural and functional networks, and discover two large modules corresponding closely to (1) bilateral hippocampal formations and (2)

bilateral extra-hippocampal structures.
Our findings represent the first comprehensive analysis of network topology of the MTL at the subregion level, and can serve as the basis for a better understanding of its physiological function. Moreover, this work can pave the way for future studies to characterize changes in MTL network architecture in disease processes involving the hippocampus.

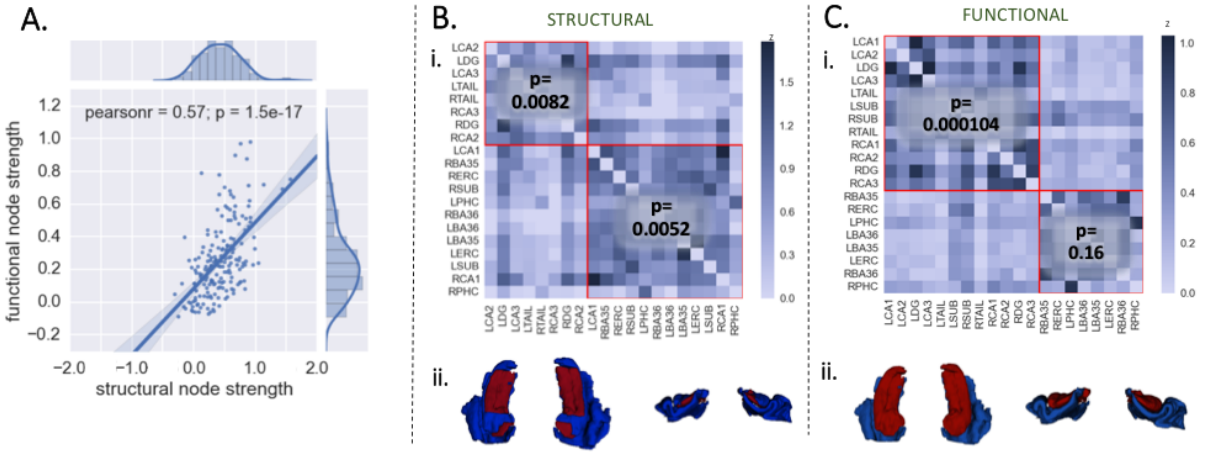

Figure 1: (A) Correlation and distribution of functional and structural connectivity matrices, with regression line and 95% confidence interval. (B) i. Detected structural modules along with significance values, ii. Visualization of structural modules mapped onto the MTL segmentation. (C) i. Detected functional modules along with significance values, ii. Visualization of functional modules mapped onto the MTL segmentation.

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Nanosymposium

017. Medial Temporal Lobe Subregion Imaging in Normal and Pathological Memory

Location: SDCC 7B

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Presentation Number: 17.04

Topic: H.02. Human Cognition and Behavior

Support: NIA R21 AG049220

P50 AG16573

P50 AG05146

NIMH R01 MH102392

NSF GRF DGE-1232825

Title: Distinct and complementary contributions of hippocampal subfields and neocortical regions to source memory and item-level pattern separation

Authors: *Z. REAGH, R. F. STEVENSON, A. P. CHUN, E. A. MURRAY, M. A. YASSA; Dept. of Neurobio. and Behavior, Univ. of California Irvine Dept. of Neurobio. and Behavior, Irvine, CA

Abstract: Episodic memories consist of items bound to contextual features, such as locations. Neural correlates of simple recognition of items have been reliably dissociated from those supporting memory for context or source. However, the link between source memory and another operation - mnemonic discrimination, driven by pattern separation of similar inputs - is poorly understood. Importantly, these features of memory are both thought to operate over detailed representations, and are both known to engage the hippocampus. We used high-resolution fMRI to simultaneously examine the contributions of hippocampal and neocortical regions to pattern separation and source memory retrieval. We found two major response profiles: discrimination-specific engagement in posterior hippocampal dentate/CA3 and perirhinal cortex, and source retrieval-specific engagement in hippocampal CA1, subiculum, and angular gyrus. We additionally observed a mixture of both types of signals in parahippocampal cortex. These findings suggest that distinct and complementary processes underlie the retrieval of conjunctive memory representations. Moreover, the present data lend an understanding of these processes under conditions of mnemonic interference.

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Nanosymposium

017. Medial Temporal Lobe Subregion Imaging in Normal and Pathological Memory

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Presentation Number: 17.05

Topic: H.02. Human Cognition and Behavior

Support: DFG Grant WE 4269/3-1

NIH Grant R37 AG011230

Title: Improving the concurrent validity of automated hippocampal subfield segmentation in older adults by direct comparison to manual tracing

Authors: *A. R. BENDER[1], A. KERESZTES*[1], N. C. BODAMMER[1], N. RAZ[1,2], M. WERKLE-BERGNER[1], Y. SHING[1,3], S. KÜHN[1,4], U. LINDENBERGER[1,5,6];

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Abstract: High-resolution magnetic resonance imaging (MRI) is increasingly used for the *in vivo* morphometry of cytoarchitectonically distinct hippocampal (HC) subfields. Demarcation of HC subfields can be achieved considerably faster with automated methods than with manual approaches, which continue to be considered as the gold standard. The validity of automated procedures is not clearly established because direct comparisons with reliable manual measurements are scarce. In addition, many automated approaches to HC subfield morphometry use atlases with boundary definitions that do not follow current recommendations for manual segmentation. The objective of the present study was to establish the concurrent validity of an improved automated method by direct comparisons to manual tracing. To this end, we built a customized HC subfield atlas that includes the subiculum, CA1/2, CA3/dentate gyrus (DG) within the HC body, as well as entorhinal cortex (ERC), and evaluated its correspondence with manually traced data. Using manually segmented, high-resolution 3T MRI images from 10 children and adolescents aged 7 to13 years, 4 younger adults aged 22 to 24 years, and 14 older adults aged 62 to 78 years, equally divided by sex, we created a customized hippocampal subfield atlas with the Automated Segmentation of Hippocampal Subfields (ASHS) software package, following current segmentation guidelines of the Hippocampal Subfields Group (Yushkevich et al., 2015). We then applied ASHS together with this customized atlas to segment subfields in an independent sample of 20 older adults aged 61 to 80 years. Comparisons to manually segmented data using intraclass correlation coefficients (ICC) showed that the validity coefficients of automated segmentation varied substantially across subfields, ranging from 0.34 to 0.91. Further inspection revealed that deviations of automated segmentation from the manual gold standard were largest at the most anterior and posterior portions of the hippocampal body. By tracing additional anterior and posterior slices during atlas creation and using manually defined ranges to truncate automated output, the validity of automated segmentation increased considerably in both hemispheres, with ICC(2) = 0.82 for bilateral ERC, and ICC(2) > .90 for subiculum, CA1/2, and CA3/DG volumes. Our results show that the validity of automated HC subfield segmentation can be improved by using a lifespan sample for atlas generation, and by manually optimizing automated procedures. These findings have strong implications for structural and functional studies of hippocampal subfields, particularly in large datasets and for lifespan comparisons.

Disclosures: **A.R. Bender:** Other; * Indicates joint first authorship. **A. Keresztes*:** Other; * Indicates joint first authorship. **N.C. Bodammer:** None. **N. Raz:** None. **M. Werkle-Bergner:** None. **Y. Shing:** None. **S. Kühn:** None. **U. Lindenberger:** None.

Nanosymposium

017. Medial Temporal Lobe Subregion Imaging in Normal and Pathological Memory

Location: SDCC 7B

Time: Saturday, November 12, 2016, 1:00 PM - 3:45 PM

Presentation Number: 17.06

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R37 AG011230

Title: Advanced age, vascular risk and inflammation exacerbate differential shrinkage of hippocampal subfields in healthy adults: a two-year longitudinal study

Authors: *A. M. DAUGHERTY[1], A. R. BENDER[2], Q. YU[3], A. T. SHAFER[4], M. ARSHAD[5], N. OFEN[6], N. RAZ[6];
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Abstract: Cross-sectional findings suggest hippocampal subfield volumes may follow different trajectories across the lifespan and are differentially vulnerable to age-related decline in vascular health. However, reliance on cross-sectional designs precludes valid estimates of change and its mediators. To our knowledge, we provide here the first longitudinal evidence of hippocampal subfield shrinkage in healthy aging. Healthy adults (N=86, age at baseline 21-80 years; M=56.35, SD=13.13) were assessed twice, two years apart. Subfield volumes within the hippocampal body were manually segmented on high-resolution MR images (0.4×0.4 mm^2 in-plane). Indicators of metabolic syndrome risk (systolic blood pressure, years of hypertension diagnosis, fasting glucose, waist-to-hip ratio, and high density lipoprotein levels) and inflammation (plasma homocysteine, C-reactive protein, and folate) were combined in latent factor composites. Latent change score models provided estimates of change and individual differences therein. After correction for multiple comparisons, older adults evidenced smaller volumes at baseline (all subfields $r \leq -0.12$, all $p > 0.06$) but no mean shrinkage over time (change = -0.01-0.07, $p \geq 0.10$). However, significant individual variability in change was observed in all regions ($\sigma = 0.06$-0.09, all $p \leq 0.01$). Models with covariates revealed that older adults with higher metabolic syndrome risk demonstrated greater CA1-2 volume decline (-0.61, $p < 0.01$; 95% CI: -0.98/-0.24), whereas greater inflammation risk worsened subiculum shrinkage in later life (-0.18, $p = 0.02$; 95% CI: -0.30/-0.05). Independent of risk factors, older adults compared to younger counterparts demonstrated steeper decline in CA3-dentate gyrus volumes (-0.07, $p = 0.08$; 95% CI: -0.14/-0.01). Thus, CA3-dentate gyrus volume decline appears to follow a non-linear trajectory that accelerates in later life, whereas vascular risk and inflammation exacerbate shrinkage of CA1-2 and subiculum in older adults. Differential

sensitivity of the hippocampal subfields supports the role of systemic risk factors in brain aging. Notably, vascular risk is a modifiable contributor to age-related decline that can be targeted to promote neural and cognitive function into senium.

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Nanosymposium

017. Medial Temporal Lobe Subregion Imaging in Normal and Pathological Memory

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Topic: H.02. Human Cognition and Behavior

Support: CIHR MOP115011

AIHS and CIHR graduate studentships

Title: Relationship between episodic memory performance and hippocampal subfields volumes in healthy cognitive aging: high resolution magnetic resonance imaging study.

Authors: *N. V. MALYKHIN[1], S. TRAVIS[2], Y. HUANG[1], F. OLSEN[1], R. CARTER[1]; [1]Biomed. Engin., [2]Neurosci. and Mental Hlth. Inst., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Background: It is unclear how each subfield within the hippocampus (HC) contributes to memory function. Animal studies indicate positive correlations between HC neurogenesis and learning performance. However it is uncertain whether this association is due to global HC volume, or constituent subfields. Additionally, it has been shown that certain tasks may have anatomical specificity within the HC subfields (Travis et al., 2014). The goal of our study was to examine the volumes of HC subfields and contrasting neuropsychological memory tests indexes in a cognitively healthy sample across the lifespan.
Methods: 140 healthy volunteers (64 M, 76 F, ages 18-85) were recruited. Exclusion criteria were neuropsychiatric disorders, and a MOCA score <26. Older subjects with Mild Cognitive Impairment (MCI) and dementia were excluded from the study. Written informed consent was obtained and the research was approved by the University of Alberta Health Research Ethics Board. Ultra-high resolution HC images were acquired using a T2-weighted, 2D FSE sequence on a 4.7T Varian scanner. HC subfields were manually segmented into the cornu amonis (CA), dentate gyrus (DG), and subiculum (Sub). Participants were administered the Wechsler Memory Scale, 4th edition (WMS). Indexes were created from WMS tasks: Auditory Memory Index (AUD), Visual Memory Index (VIS), Delayed Memory Index (DEL), and Recognition Memory

Index (REC).
Results: Age negatively correlated (all p<.001) with AUD (-.581), VIS (-.776), DEL (.758), and REC (-.627). AUD performance correlated significantly with left DG (r=.204, p=.02), while VIS performance correlated with right CA (r=.192, p=.03), left SUB (r=.212, p=.02), left DG (r=.304, p=.001), and right DG (r=200, p=.02).
DEL performance correlated significantly with left SUB (r=.219, p=.01), left DG (r=.262, p=.003), right DG (r=.195, p=.03). REC performance correlated with right CA (r=.197, p=.03), left SUB (r=.181, p=.04), left DG (r=.263, p=.003), and right DG (r=.175, p=.04). **Conclusions:** Our results that suggest that the contrasting memory indexes show a specific pattern of correlation to HC subfield volumes. Volumes of all subfields appear to be involved in the visual tasks in the WMS, whereas only volumes in the left DG are related to performance in auditory tasks. Similarly, volumes of SUB and DG are involved in delayed memory performance, and all subfield volumes measured were involved in recollection memory. Additionally, bilateral DG volumes were correlated with all memory index performances, suggesting a strong role in these episodic memory tasks.

Disclosures: N.V. Malykhin: None. **S. Travis:** None. **Y. Huang:** None. **F. Olsen:** None. **R. Carter:** None.

Nanosymposium

017. Medial Temporal Lobe Subregion Imaging in Normal and Pathological Memory

Location: SDCC 7B

Time: Saturday, November 12, 2016, 1:00 PM - 3:45 PM

Presentation Number: 17.08

Topic: H.02. Human Cognition and Behavior

Support: BRACE

ARUK

EBI

Title: Hippocampal subfield volumes and memory: investigating memory storage in healthy ageing

Authors: *H. K. ISOTALUS[1], A. R. WEARN[1], B. MCCANN[2], M. J. KNIGHT[2], D. TSIVOS[3], R. A. KAUPPINEN[2], E. J. COULTHARD[1];
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Abstract: Strengthening of the neural connections within the hippocampus (HC) has been shown to support memory storage in animals, and damage to this region is classically associated with profound memory deficits in humans. In elderly adults, global HC volume is associated with memory performance, possibly due to its involvement in consolidation. Structural correlates of different memory processes across HC subfields in humans remain unknown, however. High resolution magnetic resonance imaging (MRI) allows for a detailed investigation of HC anatomy in vivo. We measured individual HC subfield volumes and ability to retain verbal information over time in 33 healthy older participants (mean age=67.7; SD=9.9). The Hopkins Verbal Learning Task (HVLT), which is a free verbal recall task, was used to measure memory immediately and after 25-minute and 24-hour delays. Delayed memory scores were normalised to immediate memory performance across analyses to exclude encoding effects. T2-weighted MR-images were acquired on a 3 Tesla Siemens Magnetom Skyra scanner using an in-house developed CPMG-like sequence (FOV = 184 x 218 x 58mm3; slice thickness = 1.72mm; in-plane resolution = 0.34 x 0.34 mm2; TR = 5500; echo spacing = 12ms; 12 echoes). HC subfields cornu ammonis (CA)1, CA2, CA3, dentate gyrus (DG), subiculum (SUB) and stratum lacunosum/ stratum radiatum/ stratum moleculare (SL/SR/SM; grouped as one) were segmented on FSL using an in-house developed manual protocol. Across all analyses subfields were normalised for total brain volume. No associations were found between immediate memory and HC subfield volumes. High verbal memory retention score over 25 minutes (n=32) was associated with larger right CA1, DG, SL/SR/SM and total HC, while performance over 24 hours (n=22) was associated with larger right SUB volume (2-tailed $p<0.05$, uncorrected for all tests). None of the left HC subfield volumes were associated with memory retention scores. These results suggest that verbal memory storage within the HC may be right lateralised, and that the right SUB may support memory retention over longer periods. An alternative explanation is that this structure supports accessing memories that have been consolidated overnight. Future work will enlarge our sample size to confirm these findings. We will also include other MRI derived measures, such as T2-relaxation time and HC shape, to examine the relationship between memory processes and HC structure.

Disclosures: H.K. Isotalus: None. **A.R. Wearn:** None. **B. McCann:** None. **M.J. Knight:** None. **D. Tsivos:** None. **R.A. Kauppinen:** None. **E.J. Coulthard:** None.

Nanosymposium

017. Medial Temporal Lobe Subregion Imaging in Normal and Pathological Memory

Location: SDCC 7B

Time: Saturday, November 12, 2016, 1:00 PM - 3:45 PM

Presentation Number: 17.09

Topic: H.02. Human Cognition and Behavior

Support: CIHR Grant MOP-126003 to JDR

CIHR Grant MOP-115148 to MDR

NSERC Canada Graduate Scholarship to L-KY

Title: Volume reductions in the CA1 hippocampal subfield, perirhinal and anterolateral entorhinal cortices are associated with preclinical cognitive decline: implications for dementia screening

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Abstract: Neuropsychological testing is a cost-effective method for identifying individuals who are at risk for developing neurodegenerative disease. The Montreal Cognitive Assessment (MoCA) is a brief screening tool that demonstrates excellent sensitivity and specificity in detecting Mild Cognitive Impairment (MCI; Nasreddine et al., 2005; Markwick et al., 2012) and also predicts subsequent conversion to Alzheimer's Disease (AD; Julayanont et al., 2014). Here, we investigated whether MoCA score predicts structural atrophy in the medial temporal lobes of 40 community-dwelling adults without subjective memory complaints (formally assessed with the Memory Functioning Questionnaire; Gilewski et al.,1990). Manual segmentation of the hippocampal subfields and parahippocampal subregions was performed on MRI scans obtained from two age- and education-matched groups of 20 older adults that differed on the basis of their MoCA score. The two groups were defined as an "at-risk" group (mean age: 72.5 years, range: 59-81, mean education = 16.2 years, range: 12-22), consisting of individuals who scored below the recommended threshold score of 26 (indicating possible MCI, mean score = 23.4, range = 17-25), and a healthy control group (mean age: 70.3 years, range: 63-77, mean education = 16.6 years, range = 12-23), composed of individuals who scored 26 and above on the MoCA (mean score = 27.9, range = 26-30). High-resolution, oblique-coronal T2-weighted scans (voxel size: 0.4x0.4x3mm) were acquired in a plane perpendicular to the long axis of the hippocampus using a 3 Tesla MRI scanner. Hippocampal subfields (CA1, CA2/3/dentate gyrus, and subiculum) and parahippocampal subregions (entorhinal, perirhinal, and parahippocampal cortices) were manually segmented based on a previously developed protocol (Olsen et al., 2009; Olsen et al., 2013). The entorhinal cortex was further subdivided into anterolateral and posteromedial segments according the protocol of Maass, Berron and colleagues (2015). Significant group differences were observed in the CA1 subfield of the hippocampus as well as in the perirhinal and anterolateral entorhinal cortices. Thus, the brain regions that demonstrated reduced volume in individuals who fell below the recommended screening cutoff on the MoCA were highly concordant with the regions previously associated with the early stages of AD (Kahn et al., 2014; de Flores et al., 2015). These findings provide strong neuroanatomical evidence that MoCA test performance is associated with brain changes that precede subjective memory complaints and that the MoCA is a useful screening test for early detection of neurodegenerative disease.

Disclosures: **R.K. Olsen:** None. **L. Yeung:** None. **A. Noly-Gandon:** None. **M. D'Angelo:** None. **A. Kacollja:** None. **V.M. Smith:** None. **J.D. Ryan:** None. **M.D. Barense:** None.

Nanosymposium

017. Medial Temporal Lobe Subregion Imaging in Normal and Pathological Memory

Location: SDCC 7B

Time: Saturday, November 12, 2016, 1:00 PM - 3:45 PM

Presentation Number: 17.10

Topic: H.02. Human Cognition and Behavior

Support: Alzheimer's Association

Title: Tau binding measured using pet imaging correlates with mtl subregional atrophy and episodic memory performance in alzheimer's disease

Authors: ***S. DAS**, J. PHILLIPS, L. WISSE, G. STOCKBOWER, K. TERNES, C. MCMILLAN, P. YUSHKEVICH, M. GROSSMAN, I. NASRALLAH, D. WOLK;
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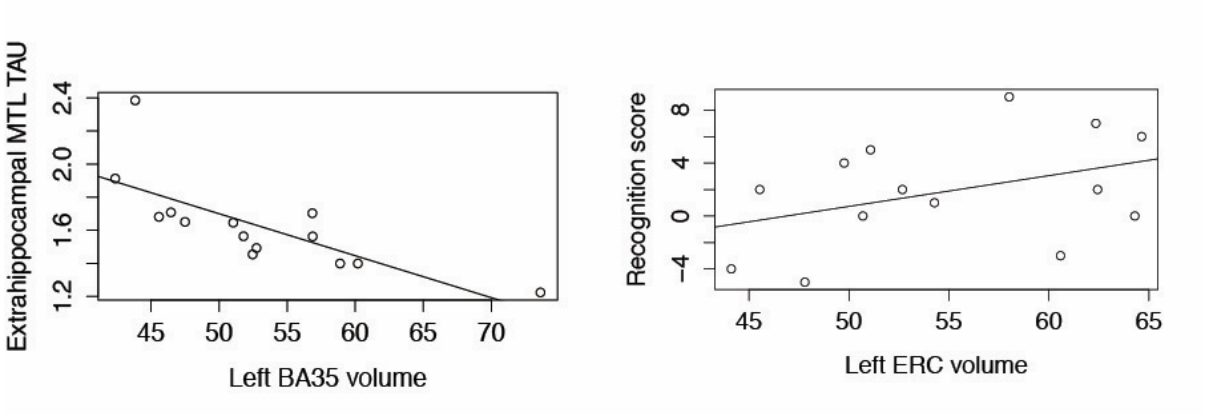
Abstract: Background: We present, for the first time, preliminary data investigating the relationship between TAU binding as measured by AV1451 PET imaging and subregional atrophy in medial temporal lobe (MTL), and how both imaging measures correlate with memory performance in Alzheimer's Disease (AD) patients.
Methods: Fourteen participants diagnosed with atypical or early onset presentations of AD underwent imaging. MTL subregions, including hippocampal subfields, perirhinal (PRC) and entorhinal (ERC) cortex were segmented in high-resolution T2-weighted MRI. Normalized TAU uptake within the hippocampus and an extrahippocampal ROI consisting of PRC and ERC, referenced against cerebellar gray matter, was measured from PET images using the AV1451 ligand. Performance in episodic memory tasks was assessed.
Results: PRC (BA35) volume was strongly correlated with TAU SUVR in extrahippocampal cortex in both sides ($p<0.01$). CA1 showed a trend. Other subregions did not show a significant correlation between volume and TAU. ERC volume was a strong predictor of both recognition and recall verbal memory performance ($p<0.01$). In the hippocampus, subiculum volume was significantly correlated with recognition and Short Delayed Recall and showed a trend with Long Delay Recall, whereas CA1 showed a trend with Short Delay Recall. TAU uptake in both hippocampus and extrahippocampal cortex did not significantly correlate with memory performance.
Conclusions: Given that TAU pathology in AD is known to progressively affect BA35, ERC and CA1, we hypothesized that atrophy in these MTL subregions will correlate with TAU binding. Both MTL showed strong correlation with BA35, the earliest site of pathology. Episodic

memory performance on tasks thought to be subserved by these subregions showed significant relationships with volumetric, but not TAU measurements. ERC showed strong correlation with all three memory scores. Despite the small sample size, these data suggest that MTL TAU pathology predicts subregional atrophy, which also has predictive value for cognitive performance in AD patients.



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Nanosymposium

017. Medial Temporal Lobe Subregion Imaging in Normal and Pathological Memory

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Topic: H.02. Human Cognition and Behavior

Support: FRSQ

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Weston Brain Institute

Alzheimer's Society

Brain Canada

Title: Hippocampus and subfield volumes are associated with CSF β-amyloid and phospho-tau and their interaction in asymptomatic individuals with parental history of Alzheimer's disease

Authors: *C. L. TARDIF[1], G. A. DEVENYI[1], P. ROSA-NETO[2], J. POIRIER[2], J. BREITNER[2], M. CHAKRAVARTY[1], T. PREVENT-AD WORKING GROUP[2];
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Abstract: Introduction Cerebrospinal fluid (CSF) biomarkers appear to be sensitive to the pathological status of individuals at high-risk for MCI or AD. Recent studies in such individuals have highlighted a synergistic interaction between amyloid-β and hyperphosphorylated tau as correlates of cortical atrophy (Fortea et al. 2014) and metabolic decline (Pascoal et al. 2016). Novel computational methods for magnetic resonance imaging (MRI) hippocampus (HC) and subfield segmentation have recently been shown to be capable of differentiating between normal and MCI or AD groups. We therefore investigated whether the interaction between CSF β-amyloid$_{1-42}$ (Aβ) and phosphorylated-tau (p-tau) can predict AD-related neuroanatomical decline at the level of the HC subfields in high-risk normal subjects.
Methods We analyzed baseline data from a longitudinal investigation of 84 individuals who had a parental history for AD but were cognitively healthy when enrolled. T1-weighted structural brain images at 1mm^3 were acquired on a 3T MRI system using the ADNI MPRAGE protocol. Preprocessed images were segmented using MAGeTbrain, a multi-atlas registration-based segmentation tool, to estimate the volume of the HC and its subfields (Pipitone et al. 2014), which were then normalized for total brain volume estimated using BeAST (Eskildsen et al. 2013). The subjects also underwent lumbar puncture to assay CSF Aβ and p-tau levels using Innotests ELISA, and were genotyped for Apolipoprotein E4 (APOE4) status. Statistical analysis was performed using general linear models in R with sex, age, APOE4 status, Aβ, p-tau, and Aβ:p-tau interaction in the model.
Results and discussion We observed negative main effects ($p<0.05$) of Aβ and p-tau, as well as an interaction between Aβ and p-tau, as predictors of right HC, and bilateral subiculum volumes. We observed modest direct effects and interaction ($p<0.10$) for right CA1, bilateral SR-SL-SM, and left HC volumes. No significant results were observed for the remaining subfields, left CA1 and bilateral CA2-CA3. The interaction suggested that lower p-tau and lower Aβ concentrations were related to decreased volumes. While we did not detect an interaction between the CSF markers and APOE4 status, APOE4 carriers with lower p-tau and higher Aβ had decreased volumes, whereas APOE E4 non-carriers with both lower p-tau and Aβ had decreased volumes. These results support the framework where a synergy between upstream Aβ and p-tau pathologies leads to neurodegeneration, potentially predictive of AD-related neuroanatomical decline. Future work will focus on whether these baseline biomarkers of pathology will predict future decline.

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Nanosymposium

018. Neural Mechanisms of Language

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IARPA 86155-Carnegi-1990360-gallant

Title: The representation of semantic information in the human brain during listening and reading

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Abstract: Extracting meaning from spoken and written language is a uniquely human ability. A recent publication from our laboratory showed that semantic information from spoken language is represented in a broad network of semantically-selective areas distributed across the human cerebral cortex. However, it is unclear which of the representations revealed in that study are specific to the modality of speech, and which are amodal. Here we studied how the semantic content of narratives received through two different modalities, listening and reading, is represented in the human brain. We used functional magnetic resonance imaging (fMRI) to record brain activity in two separate experiments while participants listened to and read several hours of the same narrative stories. We then built voxel-wise encoding models to characterize selectivity for semantic content across the cerebral cortex. We found that, in a variety of regions across temporal, parietal and prefrontal cortices, voxel-wise models estimated from one modality (e.g. listening) accurately predicted responses in the other modality (e.g. reading). In fact these cross-modal predictions only failed in sensory regions such as early auditory and visual cortices. We then used principal components analysis on the estimated model weights to recover semantic selectivity for each voxel. This revealed four important components: the first PC distinguishes between humans, social interactions (e.g. *social* and *emotional* categories) and perceptual descriptions, quantitative descriptions (e.g. *tactile* and *numeric* categories); the second PC distinguishes between perceptual (e.g. *tactile* and *visual* categories) and non-perceptual descriptions (e.g. *mental* and *professional* categories); the third PC distinguishes between

quantitative descriptions (e.g. *numeric* categories) and qualitative descriptions (e.g. *abstract* and *emotional* categories); and the fourth PC distinguishes between non-perceptual descriptions (e.g. *temporal* categories) and humans, social interactions (e.g. *communal* categories). We found strong correlations between the cortical maps of semantic content produced from listening and reading within these components (PC1: r=0.81±0.07; PC2: r=0.80±0.06; PC3: r=0.78±0.04; PC4: r=0.75±0.07, for n=7 participants). These results suggest that semantic representation of language outside of early sensory areas are not tied to the specific modality through which the semantic information is received.

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Nanosymposium

018. Neural Mechanisms of Language

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Topic: H.02. Human Cognition and Behavior

Support: Princeton Neuroscience scanning fund

Title: Similarity of social attribution to abstract, dynamic shapes predicts similarity of neural responses in default mode network

Authors: *M. NGUYEN[1], T. VANDERWAL[3], J. CHEN[1,2], U. HASSON[1,2];
[1]Dept. of Psychology, [2]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [3]Child Study Ctr., Yale Univ., New Haven, CT

Abstract: Humans have been shown to attribute animacy and mental states to even the simplest abstract shapes on the basis of their movement (Heider & Simmel, 1944). The use of abstract, somewhat ambiguous stimuli enables the comparison of neural responses to differing interpretations of the same stimuli. Are the neural representations of abstract shapes that are interpreted as animate characters similar to the neural representations of concretely described characters? To answer this question, we directly compared the neural responses to (1) a non-verbal, highly abstract audio-visual movie that used simple moving shapes to convey a complex narrative and (2) a direct verbal audio description of the same narrative. The two versions shared narrative content but varied significantly in low-level properties (non-verbal audio-visual versus verbal audio) and level of abstraction. For example, in the movie version of the narrative, a triangle might move up a diagonal line inside a box. In the audio version, the same sequence would be described as “The little boy goes upstairs to his room.” Healthy adult subjects (N = 36, 9 male) were scanned using fMRI while either watching or listening to the narrative. Following

presentation, subjects described the narrative out loud in a free recall. Similarity of recall description was assessed using Latent Semantic Analysis (LSA) to quantify each subject's degree of social attribution within and across each group. Using intersubject correlation (ISC), we identified areas of the brain that responded similarly across subjects to the abstract movie and the concrete audio narration. These areas included bilateral temporal parietal junction (TPJ), posterior superior temporal gyrus (pSTG) extending into auditory areas, precuneus along the parietal-occipital sulcus (POS), and posterior ventral visual areas. Moreover, cross-group neural similarity in the default mode network (DMN) was significantly predicted by the similarity of narrative interpretation in the abstract movie group and the concrete audio group: the more similar the attribution of animacy and intentionality in the movie group to the audio group, the more similar the neural responses ($r(17) = .781$, $p<.001$). These results suggest that telling a story using abstract visual shapes or auditory descriptions of characters activates similar patterns of neural activity, providing evidence for both modality-invariance and the strong social nature of the DMN.

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Nanosymposium

018. Neural Mechanisms of Language

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Topic: H.02. Human Cognition and Behavior

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BelSpo P7/11

KU Leuven OT/12/097

Title: Explicit retrieval of visual and non-visual properties of concrete entities: Involvement of superior temporal sulcus and anterior inferior frontal gyrus

Authors: ***A. G. LIUZZI**[1], P. DUPONT[1], R. PEETERS[2], S. DE DEYNE[4], G. STORMS[4], R. VANDENBERGHE[1,3];
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Abstract: INTRODUCTION: Critical dimensions for the organization of the semantic system are category, attribute and input-modality specificity. Within a same event-related fMRI experiment we determined the effect of these dimensions during explicit semantic retrieval.

METHODS: 18 healthy subjects participated in this fMRI study. 12 animate (mammals, birds, insects) and 12 inanimate entities (kitchen tools, clothes, music instruments) (De Deyne et al., 2008) were used. From the concept-feature matrix we selected 52 properties that were either distinctly visual or non-visual according to an on-line evaluation by 11 healthy volunteers, who judged the degree to which a property is visual or non-visual on a 1 to 7 rating scale. We selected for each subcategory 4 visual and 4 non-visual properties (further subdivided into non-visual sensory and non-sensory properties). The factorial factors in the fMRI experiment were (1) category (animate vs inanimate), (2) attribute (visual vs nonvisual) and (3) input-modality (written word vs picture). The experiment was conducted on a Philips Achieva dstream 3T equipped with a 32-channel head coil. Subjects performed a property verification task: they judged whether a given property was applicable to a given entity. The following linear model contrasts were calculated: retrieval of visual vs non-visual properties, animate vs inanimate entities and written words vs pictures. Significance level: uncorrected $p<0.001$ combined with a cluster-level corrected $p<0.05$. RESULTS: A 3-way repeated measures ANOVA with reaction times as outcome showed a main effect of input-modality (written words: 1.7s; pictures: 1.63s) ($F(1,15)=31.9$; $p=.000$) and property (non-visual: 1.69s; visual: 1.63s) ($F(1,15)=17.60$; $p=.001$). The overall accuracy of responses was 70.8%. Retrieval of non-visual compared to visual properties activated the left superior temporal sulcus, up to its most anterior end (x=-45 y=14 z=-32), anterior inferior frontal gyrus (x=-45 y=32 z=-2), superior frontal gyrus (x=-18 y=53 z=34) and posterior cingulate (x=-3 y=-52 z=22). Retrieval of visual compared to non-visual properties activated the left fusiform gyrus, left ventromedial occipitotemporal cortex, lateral occipital cortex and intraparietal sulcus bilaterally. The contrast between animate and inanimate entities yielded activations of lateral and medial fusiform gyrus respectively, in line with previous studies. CONCLUSIONS: While explicit retrieval of visual properties mainly relies on visual processing areas, the superior temporal sulcus and anterior inferior frontal gyrus appear to be preferentially involved in retrieval of non-visual properties.

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Nanosymposium

018. Neural Mechanisms of Language

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Topic: H.02. Human Cognition and Behavior

Support: Gravitational Program ‘Language in Interaction’ of the Netherland Organization for Scientific research

Title: Neural tuning to low level features of complex sound in posterior superior temporal gyrus and beyond

Authors: *J. BEREZUTSKAYA[1], Z. V. FREUDENBURG[1], U. GÜÇLÜ[2], M. A. J. VAN GERVEN[2], N. F. RAMSEY[1];
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[2]Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: It is commonly known that Heschl's gyrus and posterior superior temporal gyrus (postSTG) are involved in low level processing of complex sounds. Whether low level auditory features (LLAFs) can predict neural responses outside of these regions is poorly understood. In our study we used music and speech LLAFs to model neural responses throughout the brain and explore the difference in the LLAF tuning profiles across brain regions.
Fifteen patients with drug-resistant epilepsy participated in an electrocorticography experiment. Patients watched a series of video fragments of Pippy Longstocking (1969), 6.5 min long. The soundtrack consisted of blocks of speech and music, 30 s each. The electrode grid was placed on the left hemisphere in 12 patients. All patients had perisylvian grid coverage and most had electrodes in frontal and motor cortices.
Linear kernel ridge regression was used to model high frequency band (HFB, 60-120 Hz) responses of each electrode based on the LLAFs. The LLAFs were obtained by filtering the audio spectrogram with a bank of 2D Gabor filters and resulted in a set of different spectral (ST) and temporal (TM) modulations of the audio. All LLAFs within a lag of -500-0 ms were used to model a single time point of the HFB signal. Separate models were trained for music and speech. Affinity propagation clustering was used to reveal groupings of electrodes with similar feature tuning profiles.
We observed that music and speech produced distinct distributions over LLAFs: speech exhibited a larger spread along the TM axis (1-8 Hz) than music. Coarse SMs (<1 cyc/oct) were mostly present in speech, fine SMs (>2 cyc/oct) mostly in music.
LLAFs predicted brain responses in postSTG well for both speech and music (ρ_{max}=.51 and ρ_{max}=.26, respectively; $p < .001$, Bonf corr). For speech only, accurate predictions extended beyond postSTG to anterior STG and Broca's area (BA).Clustering of electrodes with respect to their feature tuning profiles showed that postSTG was tuned to a wide range of TMs and SMs within a lag of 0-250 ms, whereas BA was tuned to a specific range of TMs (1-8 Hz) and coarse SMs (<1 cyc/oct) within a lag of 250-500 ms. Our results suggest that postSTG is accommodated to processing various LLAFs of complex sounds. However, BA seems to be specifically tuned to slowly changing features in the auditory signal, at a coarse spectral scale. In particular, such LLAFs form a core of the speech signal and are associated with its intelligibility. We also find evidence of a progressive analysis of the complex auditory input, from processing fine-grained TMs and SMs in postSTG in 0-250 ms after sound onset, to delivering coarse sound representations to BA in 250-500 ms after sound onset

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Nanosymposium

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Presentation Number: 18.05

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01MH107513

Title: Face and language processes are integrated by a neural hub including the subcentral area.

Authors: *J. HIRSCH[1,2,3,4], J. A. NOAH[1], X. ZHANG[1], S. DRAVIDA[1];
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Abstract: Eye-to-eye contact between two humans is a universal form of social interaction and a modulator of spoken communication. However, there is no comprehensive framework for the integration of neural systems that mediate combined face and language functions. We used dual-brain functional near-infrared spectroscopy (fNIRS) in conjunction with face-to-face eye-tracking to test the hypothesis that language processes would be engaged during eye-to-eye contact with a partner relative to mutual gaze of a face picture. In this study, 38 subjects (19 dyads) participated in dual-brain experiments with blocks of eye-to-eye contact and eye-to-picture events vs. rest. BOLD signals reflecting concentrations of deoxyhemoglobin (comparable to fMRI signals) were acquired via a Shimadzu LABNIRS system with 84 channels and simultaneous whole head coverage for two subjects. Contrast results revealed an eye-to-eye effect including the subcentral area and Broca's Area, a canonical node for productive language ($p<0.025$). In further support of the hypothesis, functional connectivity based on this eye-to-eye effect and anatomical seeds implicated in the processing of faces (e.g., fusiform and middle temporal gyri) revealed distributed networks that include the subcentral area and superior temporal gyrus (STG), a canonical node for receptive language and a component of Wernicke's Area ($p<0.025$). Although not previously described, these findings are consistent with a primary role for the subcentral area in face and language processing. The subcentral area (BA43) is adjacent to the STG at the junction of the inferior end of the central sulcus and is located between the pre- and post-central gyri[1] in face-sensitive topography. The discovery of functional links between the subcentral area and Wernicke's and Broca's Areas during eye-to-eye contact suggests a neural complex that dynamically integrates meaning from faces and eyes into core systems that serve language and social processes. These findings are consistent with a model where a continuous stream of face information is integrated with hierarchical feature recognition processes to perform "on-line" evaluations, sensory-motor responses, and multiple cognitive functions.
1. Garey, L. J. (1994). *Brodmann's localization in the cerebral cortex*. London: Smith-Gordon.

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Nanosymposium

018. Neural Mechanisms of Language

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Topic: H.02. Human Cognition and Behavior

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Title: Using a novel Local Heterogeneity Regression method to index orthographic lexical representations.

Authors: ***J. J. PURCELL**, B. RAPP;
Johns Hopkins Univ., Baltimore, MD

Abstract: Studies have identified a portion of left ventral occipitotemporal cortex (vOTC) as critical for orthographic representation (Dehaene and Cohen 2011). There is typically a lower neural response for high vs. low frequency words and pseudowords in this area suggesting an association with lexical orthographic processing (Kronbichler et al. 2004). Recent work suggests that orthographic representations become sparser with learning, which is reflected in more heterogeneous neural responses (Glezer et al. 2015). We introduce a novel Local-Heterogeneity Regression (Hreg) Analysis to examine the sparcity of representations by quantifying local neural heterogeneity. We apply this approach to reading data for high frequency (HF) and low frequency (LF) words, and pseudowords (PW). We acquired block design, reading fMRI data for the following conditions (N=31): HF, LF, PW, consonants, and checkerboards. Two analyses were performed: (1) A traditional univariate analysis; (2) A whole brain, Local-Hreg search-light analysis. Within each search-light a general psychophysiological interaction analysis (gPPI; McLaren et al., 2012) was performed using the center voxel to compare with surrounding voxels. Local-Hreg indexes the mean condition-specific voxel-to-voxel interactions within each searchlight: the lower the average interaction, the higher the local heterogeneity. For both analyses we performed comparisons for HF > LF and HF>PW. Figure 1.A reports left hemisphere frontal and vOTC clusters that show greater activation for LF than HF. Figure 1.B depicts that a portion of the left vOTC cluster identified in Analysis 1 was more heterogeneous for HF than LF. A similar finding was observed for HF>PW for both analyses. In sum, despite lower BOLD response to HF, there is higher local heterogeneity for HF than LF specifically within the left vOTC. This work provides a novel approach for examining the tuning/compactness of highly practiced and well-learned orthographic representations, and has applications for probing the neural dynamics of representation and learning more generally.

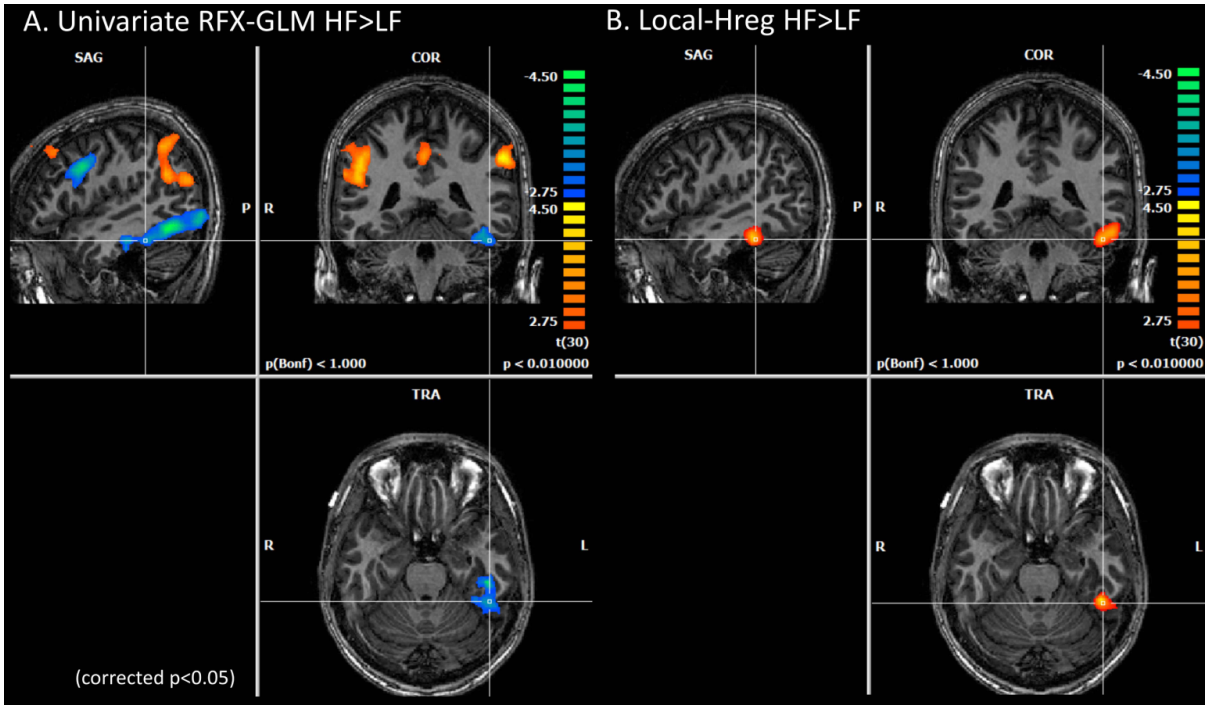

Figure 1: A. Univariate Random Effects GLM results for HF>LF. Blue clusters depict regions where LF is > than HF. B. Local-Hreg results for HF>LF. The red cluster depicts regions where HF are more heterogeneous neural responses than LF. For both A and B: Cross-hair centered on same left vOTC voxel in A and B; p<0.01 voxel-wise threshold and a cluster threshold of 100. N= 31 (neurologically intact; 17 F; mean age=25).

Disclosures: J.J. Purcell: None. **B. Rapp:** None.

Nanosymposium

018. Neural Mechanisms of Language

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Presentation Number: 18.07

Topic: H.02. Human Cognition and Behavior

Support: The Spanish Ministry of Economy and Competitiveness RYC2011-08433 and PSI2013-46334

Title: Whole-brain fMRI activity at a high temporal resolution: A novel analytic technique

Authors: *N. JANSSEN[1,2], J. HERNÁNDEZ CABRERA[1,3];
[1]Univ. De La Laguna, La Laguna, Spain; [2]Inst. of Biomed. Technologies, La Laguna, Spain; [3]Basque Ctr. for Cognition, Brain and Language, San Sebastian, Spain

Abstract: A central question in neuroscience concerns how the changing neural activity in the brain produces our thoughts, feelings, and behavior. Answering this question will require brain-imaging techniques with high spatial resolution, high temporal resolution, and with whole-brain coverage. Current human brain-imaging techniques do not readily meet these three requirements. Here we introduce a new analytic framework that for the first time reveals dynamic, time-variant videos of whole-brain functional MRI (fMRI) activity with a maximum temporal resolution on the order of several tens of milliseconds. The new method uses the veridical MRI slice acquisition times for signal extraction and thereby dramatically increases the temporal accuracy and resolution over current gold-standard methods.
Here we showcased the new framework in three studies. In the first study, we compared the new framework to the standard method on the basis of fMRI data collected from 30 participants as they performed a slow, event-related picture naming task. We extracted the fMRI signal during picture naming across a 12.5 s epoch at approximately 700 ms temporal resolution for every voxel in the brain. We show that the new technique yielded improved signal detection and a better characterization of task-based functional connectivity. In the second study, 30 different participants performed a blocked picture naming task in which stimulus presentation rate was manipulated. We extracted the whole-brain fMRI signal across a 28 s epoch at 1 second temporal resolution. We show that the temporal dynamics of activity in the dorsal Anterior Cingulate Cortex (dACC) is consistent with a context driven anticipatory role. In the final study, we examined the temporal dynamics of the task-positive and task-negative networks observed in the second study, and how such networks related to individual differences.
The new framework opens up new avenues for research. The high accuracy and temporal resolution of the proposed framework brings the possibility of integrating fMRI research with established intracranial electrophysiological recordings. The new framework provides a new opportunity for understanding how the dynamic changes in whole-brain neural activity relate to healthy behavior and neurological disease.

Disclosures: **N. Janssen:** None. **J. Hernández Cabrera:** None.

Nanosymposium

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Topic: H.02. Human Cognition and Behavior

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Title: The visual word form area is highly specialized for processing real words

Authors: *W. D. STEVENS[1], N. KHAN[1], D. J. KRAVITZ[2], C. S. PENG[2], M. H. TESSLER[2], A. MARTIN[2];
[1]Psychology, York Univ., Toronto, ON, Canada; [2]Natl. Inst. of Mental Hlth., NIH, Bethesda, MD

Abstract: The visual word form area (VWFA) is a region in the left occipitotemporal sulcus of literate individuals thought to be specialized for visual word recognition. However, its functional specificity and connectivity are debated: while there is some evidence of specificity for real words in the VWFA, other evidence suggests that it is a general visual processor, useful for visual discrimination of detailed high spatial frequency stimuli of any kind, and that it responds to a wide range of visual and non-visual word and non-word stimuli. It is a critical region for testing hypotheses about the nature of cortical functional organization, as written language is too recent an invention to have influenced genetic determinants of brain organization. If the VWFA is specialized for word processing, it should show preferential functional connectivity with language regions, critically, including the left planum temporale in the posterior superior temporal gyrus (Wernicke's area). We hypothesized that the VWFA is specialized for processing of real words by virtue of its preferential functional connectivity with Wernicke's area. We argue that the best demonstration of specialization for real words is the ability to distinguish them from pseudowords - non-word letter strings that respect the phonotactics of language - as they are indistinguishable from real words at a sublexical level. We used a combination of advanced fMRI analysis techniques - including individual functional localization, repetition suppression analysis, multivoxel pattern analysis (MVPA), and high-resolution resting-state functional connectivity (RSFC) - to assess the functional specificity and connectivity of the VWFA. An independent blocked multi-category functional localizer was used to define multiple regions of interest (ROIs), including the VWFA and several stringent control ROIs. In a separate session, participants were scanned with fMRI while performing a semantic classification task with multiple categories of pictures and words. Repetition suppression analyses and MVPA both demonstrated that the VWFA discriminated words from pseudowords better than among other categories of visual stimuli, and could do so better than the control ROIs. Further, the strength of RSFC of the VWFA with Wernicke's area predicted individual differences in performance on the semantic classification task for words, but not other categories of visual stimuli. Our results demonstrate that the VWFA is specialized for processing real words, and are consistent with the idea that experience dependent strength of functional connectivity of the VWFA with language regions drives this specialization.

Disclosures: W.D. Stevens: None. **N. Khan:** None. **D.J. Kravitz:** None. **C.S. Peng:** None. **M.H. Tessler:** None. **A. Martin:** None.

Nanosymposium

018. Neural Mechanisms of Language

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Presentation Number: 18.09

Topic: H.02. Human Cognition and Behavior

Title: Abstract concepts and neuroplasticity in bilinguals and multilinguals

Authors: *M. ORKODASHVILI;
Vanderbilt Univ., Tbilisi, Georgia

Abstract: The paper discusses the existence of higher brain plasticity in bilingual and multilingual people, especially children. The hypothesis is tested on the basis of studying abstract concept analysis and transfer, explanation and understanding of abstract concepts in different languages by multilingual and bilingual individuals.
It is assumed that transferring, explaining and understanding an abstract concept across languages is more difficult than transferring, explaining and understanding concrete concepts (i.e. concrete nouns, specific activity verbs, and so forth). Multilingual and bilingual individuals commonly encounter this challenging task of understanding and explaining abstract concepts (often so-called) *untranslatables* in different languages.
As a result of this systematic mental exercise, bilingual and multilingual individuals develop two important advantageous features of cognitive capacity: 1) quicker response time to understanding abstract concepts; and 2) multitasking ability of understanding, decision making and reacting (or proacting) to simultaneous multiple tasks, simultaneous translation, interpretation and quicker interlocutor anticipation being the most illustrative examples in this case.
The paper tests hypothesis by the method of analysing response times and multitasking abilities in 20 bilingual and 20 multilingual individuals (10 being children under the age of 18 in each group).

Disclosures: M. Orkodashvili: None.

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Alzheimer's Association BAND-9665

Title: Longitudinal decline in speech production in Lewy body spectrum disorder

Authors: *S. ASH, C. JESTER, K. FIRN, C. YORK, N. MIN, O. L. KOFMAN, C. T. MCMILLAN, M. GROSSMAN;
Neurol, Univ. Pennsylvania Sch. Med., Philadelphia, PA

Abstract: Lewy Body Spectrum Disorder (LBSD) comprises a range of conditions, including Parkinson's disease (PD), PD with dementia (PDD), and Lewy body disease (LBD), all characterized by the presence of Lewy body inclusions. These conditions are generally regarded as movement disorders, but they are often accompanied by cognitive deficits. A variety of language impairments are reported in LBSD, generally attributed to declining motor function. We hypothesize that language impairments in LBSD are due in part to cognitive deficits, not exclusively to motor deficits. To test this hypothesis, we investigated longitudinal decline in the language performance of LBSD patients and related the observed changes to neuroimaging evidence. We examined the speech production of 23 patients with Lewy body spectrum disorder, including non-demented PD patients (n=15), patients with dementia (PDD or LBD, n=8), and 20 age- and education-matched controls. All patients provided a description of the Cookie Theft picture from the BDAE (Goodglass & Kaplan, 1972) at least twice (at Time 1 and Time 2), at an average interval of about 36 months. The Cookie Theft descriptions were recorded, transcribed, and analyzed for features of grammar (mean length of utterance), discourse (adequacy of reported content), and speech fluency (speech rate, in words per minute). Average disease duration at the time of the first recording was approximately 11 years. We performed regressions of features of language performance with cortical volume in regions of interest (ROIs) that are important for language and motor functioning in a subset of all the LBSD patients (n=11). Non-demented LBSD patients did not differ from controls on speech measures. However, LBSD patients with dementia exhibited significant decline in their performance from Time 1 to Time 2 in grammar, discourse, and fluency. Regression analysis in ROIs known to be involved in language functioning showed that reduced cortical volume at Time 1 was associated with impaired language performance at Time 2. This was seen in left inferior frontal gyrus for utterance length, in left transverse temporal gyrus for content, and in right prefrontal regions for speech rate. There was no significant regression of any of the language variables with cortical

volume in motor areas. We conclude that language decline is observable in LBSD and is related to a network of brain regions that play a role in language production. Longitudinal analysis is valuable because reduced cortical volume may be a predictor of future deterioration in language capabilities.

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Nanosymposium

018. Neural Mechanisms of Language

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ANR program "Investissements d'Avenir" ANR-10-LABX-0083

Title: Perception of non-native sounds in a second language: Electrophysiological evidence of neuroplasticity in the phonological system

Authors: *K. HEIDLMAYR[1], E. FERRAGNE[1], F. ISEL[2];
[1]Paris Diderot – Sorbonne Paris Cité Univ., Paris, France; [2]Lab. MoDyCo/CNRS, Univ. Paris Ouest Nanterre la Défense – Paris Lumières, Paris, France

Abstract: Second language learners frequently encounter difficulty in perceiving specific non-native sound contrasts. This phenomenon called phonological deafness rather occurs if the second language (L2) is learned after early childhood and is quite persistent even when high L2 proficiency is attained (Dupoux et al., 2008). However, if the neuronal underpinnings of phonological processing are plastic to a certain degree, late L2 learners should be able to reach the capacity to distinguish non-native phonemic contrasts (Best & Strange, 1992; Flege et al., 1997; Iverson et al., 2012). In the present study, our goal was to examine the extent to which the phonological system in late L2 learners is adaptable. We designed an ERP experiment in which the capacity to discriminate second language phonemic contrasts mediated lexical access. We used a semantic violation paradigm in which the difference between semantically congruent and incongruent items was implemented by a phonemic contrast that was unique to the second language, English, but absent in the first language, French (e.g., /ɪ/ - /i/: *ship – sheep*). Twelve young adult native speakers of French with intermediate proficiency in English participated in the ERP experiment. Participants heard sentences that contained either a semantically congruent

item (e.g., *The anchor of the* ship *was let down*) or an incongruent one (e.g., ***The anchor of the* sheep *was let down*) and were asked to perform a grammaticality judgement. Preliminary results reveal that second language learners of English showed a larger centro-parietal negativity between 300-500 ms after the onset of semantically incongruent words as compared to congruent target words, i.e. an N400 effect. This finding indicates that L2 learners were sensitive to the semantic incongruency mediated by a phonemic contrast. Critically, the N400 effect size varied as a function of L2 proficiency, i.e. the more proficient the participants, the larger the N400 effect size. Thus, the sensitivity to phonemic contrasts of a second language seems to play a significant role in lexical access. With an increasing capacity to discriminate second language phonemic contrasts, the access to lexical information is facilitated. These findings show that even late learners of a second language can develop a perceptual sensitivity to discriminate non-native sound contrasts, at least at the segmental phonological level, which also indicates that neuroplasticity in the phonological system allows for a certain adaptation to linguistic environmental constraints. Further investigations should explore how targeted training can improve the sensitivity to second language phonemic contrasts.

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Nanosymposium

019. Genetic Techniques

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NIH P50 MH106933

NIH S10 RR028832

NIH T32 AG000222

NIH T32 GM007753

Title: Somatic mutation in single human neurons tracks developmental and transcriptional history

Authors: *M. B. WOODWORTH[1], M. A. LODATO[1], S. LEE[2], G. D. EVRONY[1], B. K. MEHTA[1], A. KARGER[3], S. LEE[2], T. W. CHITTENDEN[3], A. M. D'GAMA[1], X. CAI[1], L. J. LUQUETTE[2], E. LEE[2], P. J. PARK[2], C. A. WALSH[1];
[1]Div. of Genet. and Genomics, Children's Hosp. Boston, Boston, MA; [2]Dept. of Biomed. Informatics, [3]Res. Computing, Harvard Med. Sch., Boston, MA

Abstract: Neurons live for decades in a post-mitotic state, their genomes susceptible to DNA damage. Here we survey the landscape of somatic single-nucleotide variants (SNVs) in the human brain. We identified thousands of somatic SNVs by single-cell sequencing of 36 neurons from the cerebral cortex of three normal individuals. Unlike germline and cancer SNVs, which are often caused by errors in DNA replication, neuronal mutations appear to reflect damage during active transcription. Somatic mutations create nested lineage trees, allowing them to be dated relative to developmental landmarks and revealing a polyclonal architecture of cerebral cortex. Thus, somatic mutations in the brain represent a durable and ongoing record of neuronal life history, from development through post-mitotic function.

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Nanosymposium

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Leona M and Harry B Helmsley Charitable Trust

JPB Foundation

G Harold and Leila Y Mathers Charitable Foundation

Title: Contributions of LINE-1 retrotransposons to diversity in the primates

Authors: *A. M. DENLI[1], I. NARVAIZA[1], B. E. KERMAN[2], M. PENA[1], C. BENNER[1], M. C. N. MARCHETTO[1], J. K. DIEDRICH[1], A. ASLANIAN[1], J. MA[1], J. J. MORESCO[1], L. MOORE[1], T. HUNTER[1], A. SAGHATELIAN[1], F. H. GAGE[1];
[1]LOG-G, Salk Inst., La Jolla, CA; [2]Histology and Embryology Dept., Istanbul Medipol Univ. Sch. of Med., Istanbul, Turkey

Abstract: What are the molecular underpinnings of diversity? The answer to this question has important implications for human health and biology: variations in disease predisposition and response to pathogens and drug therapies play roles in lifespan and quality of life. A comprehensive molecular understanding of diversity and evolution requires the availability of genomic sequences, well-annotated transcriptomes and proteomes, as well as experimental models for functional genomics. This talk will summarize our recent efforts in the latter two. Transposable elements (TEs) are mobile genetic elements that can alter their chromosomal locations in the host genomes and occupy nearly 45% of the human genome. LINE-1s, as the sole autonomously active retrotransposons in humans, continue to diversify our genomes. We have recently shown that LINE-1 5'UTR contains a primate-specific open reading frame (ORF) in the antisense orientation that we named ORF0. This ORF is present in more than 3,000 loci across human and chimpanzee genomes and forms fusion proteins with proximal exons, leading to insertion-specific novel contributions to the proteome. Interestingly, expression of ORF0 is enriched in stem cells. The role of TE open reading frames in cell biology and gene regulation will be discussed.

Disclosures: **A.M. Denli:** None. **I. Narvaiza:** None. **B.E. Kerman:** None. **M. Pena:** None. **C. Benner:** None. **M.C.N. Marchetto:** None. **J.K. Diedrich:** None. **A. Aslanian:** None. **J. Ma:** None. **J.J. Moresco:** None. **L. Moore:** None. **T. Hunter:** None. **A. Saghatelian:** None. **F.H. Gage:** None.

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The Hewitt Foundation

Title: L1-associated genomic regions are mutated in somatic cells of the healthy human brain

Authors: *J. A. ERWIN[1], A. C. M. PAQUOLA[1], R. LASKEN[2], F. H. GAGE[1];
[1]Salk Inst., La Jolla, CA; [2]JCVI, La Jolla, CA

Abstract: The healthy human brain is a mosaic of varied genomes. L1 retrotransposition is known to create mosaicism by inserting L1 sequences into new locations of somatic cell genomes. Using a machine learning-based, single-cell sequencing approach, we discovered that L1-associated somatic variants (LASVs) are actually comprised of two classes: L1 retrotransposition insertions and retrotransposition-independent L1-associated variants. Retrotransposition- independent rearrangements within inherited L1s resulted in the deletion of proximal genomic regions. These rearrangements were resolved by microhomology-mediated repair, which suggests that L1-associated genomic regions are predisposed to somatic CNVs in the brain. We demonstrate that LASVs affect at least 36% of the cells in the healthy brain and that the location of germline L1s is a heritable genetic contributor to somatic mosaicism in the brain.

Disclosures: **J.A. Erwin:** None. **A.C.M. Paquola:** None. **R. Lasken:** None. **F.H. Gage:** None.

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Annette C. Merle-Smith

Title: Early life experience drives somatic mosaicism of the mouse brain

Authors: *T. A. BEDROSIAN, C. QUAYLE, N. NOVARESI, E. JENNIFER, A. PAQUOLA, F. H. GAGE;
Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Different cells within an individual contain different DNA sequences, a phenomenon known as somatic mosaicism. Such genomic variation arises from a variety of sources, including de novo copy number variants, replication errors, and DNA transposons. Somatic mosaicism was recently identified as a feature of normal, healthy brains. It has been suggested that somatic mutations contribute to brain plasticity, but it is unclear whether neuronal DNA sequences can be altered in response to environmental experience. Here we investigated whether early life experience mediates copy number of Long Interspersed Nuclear Element-1 (LINE-1 or L1) retrotransposons in specific regions of the mouse brain. L1 retrotransposons mobilize in the genome and insert copies of themselves into new locations, where they may affect gene expression or cellular function. We studied differences in maternal care as a model of early life experience. Rodents exhibit natural variations in maternal behavior that influence the neurodevelopment and adult behavior of their offspring. We developed assays for droplet digital PCR to address the hypothesis that differences in maternal care influence L1 copy number in the brain. By manipulating maternal care, we established a direct association of L1 copy number with experience. Further, we studied methylation of L1 elements in response to variations in maternal care as a potential mechanism for their expression and mobilization. Taken together, our observations indicate that early life experience drives somatic variation in the genome via L1 retrotransposons.

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH grant R01 MH100914

Title: Somatic genomic variation in developing human brain

Authors: A. ABYZOV[1], T. BAE[1], J. MARIANI[2], L. TOMASINI[2], A. AMIRI[2], B. ZHOU[4], D. FRANJIC[3], N. SESTAN[3], A. E. URBAN[4], *F. M. VACCARINO[2];

[1]Mayo Clin., Rochester, MN; [2]Child Study Ctr., [3]Neurosci., Yale Univ., New Haven, CT; [4]Stanford Univ., Palo Alto, CA

Abstract: Somatic mutations are changes in DNA sequence or copy number that occur after fertilization. Somatic mutations could cause brain disorders, add or subtract risk to single gene disorders, or play an adaptive function in brain ontogenesis and evolution. Both single nucleotide variations (SNVs) and copy number variations (CNVs) have been detected in neurons and glia of the mature brain. However, their frequency is debated, and their origin unclear. In particular, it is not known whether somatic variants occur during embryonic brain development or accumulate during aging. To answer these questions, we isolated single progenitor cells from the ventricular and subventricular zones of the cerebral cortex (n=41) and basal ganglia (n=46) from 3 prenatal human brains, and after clonal expansion we obtained the whole genome sequence (WGS) of their DNA. Comparisons of WGS between clonal populations and their respective brain tissue, as well as clonal populations with each other, revealed 1-6 CNVs, dozens of InDels and several hundred SNVs per cell. Validation experiments revealed a low rate of false positive due to sequencing error and confirmation experiments by digital PCR and locus capture and deep re-sequencing revealed that most of these mutations do not arise in vitro during clonal expansion but represent true somatic mosaicism in brain progenitor cells. The frequency spectrum of these variants ranges from 20-35% to a fraction of a percent, and in general the cortex displays an order or magnitude more somatic variants than basal ganglia. Hence, frequent and rare somatic variants are already present in all brain progenitor cells at prenatal al stages of development at similar frequencies to those detected in adulthood, suggesting an early origin for brain somatic mosaicism, perhaps as an aftereffect of the extensive cell divisions that occur in brain progenitor cells. This unsuspected richness of genomic mosaicism may play a role in normal and perhaps aberrant neuronal development.

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Support: NIH grant R01MH094740

Title: Very-deep whole-genome sequencing based detection and analysis of mosaic transposable element insertions in human brain tissue

Authors: *A. E. URBAN[1], X. ZHU[1], B. ZHOU[2], R. PATTNI[2], A.-S. FISTON-LAVIGNE[3], D. PETROV[2], M. SNYDER[2], D. LEVINSON[2];
[1]Psychiatry and Genet., [2]Stanford Univ., Palo Alto, CA; [3]Univ. of Montpellier, Montpellier, France

Abstract: Mobile elements (MEs) comprise a large portion of the human genome and some retain their capacity for transposition. Somatic retrotranspositions have been associated with several nervous system diseases, suggesting they may have a significant impact on the developing brain. Previous studies have used target capture- or single cell-sequencing methods. The results strongly suggest the presence of somatic ME mutation in human brain. However, beyond this proof of principle most fundamental questions are still unanswered. For example it is not known how frequently new mobile element insertions (MEIs) occur and during which developmental phase(s), or which proportions of cells, cell types or brain regions are affected. We developed an unbiased very-deep whole-genome sequencing-based approach to discover and quantify somatic MEIs without using target capture or whole-genome amplification methods. Using whole-genome paired-end sequencing we sequenced DNA from postmortem brain from 5 adult subjects and one fetal brain, after separating neuronal from non-neuronal cell fractions by either fluorescence activated sorting or immunopanning (we also sequenced DNA from non-neuronal tissue from each subject). We sequenced each fraction to 200x genome-wide sequencing depth (i.e. for a total of more than 100 whole-genome sequence equivalents). We extensively modified an existing ME calling algorithm to identify novel MEIs. Across the subjects we identified potentially mosaic MEIs broken down into categories of confidence as follows: more than 40 high-support MEIs, more than 800 medium-support MEIs and thousands of low-support MEIs. To validate predicted MEIs we used custom oligonucleotide capture for targeted re-sequencing of over 2700 predicted novel MEIs at >2000x depth. Initial analysis showed high mappability of the resulting captured sequence and a substantial validation rate. For example for high-support calls targeted re-sequencing showed sensitivity of 85-100% and specificity of 67-100%. Validation rates will be further evaluated with digital droplet PCR, also to quantify the degree of mosaicism for each novel MEI. This project uses very-deep whole-genome sequencing to address more comprehensively the extent and precise genomic localization of somatic retrotransposition of mobile elements in human brain cells. This phenomenon represents an additional mechanism by which genomic variation can influence brain development and disease. Methods for analysis of novel ME sequences in deep whole-genome sequencing will be needed to fully characterize this type of mutation in diverse tissues and in subpopulations of cells using cell sorting techniques.

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Title: DNA double strand breaks in human induced pluripotent stem cell-derived neurogenesis

Authors: *N. MICHEL[1,2], U. B. MAJUMDAR[2], W. M. CLARK[2], B. BANERJEE[2], M. J. MCCONNELL[2,1,3,4];
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Abstract: Somatic mosaicism is a common consequence of normal development. DNA repair is simply not perfect, and each cell's genome incurs continuous DNA damage as a consequence of transcription, replication, and other cell biological stresses. Although somatic mosaicism has been reported in many tissues, it is particularly noteworthy in the brain for two reasons. First, by contrast to other organs with regular cell replacement, the vast majority of an individual's neurons are with that individual for life. And second, neural circuits give rise directly to behavioral phenotypes. Brain somatic mosaicism, now revealed and tractable due to advances in single cell 'omic approaches, has emerged as an intriguing and unexplored aspect of neuronal diversity.
Neuronal copy number variations (CNVs), like most CNVs, are likely brought about by DNA repair mechanisms acting on transcription- or replication-induced DNA damage. Additional, and possibly related, mechanisms include retrotransposition and electrophysiological activity. In previous work, we showed that large CNVs and aneuploidy were prevalent in human induced pluripotent stem cell (hiPSC)-derived neurons, but CNVs were infrequent in hiPSC-derived neural progenitor cells (NPCs). Accumulating evidence suggests that DNA double strand break (DSB) clusters occur at long, neuronal genes, and that these may lead to "hot spots" for neuronal CNVs. Neuronal genes are over-represented among longest genes in the human genome. Moreover, many of these genes encode synaptic proteins (e.g., neurexins) that are known to effect neural circuit formation and are also associated with neuropsychiatric disease. We have quantified DNA DSB formation in hiPSC-derived NPCs and neurons in proliferative and differentiating conditions, and in response to various perturbations (e.g., topoisomerase inhibition, replication stress). Ongoing experiments aim to identify the location of distinct DNA

DSB clusters in each setting and determine if DNA DSB clusters portend CNV formation at specific loci (i.e., hot spots).

Disclosures: **N. Michel:** None. **U.B. Majumdar:** None. **W.M. Clark:** None. **B. Banerjee:** None. **M.J. McConnell:** None.

Nanosymposium

019. Genetic Techniques

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Time: Saturday, November 12, 2016, 1:00 PM - 4:15 PM

Presentation Number: 19.08

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH grant U01 MH103365

Title: Gene regulation in human iPSC-derived organoids

Authors: ***A. AMIRI**[1], G. COPPOLA[1], S. SCUDERI[1], D. FRANJIC[2], S. LIU[3], A. SZEKELY[4], N. SESTAN[2], M. GERSTEIN[3], S. WEISSMAN[4], F. M. VACCARINO[1];
[1]Child Study Ctr., [2]Neurosci., [3]Mol. Biol. and Biophysics, [4]Genet., Yale Univ., New Haven, CT

Abstract: Little is known about the genetic programs that are active during prenatal differentiation of forebrain human progenitors into neurons of the human cerebral cortex and to what extent they can be recapitulated in vitro using stem cell models. As part of the PsychENCODE project, we are validating induced pluripotent stem cells (iPSC) as models of human brain development. In particular, we are comparing cellular and molecular aspects of neuronal differentiation in iPSC-generated organoids with post-mortem human brain tissue from the same individual. Specifically we will examine (1) transcriptomes (2) gene regulatory regions (3) translated RNAs and (4) cellular phenotypes. To this end, we harvested tissue for RNA, ChIP-seq, ribosome footprinting and histology from prefrontal cortex progenitors and neurons from five human prenatal brain specimens and established induced iPSC lines from the skin of each specimen. Two iPSC clones per specimen were differentiated into telencephalic organoids using our established protocol (Mariani et al., 2015). Differential gene expression (DGE) by RNA-seq comparing the fetal fibroblast-derived iPSCs with previously generated iPSCs derived from adult fibroblasts revealed ~80 differentially expressed genes. Functional annotation of the DGE revealed extracellular region-related terms (glycoprotein, signal peptide, extracellular matrix) as significantly enriched. No enrichment was found for cell cycle, pluripotency or cell differentiation. Next, we found 604 differentially expressed genes between fetal and adult iPSC-derived organoids at terminal differentiation day 30 (n=4 lines), including a large number of histones mRNAs. Functional annotation of the DGE revealed significant enrichment for

nucleosome organization, protein-DNA complex assembly and DNA methylation. At present, we do not have any evidence supporting a differential ability of the fetal- and adult-derived iPSC to differentiate into neural tissue. In fact, immunostaining with markers for different cell types found that fetal fibroblast derived organoids have similar growth rates, proliferation, and similar number of radial glial cells and layer specific cortical neurons compared to adult fibroblast-derived organoids. Similarly, when we mapped our transcriptomes against the BrainSpan dataset, we found highest correlations with neocortex and with similar stages of prenatal brain development for both prenatal and adult iPSC-derived organoids. In conclusion, prenatal and adult fibroblasts appear be equally capable of generating pluripotent iPSC lines that can recapitulate early stages of cortical development in vitro.

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Nanosymposium

019. Genetic Techniques

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIMH Grant R21MH103877

VA Merit Grant BX002343

Title: DNA methylation differences between two major neuronal subtypes in the human prefrontal cortex

Authors: A. KOZLENKOV[1], P. ROUSSOS[1], M. WANG[2], B. ZHANG[2], Y. L. HURD[1], S. RUDCHENKO[3], M. BIBIKOVA[4], B. KLOTZLE[4], E. V. KOONIN[5], M. WEGNER[6], *S. DRACHEVA[7,1];
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Abstract: The brain is built from a large number of cell types which have been historically classified using location, morphology, and molecular markers. Recent research suggests an important role of epigenetics in shaping and maintaining cell identity in the brain. To elucidate

the role of DNA methylation in neuronal differentiation, we developed a novel method to separate neuronal nuclei from the autopsy specimens of the human prefrontal cortex into two sub-populations containing glutamatergic (GLU) projection neurons or the medial ganglionic eminence (MGE)-derived GABA neurons, respectively, using fluorescence-activated cell sorting (FACS). We detected major differences in CpG, non-CpG and hydroxymethylation (hCpG) between the neuronal subtypes. In particular, a significantly greater number of undermethylated CpG sites in GLU vs. GABA neurons were identified. These differences, however, did not directly translate into differences in gene expression. Notably, a comparable number of undermethylated non-CpG sites were identified in GLU and GABA neurons, and non-CpG methylation was a better predictor of subtype-specific gene expression compared to CpG methylation. Regions that are differentially methylated in GABA and GLU neurons were significantly enriched for schizophrenia risk loci. Collectively, our findings suggest that functional differences between neuronal subtypes are linked to their epigenetic specification.

Disclosures: **A. Kozlenkov:** None. **P. Roussos:** None. **M. Wang:** None. **B. Zhang:** None. **Y.L. Hurd:** None. **S. Rudchenko:** None. **M. Bibikova:** None. **B. Klotzle:** None. **E.V. Koonin:** None. **M. Wegner:** None. **S. Dracheva:** None.

Nanosymposium

019. Genetic Techniques

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Presentation Number: 19.10

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: 1U01MH103392

P50 MH106934

Title: Functional genomics of human brain development and autism spectrum disorder

Authors: ***N. SESTAN**[1], S. POCHAREDDY[1], M. B. GERSTEIN[1], A. C. NAIRN[1], M. STATE[2]; [1]Yale Univ. Sch. Med., New Haven, CT; [2]Univ. of California San Francisco, San Francisco, CA

Abstract: Genetic and genomic investigations have yielded important findings as to the genetic contributions to major psychiatric illnesses, illustrating significant etiological heterogeneity, as well as cross-disorder overlap. It has also become clear that understanding how this genetic variation leads to alterations in brain development and function that underlies psychiatric disease pathophysiology will be greatly advanced by a roadmap of the transcriptomic and epigenetic landscape of the human cerebral cortex across key developmental windows. As part of

PsychENCODE Consortium, we performed time-, and region-, specific molecular profiling of control and autism spectrum disorder brains, including transcriptome, *cis*-regulatory elements and proteome. We analyzed and integrated these datasets to identify regional, developmental, and ASD-related processes to gain insight into underlying mechanisms. We are also harmonizing these multi-omic data with other psychENCODE studies, as well as other large scale data sets, such as BrainSpan, ENCODE, GTEx and Roadmap Epigenomics Project. We hope that this well-integrated resource across human brain development that will provide greater insights into pathophysiological understanding of autism spectrum disorder and other related neuropsychiatric disorders.

Disclosures: **N. Sestan:** None. **S. Pochareddy:** None. **M.B. Gerstein:** None. **A.C. Nairn:** None. **M. State:** None.

Nanosymposium

019. Genetic Techniques

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Presentation Number: 19.11

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH grant U01 MH103365

NARSAD Young Investigator Award to ST

Title: Molecular phenotyping of human iPSC organoids using CLARITY and 2-photon microscopy

Authors: ***S. TOMASI**, S. SCUDERI, A. AMIRI, G. G. ALTOBELLI, J. MARIANI, C. DAMBROT, G. COPPOLA, F. M. VACCARINO;
Child Study Ctr., Yale Univ., New Haven, CT

Abstract: To understand the role of gene regulation in human brain development and neuropsychiatric disorders, it is essential to develop cellular models of the human brain. Induced pluripotent stem cells (iPSCs)-derived brain organoids can be used to investigate the role of gene regulatory elements, noncoding RNA, and in general, noncoding disease associated gene variants in brain development and function. Organoids enable gradients of morphogenes and other extracellular cues to build up in the intercellular milieu and to interact with the genetic and epigenetic background of a given progenitor cell during the course of brain development. We have developed an iPSC-derived organoid model of the early human forebrain, where differentiation of cortical excitatory and inhibitory neurons can be studied in a reproducible

fashion, enabling a more precise identification of molecular events crucially involved in the specification of distinct neuronal subtypes. However, a precise assessment of protein and RNA expression in intact organoids is hampered by the limited penetration of molecular probes, therefore requiring the preparation of thin sections and greatly limiting the capacity of exploring molecular and cellular features in a 3D environment. Here, we labeled telencephalic excitatory and inhibitory lineages in using pLenti-CAMKII-GFP and pLenti-DlxI12b-BG-DsRed vectors, then used two-photon microscopy to image the genetically-encoded fluorescence at higher resolution in live forebrain organoids. Next, we used CLARITY to clear the organoids and perform immunostainings on the intact cell aggregates. Our current protocol enables a 3D reconstruction of GFP/DsRed filled cells allowing the analysis of axonal and dendritic arborization, dendrite length, synapse and spine distribution, as well as stereological counts of structures labeled by specific markers. Using these combined approaches, we aim at comparing intra-organoid layer cytoarchitecture and its emerging connectivity with parallel data from RNA-seq and ChIP-seq experiments, and develop new tools for linking the molecular and cellular features of organoids derived from different individuals.

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Nanosymposium

019. Genetic Techniques

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIMH U01 MH103392

Brain & Behavior Research Foundation

Title: Histone modification profiling in human brain

Authors: ***S. AKBARIAN**[1], Y. JIANG[2], M. KUNDAKOVIC[3], D. KAVANAGH[4], M. FROMER[4], S. SIEBERTS[5], B. LIPSKA[6], M. PETERS[5], P. SKLAR[4];
[1]Psychiatry, Icahn Sch. of Med. At Mount Sinai, New York, NY; [3]Psychiatry, [4]Genet. and Genomic Sci., [2]Icahn Sch. of Med. at Mount Sinai, New York, NY; [5]SAGE Bionetworks, Seattle, WA; [6]Brain Collection Core, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Background: The nervous system includes hundreds of residue-specific post-translational modifications (PTMs) of histones forming the nucleosome core that are often

regulated in cell type-specific manner. On a genome-wide scale, some of the histone PTM landscapes show significant overlap with the genetic risk architecture for several psychiatric disorders, fueling *PsychENCODE* and other large scale efforts to comprehensively map neuronal and non-neuronal epigenomes in hundreds of specimens. However, practical guidelines for efficient generation of histone ChIP-seq (chromatin immunoprecipitation followed by deep sequencing) datasets from postmortem brains are needed. **Methods**: Protocols and quality controls are given for the following: 1.) extraction, purification, and NeuN neuronal marker immunotagging of nuclei from adult human cerebral cortex, 2.) fluorescence-activated nuclei sorting, 3.) preparation of chromatin by micrococcal nuclease digest, 4.) ChIP for open chromatin-associated histone methylation and acetylation, and 5.) generation and sequencing of ChIP-seq libraries. **Results**: We present a ChIP-seq pipeline for epigenome mapping in the neuronal and non-neuronal nuclei from the postmortem brain. This includes a step-wise system of quality controls and user-friendly data presentation platforms. **Conclusion**: Our practical guidelines will be useful for projects aimed at histone PTM mapping in chromatin extracted from hundreds of postmortem brain samples in cell type-specific manner.

Disclosures: S. Akbarian: None. **Y. Jiang:** None. **M. Kundakovic:** None. **D. Kavanagh:** None. **M. Fromer:** None. **S. Sieberts:** None. **B. Lipska:** None. **M. Peters:** None. **P. Sklar:** None.

Nanosymposium

019. Genetic Techniques

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Presentation Number: 19.13

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Paul G. Allen

Title: An automated platform for single-cell electrophysiology and perturbation *In vivo*.

Authors: *L. LI[1], B. OUELLETTE[1], W. A. STOY[2], E. GARREN[1], T. DAIGLE[1], C. FOREST[2], H. ZENG[1];
[1]Allen Inst. For Brain Sci., Seattle, WA; [2]Georgia Inst. of Technol., Atlanta, GA

Abstract: Single-cell characterization and perturbation of neurons will provide knowledge critical to addressing fundamental neuroscience questions including the structure-function relationship and neuronal cell-type classification. Here we report a robotic platform for automatically performing single-cell experiments in vivo in deep brain tissues optically difficult to access. This platform automates ‘blind’ (non-visually guided) electrophysiology and single-

cell electroporation (SCE) to characterize in vivo neural responses and/or drive expression of extraneous genes in single neurons. Tested in mouse brain, our platform successfully revealed the full morphology of single infragranular neurons recorded in multiple neocortical regions, as well as deep brain structures, for example, ventral hippocampus with a much improved efficiency (47%), about 3-fold to manual experiments. Our platform thus can be used to obtain in vivo full morphology and electrophysiology and correlate them with genetics of single neurons in the brain.

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Nanosymposium

107. Mechanisms of Neurotransmitter Release

Location: SDCC 25A

Time: Sunday, November 13, 2016, 8:00 AM - 11:00 AM

Presentation Number: 107.01

Topic: B.06. Neurotransmitter Release

Support: National Basic Research Program of China

Natural Science Foundation of China

Title: Neuronal signaling at single active zone contained central synapses

Authors: ***J. SUN**[1,2], J. DAI[2], Q. ZHU[2], K. MA[2], P. SAH[3];
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Abstract: In the light of the Katz's quantal theory, the small all-or-none unit, termed miniature postsynaptic potential or current (mini), corresponding to presynaptic vesicle fusion,represents the building element or quantum of synaptic signal. However, justification of the nature of minis has never been completed as minis or single vesicle membrane capacitance is not identical in size and never follows the principle of invariance. It was proposed that each Katz's quantum could be composed of smaller subunits. However, this subquantal hypothesis was rigorously debated and ended in suspense because all the experimental and theoretical studies were not sufficient to support or exclude the existence of the subquantal element. The major arguments are: all the studies were based on histogram analyses and no confidence limits have been given to evaluate the significance of these subunits; increasing the sample size of recordings decreased, the prominence of subunit in histogram; the subunit sizes estimated from the different subsets of the same recording were different. We attribute these discrepancies to the less quantitative

analysis of quantal signals due to space-clamp problems and the large variation of the analyzed minis from multiple synapses. Here, we selectively observed the miniatures from single active zone contained axo-soma synapses and took the advantage of well electrical access of the somatic whole cell recording to quantitatively analyze single quantal events with much less variability of postsynaptic response. We recorded spontaneous mEPSCs at single synaptic boutons of early age developing calyx synapse, micrometer size Ca^{2+} uncaging induced mEPSCs at single boutons of juvenile calyx synapse and micrometer size channelrhodopsin-2 activation evoked mEPSCs at single boutons of mature PB-CeAL synapse and revealed the significant subquantalized synaptic transmission and thus calls for re-evaluating the precision and capacity of information procession at synapses.

Disclosures: **J. Sun:** None. **J. Dai:** None. **Q. Zhu:** None. **K. Ma:** None. **P. Sah:** None.

Nanosymposium

107. Mechanisms of Neurotransmitter Release

Location: SDCC 25A

Time: Sunday, November 13, 2016, 8:00 AM - 11:00 AM

Presentation Number: 107.02

Topic: B.06. Neurotransmitter Release

Title: Dissecting molecular mechanisms of synaptic and large dense-core vesicle docking and priming.

Authors: ***C. IMIG**, K.-N. M. MAN, J. RHEE, N. BROSE, S. M. WOJCIK, B. H. COOPER; Mol. Neurobio., Max Planck Inst. of Exptl. Med., Goettingen, Germany

Abstract: Secretory vesicle docking, priming and fusion are mediated by a complex molecular machinery. The precise molecular mechanisms that orchestrate membrane attachment of vesicles to the plasma membrane are experimentally challenging to dissect: Past studies have employed various preparation and culture methods, model organisms, cell types, fixation protocols, imaging and analysis approaches, and terminology, potentially explaining the many inconsistencies in the field. An accurate analysis of vesicle docking requires electron microscopy to measure intermembrane distances in the nanometer range. To assess the molecular mechanisms of large dense-core vesicle (LDCV) and synaptic vesicle (SV) docking, we prepared acute adrenal gland slices and hippocampal organotypic slice cultures from mouse mutants lacking key presynaptic proteins. Using a systematic approach combining rapid cryofixation, freeze-substitution, and three-dimensional electron tomography, we identified sequential steps in vesicle recruitment (tethering) and membrane attachment (docking). In synapses, SV docking is mediated by members of the Munc13/CAPS families and the neuronal SNAREs Synaptobrevin-2, SNAP-25 and Syntaxin-1, indicating that docked SVs comprise the readily-releasable pool

(RRP) of primed vesicles. In chromaffin cells, however, loss of Munc13 proteins has no effect on LDCV docking, indicating that the majority of docked LDCVs are not fusion-competent and that the functional RRP cannot be distinguished morphologically in this system. Our data therefore demonstrate distinct molecular mechanisms of LDCV and SV docking in chromaffin cells and hippocampal glutamatergic synapses, respectively.

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Nanosymposium

107. Mechanisms of Neurotransmitter Release

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Presentation Number: 107.03

Topic: B.06. Neurotransmitter Release

Support: NIH M060600

Title: Syntaxin-1a is in a closed conformation and bound to Munc18 where secretory granules dock in live cells.

Authors: ***W. ALMERS**, X. CHEN, L. WAN;
VOLLUM INSTITUTE, Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: When a secretory granule docks, it assembles a cluster of 50-70 syntaxin molecules in the plasma membrane (Barg S, PNAS 107, 20804). By mutations, we wished to learn which portions of syntaxin are important for its inclusion into clusters, and whether syntaxin exists in its closed or its open conformation. Granules were labeled with neuropeptide-Y-mCherry and their associated syntaxin clusters with syntaxin 1a-GFP. Live PC12 cells were imaged by TIRF microscopy. Syntaxin-1a-GFP or its mutants were expressed at low levels and competed for inclusion into clusters with a 2-fold excess of endogenous syntaxin. The brightness of clusters was measured and assumed to assay how effectively mutants competed. All mutants known to inhibit binding to Munc18 *in vitro* (Burkhardt P, 2008, EMBO J. 27, 923) also diminished the inclusion of syntaxin into clusters to varying degrees. This is consistent with syntaxin being in a complex with Munc18. Deletion of residues 2-19 (the so-called N-peptide) had only a modest effect. Deletions of the SNARE or the Habc domains caused clusters to be barely detectable (SNARE domain) or undetectable (Habc domain), implying that these mutants were almost excluded from clusters. To learn which syntaxin residues in the two domains were the most important, we considered the structure of a syntaxin-Munc18 complex (Misura KM, 2000, Nature 404, 355) and replaced residues known to contact Munc18 with alanines. Of the 16

Munc18-contacting residues in the SNARE domain, replacement of I233 was the most effective. The 13 Munc18-contacting residues in the Habc domain were mutated next. The most effective mutation was a double replacement that diminished the inclusion into clusters by 9 fold. Double replacements of other Munc18-facing residues had only modest effects, and those of residues facing outwards from the syntaxin-Munc18 complex had no effect. We next explored the interface between the SNARE and the Habc domains. A triple mutation of three residues in the SNARE domain strongly reduced syntaxin's inclusion into clusters. Inclusion could be rescued by a complementing triple mutation in the Habc domain. The rescue implies that the SNARE and Habc domains are in contact, and that syntaxin is in a closed conformation.

Disclosures: **W. Almers:** None. **X. Chen:** None. **L. Wan:** None.

Nanosymposium

107. Mechanisms of Neurotransmitter Release

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Topic: B.06. Neurotransmitter Release

Support: NeuroCure Cluster of Excellence Exc257

ERC advanced grant SynVGlut

German Research Council collaborative research grant SFB958

Title: Distinct functions of syntaxin-1 in neuronal maintenance, synaptic vesicle docking and fusion in mouse neurons

Authors: ***G. VARDAR**[1,2], S. CHANG[1,2], M. ARANCILLO[2], Y.-J. WU[2], T. TRIMBUCH[1,2], C. ROSENMUND[1,2];
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Abstract: Neurotransmitter release requires the formation of SNARE complexes by SNARE proteins syntaxin-1 (Stx1), synaptosomal-associated protein 25 (SNAP-25), and synaptobrevin-2 (Syb2). In mammalian systems, loss of SNAP-25 or Syb2 severely impairs neurotransmitter release; however, complete loss of function studies for Stx1 have been elusive due to the functional redundancy between Stx1 isoforms, Stx1A and Stx1B, and the embryonic lethality of Stx1A/1B double knockout (DKO) mice. Here we studied the roles of Stx1 in neuronal maintenance and neurotransmitter release in mice with constitutive or conditional deletion of Stx1B on an Stx1A null background. Both constitutive and postnatal loss of Stx1 severely

compromised neuronal viability *in vivo* and *in vitro* indicating an obligatory role of Stx1 for maintenance of developing as well as mature neurons. Loss of Munc18-1, a high-affinity binding partner of Stx1, also showed severely impaired neuronal viability but with a slower time course compared to Stx1A/1B DKO neurons, and exogenous Stx1A or Stx1B expression significantly delayed Munc18-1 dependent lethality. Additionally, loss of Stx1 completely abolished fusion-competent vesicles and severely impaired vesicle docking, demonstrating its essential roles in neurotransmission. Putative partial SNARE complex assembly with the SNARE motif mutant $Stx1A^{AV}$ (A240V,V244A) was not sufficient to rescue neurotransmission despite full recovery of vesicle docking and neuronal survival. All together, these data suggest that Stx1 has independent functions in neuronal maintenance and neurotransmitter release and complete SNARE complex formation is required for vesicle fusion and priming, whereas partial SNARE complex formation is sufficient for vesicle docking and neuronal maintenance.

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Nanosymposium

107. Mechanisms of Neurotransmitter Release

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Discovery Project Grant DP1093968

Title: *In vivo* single molecule imaging of syntaxin-1A reveals change in lateral mobility required for neurotranmsitter release

Authors: *A. D. BADEMOSI[1], L. ODIERNA[1], Y. CHAI[1], A. PAPADOPULOS[1], E. LAUWERS[2], O. ZALUCKI[1], M. TROUP[1], R. GORMAL[1], B. KOTTLER[1], J. STEVES[1], P. VERSTREKEN[2], V. ANGGONO[1], B. VAN SWINDEREN[1], F. MEUNIER[1];
[1]Univ. of Queensland, Brisbane, Australia; [2]VIB Ctr. for Biol. of Dis., Leuven, Belgium

Abstract: Syntaxin1A is a key protein involved in mediating synaptic transmission through its ability to form the SNARE complex with cognate partners - SNAP-25 and VAMP2. Syntaxin1A

molecules have been shown to be organized in nano-clusters in neuro-secretory cells that have important role in docking and priming of secretory vesicles. How molecules of Syntaxin1A are entering and exiting these nanoclusters by lateral diffusion and how stimulation affects their dynamic equilibrium in pre-synapse *in vivo* in unknown. To image single molecules of Syntaxin1A in live synapses, we generated a drosophila line constitutively expressing fluorescently tagged syntaxin1A (syx1A-mEos2) to carry out single particle tracking Photoactivated Localization Microscopy (sptPALM) on live drosophila larva neuromuscular junction (NMJ) using slightly oblique Total Internal Reflection Florescence (TIRF) microscopy. We examined the change in mobility and micro-domain organisation elicited by increased pre-synaptic activity using both optogenetic and thermogenetic tools. Here, we show that in drosophila larva motor nerve terminals, syx1A is also organized in nanoclusters. In sharp contrast to PC12 cells, the overall mobility of syx1A molecules were lower in live resting motor nerve terminals. Opto- and thermo-genetic stimulation led to an increase in syx1A mobility at the motor nerve terminal. Importantly, this change at the motor terminal was also promoted by concomitant expression of TeTx/LC. Our results suggests that the relative immobility of syx1A molecules within synapses is indicative of a high level of primed vesicles in live motor nerve terminals when compared with cultured neurosecretory cells. To further investigate the clinical role of syntaxin1A in general aesthetic, we perfused PC12 cells expressing syx1A-mEos2 with clinical doses of the general anesthetic - propofol (3uM); we observed a significant decrease it syx1A mobility. Co-expression of a truncated syx1A mutant lacking the transmembrane domain which produces behaviour resistance to propofol in *Drosophila* flies blocked the propofol effects on syx1A mobility. Our results suggest that changes in Syntaxin1A mobility are essential to underpin neurotransmitter release and decoy- or neurotoxin-induced manipulation leads to severe clinical defects in neurotransmission.

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Nanosymposium

107. Mechanisms of Neurotransmitter Release

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Topic: B.06. Neurotransmitter Release

Support: ISF 234/14

Title: The polybasic juxtamembrane stretch of syntaxin 1 has a role in Ca^{2+}-dependent evoked neurotransmitter release in PC12 cells

Authors: *I. LOTAN, D. SINGER-LAHAT, N. BARAK-BRONER, D. CHIKVASHVILI; Tel-Aviv Univ., Tel-Aviv, Israel

Abstract: Exocytosis mediated by the SNARE complex formation is a highly regulated process. Although the basic elements of the underlying machinery are well studied, the detailed mechanics are still poorly understood. It is well accepted that in order to enter SNARE complexes syntaxin must resume an 'open' conformation. Recently, we have developed a syntaxin 1A- based Fluorescence Resonance Transfer (FRET) probe that is able to be incorporated within endogenous SNARE complexes and reports transition of syntaxin between 'closed' and 'open' conformations related to exocytosis both in PC12 cells and hippocampal neurons. Using this probe we resolved two distinct syntaxin conformational transitions during membrane depolarization-induced exocytosis in PC12 cells: a partial 'opening' in the absence of Ca2+ entry and an additional 'opening' upon Ca2+ entry. The Ca2+-dependent 'opening' is abolished upon neutralization of a stretch of basic charges in the juxtamembrane region of syntaxin. Importantly, this stretch has been shown to interact with polyphosphoinositides including PI(4,5)P2 and has been implicated in clustering and segregation of syntaxin into distinct microdomains where synaptic vesicles undergo exocytosis.
Here, combining dynamic FRET measurements with ameprometric measurements and biochemical analysis in PC12 cells, we show that the polybasic stretch, beyond its involvement in the final phase of SNARE complex assembly, has a significant role in the regulation of Ca2+-dependent evoked neurotransmitter release via interaction with PI(4,5)P2 . Furthermore, phosphorylation of syntaxin by Casein kinase II may be involved.

Disclosures: I. Lotan: None. **D. Singer-Lahat:** None. **N. Barak-Broner:** None. **D. Chikvashvili:** None.

Nanosymposium

107. Mechanisms of Neurotransmitter Release

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Topic: B.06. Neurotransmitter Release

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Title: Molecular steps between Ca^{2+} influx, a conformational change of SNAP25, and vesicle fusion

Authors: Y. ZHAO[1], Q. FANG[1], S. SHARMA[1], *M. LINDAU[2,1];
[1]Nanoscale Cell Biol., Max-Planck-Institute for Biophysical Chem., Goettingen, Germany;
[2]Cornell Univ., Ithaca, NY

Abstract: The SNARE complex, including synaptobrevin-2, syntaxin and SNAP25, is thought to execute a large conformational change as it provides force and energy to drive the membrane fusion and exocytosis. A SNAP25 based FRET construct (SCORE: Snare COmplex REporter, a fusion protein containing SNAP-25, CFP and Venus) reported a transient conformational change indicated by rapid FRET increase preceding fusion and reversal of the increase within ~5 s after fusion (Zhao et al. 2013 PNAS 110: 14249). An improved version of SCORE (SCORE2) was generated replacing CFP by mCerulean-3. Coarse grained molecular dynamics simulations of SCORE2 in complex with syntaxin revealed an average distance of ~5.5 nm and κ^2 of ~0.27 between the fluorophores of the FRET pair. In SNAP25 KO embryonic mouse chromaffin cells stimulated in the whole cell patch clamp configuration by 200 ms depolarization pulses, viral expression of SCORE2 fully rescued the capacitance change to the level observed in wt cells, indicating that it fully supports fusion. In synaptobrevin $2^{+/+}$ cellubrevin$^{-/-}$ embryonic mouse chromaffin cells expressing SCORE2, 200 ms long depolarization pulses produced a robust capacitance increase and a transient FRET increase measured in TIRF excitation mode, with similar duration as that associated with individual fusion events. In contrast, no FRET change was detectable in synaptobrevin 2 / cellubrevin DKO cells, which also produced no detectable capacitance change. The conformational change following pulse stimulation reported by SCORE/2 therefore critically depends on the v-SNARE.
Using short (10 ms or 25 ms) pulses it was found that the FRET increase occurs coincident with the Ca^{2+} increase, which is maximal near the end of the pulse. To overcome the low time resolution of the imaging frames needed to collect sufficient signal intensity, event correlation microscopy (ECOM) was applied, which revealed that the FRET change and intracellular Ca^{2+} concentration increase are simultaneous within <5 ms. Our results suggest that following the Ca^{2+} stimulus, a rapid conformational change of SNAP25 is produced involving the v-SNARE synaptobrevin 2 followed by fusion, possibly with a Ca^{2+} dependent delay.

Disclosures: **Y. Zhao:** None. **Q. Fang:** None. **S. Sharma:** None. **M. Lindau:** None.

Nanosymposium

107. Mechanisms of Neurotransmitter Release

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Presentation Number: 107.08

Topic: B.06. Neurotransmitter Release

Support: R37MH63105

HCIA

Title: Molecular mechanism of the synaptotagmin-SNARE interaction in Ca^{2+}-triggered synaptic neurotransmitter release

Authors: *Q. ZHOU[1], A. BRUNGER[2];
[1]Mol. and Cell. Physiol., [2]Mol. and Cell. Physiology, Neurol. and Neurolog. Sciences, Structural Biol., Stanford Univ. / HHMI, Stanford, CA

Abstract: Synaptotagmin-1 and neuronal SNARE proteins have central roles in evoked synchronous neurotransmitter release; however, it is unknown how they cooperate to trigger synaptic vesicle fusion. Here we report crystal structures of Ca^{2+}- and Mg^{2+}-bound complexes between synaptotagmin-1 and the neuronal SNARE complex. All these structures share a large, specific, Ca^{2+}-independent and conserved interface. Tests of this interface by mutagenesis suggest that it is essential for Ca^{2+}-triggered neurotransmitter release in mouse hippocampal neuronal synapses and for Ca^{2+}-triggered vesicle fusion in a reconstituted system. We propose that this interface forms before Ca^{2+}-triggering, moves *en bloc* as Ca^{2+}-influx promotes the interactions between synaptotagmin-1 and the plasma membrane, and consequently remodels the membrane to promote fusion.

Disclosures: Q. Zhou: None. **A. Brunger:** None.

Nanosymposium

107. Mechanisms of Neurotransmitter Release

Location: SDCC 25A

Time: Sunday, November 13, 2016, 8:00 AM - 11:00 AM

Presentation Number: 107.09

Topic: B.06. Neurotransmitter Release

Support: 1U54NS083924-01 NINDS-NIH

Title: Complexin increases Ca^{2+} coupling in vesicle fusion at nano and micro domains

Authors: *R. A. JORQUERA[1,2], E. QUIROZ[1], A. GONZALEZ-RUIZ[1];
[1]Neurosci., Univ. Central Del Caribe, Sch. of Med., Bayamon, PR; [2]Inst. of Biomed. Sci., Univ. de Chile, Santiago, Chile

Abstract: Complexin (Cpx) is a key neuronal protein with a dual function, inhibits spontaneous synaptic vesicle (SV) fusion and promotes nerve-evoked SV fusion. Decreased Cpx levels correlate with several neuronal dysfunctions and schizophrenia and Parkinson's diseases patients. Nevertheless, the role of Cpx in neurological condition is unknown. Combining electrophysiology, genetic and chemical manipulation, we study the synaptic transmission at the *Drosophila* larval neuromuscular junction. Our work shows that there is a tight coupling of SVs with calcium influx in this synapse that is promoted by Cpx, modifying the kinetics of nerve-evoked EPSCs. Cpx and intracellular calcium buffering suppressed spontaneous SVs fusion whereas calcium channel inhibition did not. In addition, trains of nerve stimulation and deconvolution analysis indicated that synchronous and asynchronous SVs pools are modulated by Cpx. Our results show a crucial role of Cpx in coupling and fusion, regulating the availability of synchronous and asynchronous SVs, which are relevant in synaptic physiology.

Disclosures: R.A. Jorquera: None. **E. Quiroz:** None. **A. Gonzalez-Ruiz:** None.

Nanosymposium

107. Mechanisms of Neurotransmitter Release

Location: SDCC 25A

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Presentation Number: 107.10

Topic: B.06. Neurotransmitter Release

Support: German Research Foundation: Collaborative Research Center 889

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Bernstein Center for Computational Neuroscience 01GQ1005A

Title: Nanoscale imaging of presynaptic calcium signaling

Authors: *N. T. URBAN[1,4], J. NEEF[5,7,8,2], T.-L. OHN[5,7], T. FRANK[5], S. W. HELL[3,4], K. I. WILLIG[4,7,6,3], T. MOSER[5,6,9,7,8];

[2]Synaptic Nanophysiology Group, [3]Dept. of NanoBiophotonics, [1]Max-Planck-Institute for Biophysical Chem., Goettingen, Germany; [4]Ctr. for Nanoscopy and Mol. Physiol. of the Brain, Goettingen, Germany; [5]Inst. for Auditory Neurosci. and InnerEarLab, [6]Optical Nanoscopy in Neurosci., Univ. Med. Ctr. Goettingen, Goettingen, Germany; [7]Collaborative Res. Ctr. 889, Univ. of Goettingen, Goettingen, Germany; [8]Bernstein Focus for Neurotechnology, Univ. of Goettingen, Goettingen, Germany; [9]Bernstein Ctr. for Computat. Neuroscience, Univ. of Goettingen, Goettingen, Germany

Abstract: The influx of calcium at the presynaptic active zone (AZ) triggers the priming and release of synaptic vesicles, initiating synaptic transmission. Observing this process is tricky, as it is both very fast and occurs in areas that are nanoscopic in size. Precise details of this event are necessary, however, to correctly understand how this part of the synapse functions. Therefore, we combined patch-clamping with high-resolution fluorescence microscopy techniques, either confocal microscopy or diffraction-unlimited STED nanoscopy, enabling us to quantify these fast processes inside living tissue with both the necessary temporal and spatial resolution. With these methods we characterized the presynaptic active zones of sensory inner hair cells (IHCs) in the mouse cochlea, measuring not only the number and distribution of calcium channels at the AZ, but also the actual amount and spread of Ca^{2+} itself during influx.
For the experiments we used C57B6/N wildtype mice and mice carrying a deletion of exons 4 and 5 of the Bassoon gene (*Bsn*$^{\Delta Ex4/5}$), at the age of postnatal day 15 (P15) to P18 or P26 to P33. We measured the size and distribution of immunohistochemically labeled $Ca_v1.3$ channel clusters in IHCs of wildtype mice using three dimensional STED nanoscopy of fixated samples, revealing mostly linear arrangements typically 60 nm wide and 300-700 nm in length. Using two independent methods we then quantified the number of Ca^{2+} channels at the AZ using both quenching of the Ca^{2+} influx of individual synapses by local iontophoretic application of EGTA, as well as optical fluctuation analysis. For this we used patch-clamping both for evoking and recording Ca^{2+} currents, as well as for introducing the Ca^{2+} indicator dye into the cell. Simultaneously we used high-resolution Ca^{2+} imaging to record the changes in Ca^{2+} indicator fluorescence. We further observed the size of the depolarization-induced presynaptic Ca^{2+} domains using a low-affinity Ca^{2+} indicator and STED nanoscopy. The spread of Ca^{2+} closely matched the distribution suggested by simulations based on the prior information about the dimensions of Ca^{2+} channel clusters at the AZ, whereas the spread in Bassoon-mutant mice deviated strongly from expectations based on fixated samples, and was of much larger scale than in wildtype mice. The local Ca^{2+} concentrations at the active zone during depolarization were measured by analyzing the fluorescence lifetime of the Ca^{2+} indicator with both confocal and STED resolution.
In conclusion, we used several novel approaches to characterize the number, distribution and function of voltage gated calcium channels at presynaptic active zones in hitherto unprecedented detail.

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Nanosymposium

107. Mechanisms of Neurotransmitter Release

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Presentation Number: 107.11

Topic: B.06. Neurotransmitter Release

Support: NIH Grant MERIT R37 MH063105-14

Mathers Fdn

HHMI

Title: An *In vitro* fusion assay utilizing endogenous excitatory synaptic vesicles

Authors: *J. LEITZ[1,2], J. J. PETERS[1,2], A. L. WANG[1,2], R. A. PFUETZNER[1,2], J. DIAO[3], A. T. BRUNGER[1,2];
[1]Cell. and Mol. Physiol., Stanford Univ., Stanford, CA; [2]Howard Hughes Med. Inst., Stanford, CA; [3]Cancer Biol., Univ. of Cincinnati Col. of Med., Cincinnati, OH

Abstract: Here we build upon a previous *in vitro* synthetic synaptic vesicle fusion assay to utilize endogenous synaptic vesicles isolated from mouse brain tissue. We use this system to investigate the action of cytosolic proteins on synaptic vesicle fusion kinetics.

Disclosures: J. Leitz: None. **J.J. Peters:** None. **A.L. Wang:** None. **R.A. Pfuetzner:** None. **J. Diao:** None. **A.T. Brunger:** None.

Nanosymposium

107. Mechanisms of Neurotransmitter Release

Location: SDCC 25A

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Presentation Number: 107.12

Topic: B.06. Neurotransmitter Release

Support: LSUHSC REF

Title: Identification of an activity-induced, calcium-dependent glutamine transporter in hippocampal axon terminals

Authors: *J. D. ERICKSON;
Neurosci., LSUHSC, New Orleans, LA

Abstract: Excessive presynaptic glutamatergic transmission is thought to be involved in various human disorders including epilepsy. Seizure activity or intense glutamatergic transmission requires import of glutamine into axon terminals from glia to maintain vesicular glutamate stores for continued release. This concept of glutamate-glutamine cycling for replenishment of the neurotransmitter glutamate pool originated from 'classic' biochemical experiments performed nearly 40 years ago; however, a critical barrier to progress in this field has been the lack of direct functional evidence for activity-induced, Ca^{2+}-dependent glutamine transport activity in hippocampal neurons and any evidence for the presence of such a glutamine transporter in axon terminals. Here, I present the functional identification and characterization of a novel activity-induced, Ca^{2+}-dependent glutamine transporter in mature hippocampal neurons. Activity-induced transport is observed in hippocampal neurons and not astrocytes, requires exogenous Ca^{2+}, and is blocked by inhibition of P-type voltage-gated Ca^{2+} channels (verapamil; 20 μM). Preferred endogenous substrates likely include alanine, proline, histidine, and glutamine; although glutamine is the most physiologically relevant substrate as it is present at 10-fold higher concentration in extrasynaptic space, compared to all others. This activity-induced, Ca^{2+}-dependent glutamine transport system in neurons saturates at 200 μM and it displays a substrate affinity (Km) of 30 +/- 4 μM. Sarcosine, an effective anti-seizure compound, abolishes transport when present at 1 mM concentration. These results support the concept that a relatively high affinity, activity-induced glutamine transporter operates maximally following Ca^{2+}-dependent exocytosis of this transporter to the plasma membrane. The kinetics of this neuronal glutamine transport system differentiate it from established neuronal glutamine transporters SNAT1 and SNAT2, which display much lower affinity (Km ~0.4 millimolar). In addition, SNAT1 and SNAT2 are restricted to neuronal cell bodies and dendrites. On the other hand, immunohistochemical labeling with antibodies that were raised against the activity-induced glutamine transporter is restricted to axons and axon terminals in the hippocampus. Hence, this novel activity-induced and Ca^{2+}-dependent glutamine transporter in hippocampal axon terminals represents a new potential target in disorders of excessive presynaptic glutamatergic transmission and glutamate excitotoxicity.

Disclosures: J.D. Erickson: None.

Nanosymposium

108. Energy Metabolism and Mitochondria Function in Health, Disease, and Aging

Location: SDCC 32B

Time: Sunday, November 13, 2016, 8:00 AM - 10:15 AM

Presentation Number: 108.01

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Swiss Anorexia Nervosa Foundation

Novartis Foundation for Biomedical Research

Title: A glucose-sensing circuit in the insular cortex

Authors: *I. DE ARAUJO SALGADO, N. BENFREDJ, C. LAMY;
Univ. of Fribourg, Fribourg, Switzerland

Abstract: The insular cortex (IC) plays an important role in integrating body metabolic signals and in adaptive behaviors. Neuroimaging studies and previous work from our lab has shown that it is affected by feeding states. We asked whether nutrient sensing mechanisms exist in IC that could account for its ability to monitor body energy levels. We established a transgenic activity reporter system to target putative glucose-responsive neurons in vitro. Whole-cell recordings performed in this model revealed a population of glucose-sensing neurons that respond to glucose with a cell-autonomous glucose-inhibited pattern. Their response was driven by the opening of 2-pore domain potassium channels. Morphological reconstructions after recordings showed that those cells are a homogeneous population of thick-tufted layer 5 pyramidal cells. Our results suggest a mechanism by which glucose could affect IC functional outputs and IC-dependent behaviors in relation to the organism's metabolic status.

Disclosures: I. De Araujo Salgado: None. **N. BenFredj:** None. **C. Lamy:** None.

Nanosymposium

108. Energy Metabolism and Mitochondria Function in Health, Disease, and Aging

Location: SDCC 32B

Time: Sunday, November 13, 2016, 8:00 AM - 10:15 AM

Presentation Number: 108.02

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: MRC A6545QB40

Title: Bi-directional manipulation of ventromedial hypothalamic SF1 neuron activity has opposing effects on feeding and adiposity

Authors: *P. VISKAITIS, E. E. IRVINE, M. A. SMITH, S. M. A. PEDRONI, A. CHOUDHURY, J. GLEGOLA, D. HARDY, M. PAIVA PESSOA, L. KATSOURI, D. J. WITHERS;
MRC Clin. Sci. Ctr., Imperial Col. London, London, United Kingdom

Abstract: The ventromedial hypothalamus (VMH) has historically been viewed as one of the centres in the central nervous system involved in integrating the control of feeding, energy metabolism and behaviours like aggression and fear. Recent studies using cell type specific functional approaches (DREADD and optogenetics) have focused on behavioural phenotypes (Wang et al. 2015; Lin et al. 2011; Kunwar et al. 2015; Silva et al. 2013) or glucose homeostasis (Toda et al. 2016; Garfield et al. 2014). In this study we analysed the interaction between feeding and metabolism using chemogenetic approaches in the steroidogenic factor 1 (SF1) expressing neurons of the VMH. We show that acute DREADD-mediated modulation of the SF1 expressing neurons had bi-directional effects on feeding, metabolic rate and adiposity. Acute activation of SF1 neurons with hM3Dq significantly decreased food intake ($p < 0.0001$) and significantly reduced respiratory exchange ratio (RER, $p<0.01$). Inhibition of SF1 neurons with hM4Di had the opposite effect on feeding with a significant increase in food intake observed over 24h ($p<0.001$) without altering the RER. Chronic DREADD-mediated activity of SF1 neurons inversely correlated with changes to bodyweight and fat mass which were independent of feeding. After chronic oral CNO administration, hM3Dq-mediated activation significantly reduced adiposity (fat/lean ratio, $p<0.01$) while hM4Di driven inhibition resulted in opposing effects on this measure ($p<0.0001$). Moreover, prolonged activation of SF1 neurons protected from, while inhibition enhanced, the effects of high fat feeding on adiposity. In summary, we show that modulating the activity of SF1 expressing neurons in the VMH affects feeding, adiposity and susceptibility to high fat diet.

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Nanosymposium

108. Energy Metabolism and Mitochondria Function in Health, Disease, and Aging

Location: SDCC 32B

Time: Sunday, November 13, 2016, 8:00 AM - 10:15 AM

Presentation Number: 108.03

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Hypothermia in mouse is caused by AMP and A1 and A3 adenosine receptor agonists via three distinct mechanisms

Authors: *J. CARLIN[1], D. K. TOSH[2], S. JAIN[3], K. A. JACOBSON[2], O. GAVRILOVA[3], M. L. REITMAN[1];
[1]DEOB, Natl. Inst. of Hlth., Bethesda, MD; [2]Mol. Recognition Section, Lab. of Bioorganic Chem., [3]Mouse Metabolism Core, NIH, Bethesda, MD

Abstract: Background: Adenosine and 5'-adenosine monophosphate (AMP) have been implicated in triggering the torpor response seen in small mammals during hibernation or fasting. Adenosine is thought to act through A_1AR in the nucleus of the solitary tract or pre-optic area to elicit hypothermia in rodents. AMP is also proposed to be a natural regulator of torpor. However, AMP-induced torpor was intact in mice lacking any one of the adenosine receptors (A_1AR, A_{2A}AR, A_{2B}AR, or A_3AR). Our lab has characterized a third mechanism to achieve hypothermia through A_3AR mast cell degranulation and central histamine H_1 receptor activation
Methods: Adenosine agonists were administered intraperitoneally (i.p.) or intracerebroventricularly (i.c.v.) and core body temperature and physical activity were monitored by telemetry in freely active mice. Wild type mice, mice lacking A_1AR, A_3AR, or both, and mice lacking mast cells (*Kit*$^{W\text{-}sh/W\text{-}sh}$) were studied.
Results: We found that putative A_1AR agonists N6-cyclopentyl adenosine (CPA 0.3 mg/kg) and N6-cyclohexyladenosine (CHA 0.05 mg/kg i.p) at commonly used doses are non-selective; working via both A_1AR and A_3AR to cause hypothermia. (±)-5'-Chloro-5'-deoxy-ENBA (Cl-ENBA 3mg/kg) was the most specific A1AR tested. Cl-ENBA (15ug/mouse, i.c.v) caused hypothermia in WT mice that is lost in *Adora1*-/- mice. Cl-ENBA (3mg/kg, i.p.) elicited hypothermia in *Adora3*-/- mice and was associated with reduced energy expenditure, reduced activity, and behavior in a temperature gradient test. AMP (100mg/kg, i.p.) caused hypothermia and reduced activity. AMP (100ug/mouse, i.c.v) caused hypothermia in WT mice that remains intact in *Adora1*-/- mice. AMP (100mg/kg) is also associated with reduced energy expenditure, reduced activity, and behavior in a temperature gradient test. Cl-ENBA and AMP hypothermia remained intact in *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice and after pretreatment with pyrilamine. Finally, we show the torpor response after a 24 hour fast occurs in mice lacking A_1AR, A_3AR, or both, demonstrating that these receptors are not required for this effect.
Conclusion: Together, these data demonstrate at least three distinct mechanisms (A_1AR, A_3AR, AMP) that cause a centrally mediated hypothermia response in mice. A_3AR hypothermia acts via mast cell histamine release and activation of central histamine receptors. A_1AR and AMP cause hypothermia through a central mechanism that is not dependent on H_1R or mast cells. Finally, A_1AR and A_3AR are not necessary for fasting induced torpor response.

Disclosures: J. Carlin: None. **D.K. Tosh:** None. **S. Jain:** None. **K.A. Jacobson:** None. **O. Gavrilova:** None. **M.L. Reitman:** None.

Nanosymposium

108. Energy Metabolism and Mitochondria Function in Health, Disease, and Aging

Location: SDCC 32B

Time: Sunday, November 13, 2016, 8:00 AM - 10:15 AM

Presentation Number: 108.04

Topic: C.01. Brain Wellness and Aging

Support: UGC Grant

Title: Attenuation of d-galactose induced cognitive impairment by Trigonelline via its anti-oxidative, anticholinesterase & antiglycative pathways.

Authors: *A. A. CHOWDHURY, N. GAWALI, V. BULANI, P. KOTHAVADE, A. R. JUVEKAR;
Dept. of Pharmaceut. Sci. and Technol., Inst. of Chem. Technol., Mumbai, India

Abstract: Alzheimer's disease (AD), a progressive neurodegenerative disorder is characterized by senile plaque deposition, neurofibrillary tangles & cognitive impairment. Recent research indicates that advanced glycation end products (AGEs) interact with receptors for AGEs (RAGE) & lead to the pathological changes of AD. Further evidence reveals that the interaction between AGEs & RAGE elicits intracellular oxidative stress, proinflammatory apoptosis, and oxidative stress responses, which lead to nerve cell damage. D-galactose (D-gal), a reducing sugar is metabolized at normal concentration. However, at high levels, it reacts readily with the free amines of amino acids in proteins and peptides in vivo to form advanced glycation end products (AGEs). Literature sources demonstrate that continuous subcutaneous injection of D-gal in rodent induced production of free radicals and deterioration of learning & memory function.Present study was conducted to explore the possible role of Trigonelline, a plant alkaloid with known antidiabetic properties against D-Gal induced amnesia & its possible mechanism of action. Chronic administration of D-galactose (150 mg/kg) for 6 weeks significantly impaired cognitive performance (both in Morris water maze and Y maze), oxidative defence & displayed elevated AGE levels as compared to sham group. Trigonelline (50 and 100 mg/kg) treatment significantly ameliorated cognitive performance, oxidative defence & restored AGE levels as compared to control (D-galactose). Further Trigonelline treatment significantly attenuated acetylcholine esterase activity in D-gal treated mice. In conclusion, present study highlights the potential role of trigonelline against D-galactose induced cognitive impairment due to its antioxidant, antiglycative and anticholinesterase properties.

Disclosures: A.A. Chowdhury: None. **N. Gawali:** None. **V. Bulani:** None. **P. Kothavade:** None. **A.R. Juvekar:** None.

Nanosymposium

108. Energy Metabolism and Mitochondria Function in Health, Disease, and Aging

Location: SDCC 32B

Time: Sunday, November 13, 2016, 8:00 AM - 10:15 AM

Presentation Number: 108.05

Topic: C.01. Brain Wellness and Aging

Title: Improving mitochondrial function to treat age-related visual decline

Authors: ***N. M. ALAM**[1], S. LI[2], W. C. MILLS, 3rd[2], Y. SOONG[2], H. H. SZETO[2], G. T. PRUSKY[1,2];
[1]Burke Med. Res. Inst., White Plains, NY; [2]Weill Cornell Med. Col., New York, NY

Abstract: Age-related visual decline is a major source of disability with no remediation. To investigate the cause and treatment of visual decline with age we characterized photopic spatial visual function over the lifespan of C57BL/6 mice, using measures of optokinetic tracking in a virtual-reality optokinetic system (OptoMotry). We found that spatial frequency thresholds were stable from 4-18 months, but by 24 months, were reduced by ~15%. Function rapidly declined after 24 months, such that by 32 months, thresholds were decreased by ~50%. Visual decline with age was paralleled by a reduction in the length of photoreceptor outer segments, and disorganization of the contents of outer segment disks, which serve to concentrate, stabilize and orient opsin molecules for photon capture. Evidence of augmented outer segment shedding, in the form of increased pigment granules in the retinal pigment epithelium and choroid, was also evident. Outer segments undergo daily shedding and must be sustained by a high rate of lipid and protein synthesis, which is enabled by very long mitochondria in the inner segment ellipsoid. Since a decrease in the number and length of inner segment mitochondria was also present with age, it indicated that mitochondrial dysfunction is the origin of the age-related retinal pathology and visual decline. To examine this possibility, we treated aging mice daily with eye drops containing the peptide SS-31, which selectively targets mitochondria and improves mitochondrial function by increasing ATP production and preventing the formation of reactive oxygen species. Indeed, the treatment was able to fully reverse visual dysfunction within 5 weeks when initiated at 24 months, and partially restore function when initiated at 32 months. SS-31 was also able to normalize the length and internal organization of outer segments, and the length and number of inner segment mitochondria. This is the first report of a mitochondrial targeting agent that can reverse visual dysfunction in aging mammals. Since SS-31 has been well tolerated in multiple human clinical trials, and can be delivered by eye drops rather than intravitreal injection, it has great potential to reduce disability by extending the visual heathspan.

Disclosures: **N.M. Alam:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CerebralMechanics Inc. **S. Li:** None. **W.C. Mills:** None. **Y. Soong:** None. **H.H. Szeto:** None. **G.T. Prusky:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CerebralMechanics Inc.

Nanosymposium

108. Energy Metabolism and Mitochondria Function in Health, Disease, and Aging

Location: SDCC 32B

Time: Sunday, November 13, 2016, 8:00 AM - 10:15 AM

Presentation Number: 108.06

Topic: C.01. Brain Wellness and Aging

Support: German Federal Ministry for Economic Affairs and Energy KF2118004CS3

Title: Effects of long-term Rice Bran Extract supplementation on survival, cognition and brain mitochondrial function in aged NMRI mice

Authors: *G. P. ECKERT[1], H. ASSEBURG[1], M. HEINRICH[2], N. SUS[3], E.-M. BLUMRICH[4], R. DRINGEN[4], J. FRANK[3], S. HAGL[2];
[1]Inst. for Nutritional Sci., Justus-Liebig-University, Giessen, Germany; [2]Pharmacol., Goethe-University Frankfurt, Frankfurt, Germany; [3]Nutritional Sci., Univ. Hohenheim, Stuttgart, Germany; [4]Neurobiochemistry, Univ. Bremen, Bremen, Germany

Abstract: Aging represents a major risk factor for the development of neurodegenerative diseases like Alzheimer's disease (AD). As mitochondrial dysfunction plays an important role in brain aging and occurs early in the development of AD, the prevention of mitochondrial dysfunction might help to slow brain aging and the development of neurodegenerative diseases. Rice bran extract (RBE) contains high concentrations of vitamin E congeners and γ-oryzanol. We have previously shown that RBE increased mitochondrial function and protected from mitochondrial dysfunction *in vitro* and in short-term *in vivo* feeding studies. To mimic the use of RBE as food additive we have now investigated the effects of a long-term (6 months) feeding of RBE on survival, behavior and brain mitochondrial function in aged NMRI mice. RBE administration significantly increased survival and performance of aged NMRI mice in the passive avoidance and Y-Maze test. Furthermore, brain mitochondrial dysfunction found in aged mice was ameliorated after RBE administration. Furthermore, data from mRNA and protein expression studies revealed an up-regulation of mitochondrial proteins in RBE-fed mice, suggesting an increase in mitochondrial content which is mediated by a peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC1α)-dependent mechanism. Our findings suggest that a long-term treatment with a nutraceutical containing RBE could be useful for slowing down brain aging and the progression of AD.

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Nanosymposium

108. Energy Metabolism and Mitochondria Function in Health, Disease, and Aging

Location: SDCC 32B

Time: Sunday, November 13, 2016, 8:00 AM - 10:15 AM

Presentation Number: 108.07

Topic: C.01. Brain Wellness and Aging

Support: Research award from Hoansha foundation (KA)

Grants in aid for Scientific Research 15K06712 (KA)

Takeda Science Foundation (KA)

NIG Collaborative Research Program B (KA)

Title: Reduction in ATP levels in the axon during aging and the role of mitochondrial distribution

Authors: *M. OKA[1], E. SUZUKI[2], S.-I. HISANAGA[1], K. M. IIJIMA[3], K. ANDO[1];
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Abstract: Neurons have highly polarized structure and their functions need to be supported by local energy supply. ATP in neurons is produced by either glycolysis or oxidative phosphorylation, and both play important roles in local ATP homeostasis. Mitochondria are actively transported to the axon, and glycolytic enzymes diffuse throughout the axon. Mitochondrial function and transport reduces during aging, which may underlie loss of functional and structural integrity of the brain during aging. However, it is not fully understood how local ATP levels in the axon changes during aging, and to what extent dysregulation of mitochondrial distribution contribute to it.

Here we investigated age-dependent changes in ATP levels in the axon in Drosophila brain. To analyze local ATP levels, genetically encoded fluorescent ATP Biosensor under the control of UAS promoter was expressed in neurons by using the pan-neuronal elav-GAL4 driver. Analyses were focused on the mushroom body structure, where the cell body (Kenyon cell region), dendrites (calyx) and axons (lobes) are easily identified. Comparison between the young flies (at the age of 5 day-after-eclosion (dae)) and old flies (30 dae) showed that ATP levels reduced in the cell body, dendrites and axons during aging.

Next, we asked the contribution of mitochondria in this age-dependent change in the axon by using genetic depletion of axonal mitochondria. Mitochondria are transported to the axon on microtubules via kinesin motors, and milton, which regulates attachment of mitochondria to kinesin heavy chain, is essential for this process. RNAi-mediated knockdown of milton in

neurons depletes mitochondria from the axon terminals but not from the soma. We found that milton knockdown caused a reduction in ATP levels in the axon in the young flies. Interestingly, ATP levels reduced further during aging in the cell body, but not in the axons, of milton knockdown fly neurons. These results suggest that age-dependent decline in ATP levels in the axon maybe attributable to reduction in the number and/or functions of axonal mitochondria. Reduction in the number and/or function of mitochondria in synaptic terminals has been associated with a number of age-related neurodegenerative diseases, and these results also suggest that local energy deficiency caused by depletion of mitochondria from the axon might contribute to the pathogenesis of those diseases. Further studies are expected to reveal the mechanisms underlying regulation of long-term energy homeostasis during aging and in disease.

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Nanosymposium

108. Energy Metabolism and Mitochondria Function in Health, Disease, and Aging

Location: SDCC 32B

Time: Sunday, November 13, 2016, 8:00 AM - 10:15 AM

Presentation Number: 108.08

Topic: C.01. Brain Wellness and Aging

Support: IGF project 18239 N

Title: Dissecting mitochondrial dysfunction in alzheimer´s disease by metabolic imaging

Authors: ***P. M. SCHAEFER**[1], B. VON EINEM[1], E. CALZIA[2], A. RÜCK[3], C. A. F. VON ARNIM[1];
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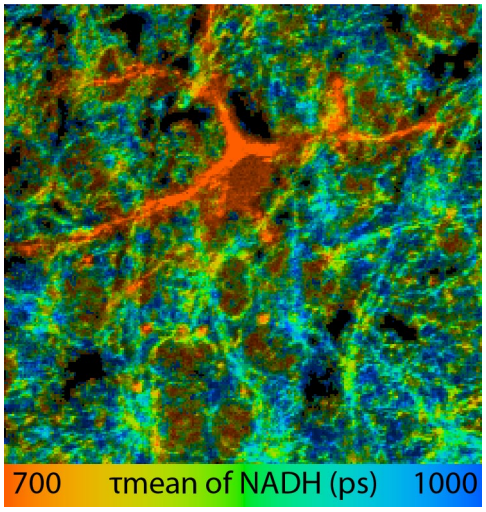
Abstract: Alzheimer´s disease (AD) is characterized by alterations in brain metabolism, which are thought to be one underlying factor of neuronal death. These metabolic disturbances encompass mitochondrial defects mainly caused by an aberrant sorting of amyloid beta. Interestingly, amyloid beta transport and localization determine its toxic effect on mitochondria. Likewise, mitochondrial dysfunction in Alzheimer´s disease is not uniform in the brain but there is a selective vulnerability of different brain regions, cell types and even mitochondrial populations. Current methods in assessing mitochondrial function are limited with respect to their capability of spatially separating these metabolic differences. Thus, the aim of our study was to establish a metabolic imaging technique to screen for mitochondrial function on the cellular level in intact systems maintaining the complex metabolic interplay between different

cell types.
For this purpose we determined NADH redox state by measuring its autofluorescence lifetime. In general NADH lifetime becomes shorter when cells mainly do glycolysis and is elongated when cells perform mitochondrial respiration. As the NADH lifetime is also dependent on the pH, we accomplished this by a parallel measurement of mitochondrial matrix pH.
Here we demonstrate the suitability of this highly innovative approach for determining mitochondrial function with a high spatial and temporal resolution on the single cell level in primary neurons, astrocyte-neuron co-cultures and brain slices. Thereby we provide a novel insight into the complex energy metabolic interactions between cell types of the brain. In addition we present first applications on Alzheimer´s disease underlining the importance of intracellular amyloid beta in AD-associated mitochondrial failure. Consequently, our metabolic imaging technique will allow for dissecting mitochondrial deficits in a range of neurodegenerative diseases shedding light into the associated bioenergetic failures thereby rendering possible new therapeutic approaches.



Disclosures: **P.M. Schaefer:** None. **B. von Einem:** None. **E. Calzia:** None. **A. Rück:** None. **C.A.F. von Arnim:** None.

Nanosymposium

108. Energy Metabolism and Mitochondria Function in Health, Disease, and Aging

Location: SDCC 32B

Time: Sunday, November 13, 2016, 8:00 AM - 10:15 AM

Presentation Number: 108.09

Topic: A.04. Transplantation and Regeneration

Support: DFG BE-5136/1-1

Title: Mitochondrial metabolism controls early lineage progression and ageing phenotypes in adult hippocampal neurogenesis

Authors: R. BECKERVORDERSANDFORTH[1], B. EBERT[2], *L. DIMOU[3,4], I. SCHÄFFNER[1], C. FIEBIG[1], J. SHIN[5], J. MOSS[6], D. MOORE[7], L. GOSH[7], M. F. TRINCHERO[8], C. STOCKBURGER[9], K. FRIEDLAND[9], S. KEINER[10], R. JAGASIA[11], A. F. SCHINDER[8], S. JESSBERGER[7], G.-L. MING[5], H. SONG[5], N. TONI[6], D. C. LIE[1];
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Abstract: Precise regulation of cellular metabolism is hypothesized to constitute a vital component of the developmental sequence underlying the life-long generation of hippocampal neurons from quiescent neural stem cells. The identity of stage-specific metabolic programs and their impact on neurogenesis, however, are largely unknown. We show that activity of mitochondrial complexes functionally demarcates the transition from activated neural stem cells to intermediate neural progenitors. Intriguingly, perturbation of the function of mitochondrial complexes by ablation of the mitochondrial transcription factor A (Tfam) reproduces multiple hallmarks of ageing in hippocampal neurogenesis, whereas pharmacological enhancement of mitochondrial function ameliorates age-associated neurogenesis defects. These data link mitochondrial complex function to lineage progression of adult neural stem cells and identify mitochondrial function as a potential target to restore neurogenesis in the ageing hippocampus.

Disclosures: R. Beckervordersandforth: None. **B. Ebert:** None. **L. Dimou:** None. **I. Schäffner:** None. **C. Fiebig:** None. **J. Shin:** None. **J. Moss:** None. **D. Moore:** None. **L. Gosh:** None. **M.F. Trinchero:** None. **C. Stockburger:** None. **K. Friedland:** None. **S. Keiner:** None. **R. Jagasia:** None. **A.F. Schinder:** None. **S. Jessberger:** None. **G. Ming:** None. **H. Song:** None. **N. Toni:** None. **D.C. Lie:** None.

Nanosymposium

109. Glia and Immune Responses in Alzheimer's Disease

Location: SDCC 33C

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Presentation Number: 109.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Science Foundation - Graduate Research Fellowship Program & Graduate Research Opportunities Worldwide

Alzheimer's Association Zenith Fellows Award

AFAR/Ellison Medical Foundation Julie Martin Mid-Career Award in Aging Research

5R01NS076794-02

Title: Modeling chronic inflammation mediated by microglial Trem2 expression

Authors: *C. J. MILLER[1], B. P. LEUNG[1], K. R. DOTY[2], T. TOWN[2];
[1]Dana and David Dornsife Col. of Letters, Arts and Sci., USC, Los Angeles, CA; [2]Zilkha Neurogenetic Inst., Keck Sch. of Med. of USC, Los Angeles, CA

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder that accounts for the majority of dementia cases. Pathological hallmarks include amyloid β (Aβ) plaques, neurofibrillary tangles, and low-level, chronic neuroinflammation. The amyloid cascade hypothesis purports that AD pathogenesis is driven by Aβ accumulation leading to neuronal death. Recent genome-wide association studies have shown that Triggering Receptor Expressed on Myeloid cells 2 (*TREM2*) is associated with risk for late onset AD. We have found that TREM2 protein expression is upregulated in LOAD patient brain lysates compared to aged-matched controls. To explore this further, using cultured murine and human microglia, we developed a chronic stimulation model - overnight high dose Aβ stimulation with subsequent low dose Aβ stimulation. Our data show that chronic Aβ exposure increases *TREM2* expression, perturbing microglial homeostasis.

Disclosures: **C.J. Miller:** None. **B.P. Leung:** None. **K.R. Doty:** None. **T. Town:** None.

Nanosymposium

109. Glia and Immune Responses in Alzheimer's Disease

Location: SDCC 33C

Time: Sunday, November 13, 2016, 8:00 AM - 10:45 AM

Presentation Number: 109.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NEI EY006311

NIA AG18031

NIA AG038834

Title: Over-expressed pathogenic miRNAs in Alzheimer's disease (AD) and prion disease (PrD) drive deficits in TREM2-mediated Aβ42 peptide clearance

Authors: *W. J. LUKIW, Y. ZHAO, V. JABER;
Neurosci. Ctr., LSU NEUROSCIENCE CENTER, New Orleans, LA

Abstract: One prominent and distinguishing feature of progressive, age-related neurological diseases such as Alzheimer's disease (AD) and prion disease (PrD) is the gradual accumulation of amyloids into dense, insoluble end-stage protein aggregates. These polymorphic proteolipid lesions are known to contribute to immunogenic and inflammatory pathology in these insidious and fatal disorders of the human central nervous system (CNS). For example, the evolution of self-aggregating amyloid-beta (Aβ) peptides, such as the 42 amino acid Aβ42 peptide monomer into higher order aggregates are largely due to: **(1)** the inability of natural processes to clear them from the cellular environment; and/or **(2)** the overproduction of these amyloid monomers which rapidly mature into higher order oligomers, fibrils and insoluble, end-stage senile plaques. Cells of the CNS such as microglial (MG) cells have evolved essential homeostatic mechanisms to clear Aβ peptides to avoid their accumulation, however when defective, these clearance mechanisms become overwhelmed and excessive deposition and aggregation of these amyloids result. This paper will highlight some emerging concepts on the up-regulation of an inducible microRNA-34a in AD and PrD that drives the down-regulation of the amyloid sensing- and clearance-receptor protein TREM2 (the triggering receptor expressed in myeloid/microglial cells). The impairment of this TREM2- and MG-cell based amyloid clearance mechanism may be due in part to increases of an intrinsic miRNA-34a-regulated amyloid clearance system that contributes to amyloidogenesis associated with both AD and PrD.

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Nanosymposium

109. Glia and Immune Responses in Alzheimer's Disease

Location: SDCC 33C

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Presentation Number: 109.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: grant FONDECYT 1131025

fellowship CONICYT 21120013

Title: Aging-related dysregulation of glial cells: untangling their participation in Alzheimer's disease

Authors: *R. VON BERNHARDI, F. CORNEJO, F. HEREDIA, J. CARDENAS; Pontificia U Catolica De Chile, Fac Med., Santiago, Chile

Abstract: Aging is the main risk factor for late onset Alzheimer’s disease (AD), which corresponds to the great majority of AD patients. Our “glia-dysregulation” hypothesis proposes that age-related impairment of microglia regulation is a key event in AD pathogenesis, with aberrant microglia activation leading to the establishment of a deleterious environment, neuroinflammation and increased cytotoxicity. In aged mice, microglia show increased expression of cytokines and an exacerbated inflammatory response to pathological changes. Interestingly, whereas the regulatory cytokine TGFβ1 is also increased in the aged brain, neuroinflammatory activation persists. Regarding this apparent contradiction, we reported that TGFβ1 induction and activation of Smad3 signaling after inflammatory stimulation are reduced in adult mice. Here, we evaluated the participation of TGFβ1 signal transduction pathways, on the regulation of glia activation at different ages. We assessed *in vitro* and *in vivo* production of inflammatory mediators, expression of scavenger receptors (SR), phagocytosis, induction of neurotoxicity and neurobehavioral performance. The reduced activation of TGFβ1-Smad was associated with functional changes on the activation of microglia and included impaired expression of SR-A, which in turn associated with altered cytokine profiles in the plasma and in the hippocampus as mice aged. We observed that LPS induced a robust production of reactive oxygen species (ROS) in microglia obtained from older mice, compared with the response of young mice, which could result in increased oxidative stress. Protective functions, such as phagocytosis of Aβ, was also induced by TGFβ through a Smad-dependent mechanism. In fact, although phagocytosis was observed in glial cells obtained from aged animals, induction by inflammatory stimuli and TGFβ1 was impaired in microglia from older animals compared with the response obtained in young animals. Modulation was partially dependent on Smad3 pathway and was impaired by inflammatory preconditioning. Our results show that changes in TGFβ1-Smad3 signaling affects the expression of scavenger receptors and the activation pattern of microglia, which is impaired in aging and chronic inflammatory preconditioning. Those changes

could at least, partially mediate age-associated microglial cell changes, and could result in the facilitation of the cytotoxic activation of microglia and promotion of neurodegenerative changes.

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Nanosymposium

109. Glia and Immune Responses in Alzheimer's Disease

Location: SDCC 33C

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Presentation Number: 109.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG000538

Title: Long lifespan of microglia mitigates the ability to temporally induce Cre^{ERT} activity but definitively demonstrates microglia as the dominant source of C1q in a mouse model of Alzheimer's disease

Authors: ***M. I. FONSECA**[1], S.-H. CHU[1], M. X. HERNANDEZ[2], M. FANG[1], L. MODARRESI[1], A. J. TENNER[3];
[1]Mol. Biol. and Biochem., [2]Pathology and Lab. Med., [3]Neurobio. and Behavior, Univ. California, Irvine, CA

Abstract: Alzheimer's disease (AD) is a dementia characterized by the accumulation of amyloid plaques, neurofibrillary tangles, neuronal loss, and neuroinflammation. C1q (first component of complement cascade) can play a detrimental role in AD progression via activating the complement cascade inducing neuroinflammation and synapse loss. However, *in vitro* C1q has direct neuroprotective effects which may be beneficial at early stages of neurological disease, and thus has implications for therapeutic control of complement activation in neurodegenerative disease. To investigate the cellular source of C1q in brain, $C1qa^{FL/FL}$ mice were crossed to $Cx3cr1^{CreERT}$ and to $Thy1^{CreERT}$ mice to enable inducible cell specific ablation of the C1q gene. Strong C1q immunoreactivity was evident in the molecular layer of hippocampus (ML), and inside cell bodies of microglia and subsets of interneurons in $C1qa^{FL/FL}$, $Cx3cr1^{CreERT}$ and $Thy1^{CreERT}$, comparable to wild type (WT) mice. Since both Cre constructs also direct YFP expression, C1q could be seen colocalized with intracellular YFP and Cre in microglia of the $Cx3cr1^{CreERT}$, but not in YFP neurons of $Thy1^{CreERT}$mice. After tamoxifen treatment to induce cell specific Cre recombinase gene ablation, the $C1qa^{FL/FL}$:$Thy1^{CreERT}$ mice showed no decrease in C1q expression. In contrast, tamoxifen treated 5 month old $C1qa^{FL/FL}$:$Cx3cr1^{CreERT}$ were devoid of C1q in microglia as well as in the neuropil particularly in the ML. However,

surprisingly, the dramatic loss of C1q immunoreactivity was seen also in the vehicle-treated $C1qa^{FL/FL}$:$Cx3cr1^{CreERT}$ mice. Immunohistochemical results were confirmed by Western blots analysis. Neonatal derived microglia showed comparable levels of C1q in both $C1qa^{FL/FL}$:$Cx3cr1^{CreERT}$ and WT littermates, but by 1 month of age the decrease in C1q expression in the $C1qa^{FL/FL}$:$Cx3cr1^{CreERT}$ was near knock out levels at both the protein and mRNA levels. In contrast, no differences in the levels of C1q was detected in either liver or kidney from $C1qa^{FL/FL}$:$Cx3cr1^{CreERT}$ mice relative to WT or $C1qa^{FL/FL}$ littermates. $C1qa^{FL/FL}$:$Cx3cr1^{CreERT}$ mice had only a partial, if any, reduction in C1q plasma levels. These results caution that in this model, Cre^{ERT} gains access to the nucleus independently of tamoxifen, perhaps due to high expression levels of the recombinase under the Cx3cr1 promoter in microglia, inducing recombination and deletion of floxed genes during the long life of the microglia. Nevertheless, our finding that the specific deletion of C1q in microglia caused the elimination of C1q in brain but not in the periphery, provide unequivocal evidence that the microglia is the dominant source of C1q in the brain.

Disclosures: **M.I. Fonseca:** None. **S. Chu:** None. **M.X. Hernandez:** None. **M. Fang:** None. **L. Modarresi:** None. **A.J. Tenner:** None.

Nanosymposium

109. Glia and Immune Responses in Alzheimer's Disease

Location: SDCC 33C

Time: Sunday, November 13, 2016, 8:00 AM - 10:45 AM

Presentation Number: 109.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Microglial PD-1 modulates neuroinflammation and AD pathology by astrocytic PD-L1

Authors: ***M. P. KUMMER**[1], C. KUMMER[1], H. SARLUS[2], A. GRIEP[2], S. SCHWARTZ[1], A. VIEIRA-SAECKER[1], M. BRÜCKNER[3], A. HALLE[4], K. HÄNDLER[1], M. BEYER[1], J. SCHULTZE[1], E. LATZ[1], M. HENEKA[1];
[1]Univ. of Bonn, Bonn, Germany; [2]DZNE, Bonn, Germany; [3]Ceasar, Bonn, Germany; [4]Caesar, Bonn, Germany

Abstract: Neuroinflammation, a component of Alzheimer disease (AD), may limit the ability of the brain to clear deposits and debris. Tight control of the immune system may therefore be key to sustain the ability of the brain for repair and clearance. Here we report that PD-L1 on astrocytes and its receptor PD-1 on microglia, known for its inhibitory immune function, are upregulated around amyloid plaques in AD and APP/PS1 mice. Juxtamembrane shedding of PD-L1 was observed from astrocytes suggesting ectodomain signaling to microglial PD-1. Deletion of PD-1 in microglia evoked an inflammatory response and compromised Aβ uptake. Likewise,

in APP/PS1 PD-1(-/-) mice increased deposition of the amyloid β (Aβ), reduced microglial Aβ uptake, and decreased expression of the Aβ receptor CD36 on microglia were detected. Therefore, ineffective immune regulation by the PD-1/PD-L1 axis contributes to Aβ plaque deposition during chronic, unresolved neuroinflammation in AD.

Disclosures: **M.P. Kummer:** None. **C. Kummer:** None. **H. Sarlus:** None. **A. Griep:** None. **S. Schwartz:** None. **A. Vieira-Saecker:** None. **M. Brückner:** None. **A. Halle:** None. **K. Händler:** None. **M. Beyer:** None. **J. Schultze:** None. **E. Latz:** None. **M. Heneka:** None.

Nanosymposium

109. Glia and Immune Responses in Alzheimer's Disease

Location: SDCC 33C

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Presentation Number: 109.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Department of Veteran Affair VA merit/1I01BX002572-01A2

Title: Pathological activation of the spleen tyrosine kinase in microglia and neurons of different mouse models of alzheimer's disease

Authors: ***J. E. SCHWEIG**, D. BEAULIEU-ABDELAHAD, Y. LIN, M. MULLAN, F. CRAWFORD, D. PARIS;
Roskamp Inst., Sarasota, FL

Abstract: Besides its well-known role as a mediator of inflammatory responses in immune cells, the spleen tyrosine kinase (Syk) has been suggested to mediate microglial activation induced by Aβ *in vitro*. We have previously shown that Syk regulates both Aβ production and tau phosphorylation. Therefore, we investigated the immuno-localization of activated Syk (p-Syk Y525/526) in the brain of different transgenic mouse models of Alzheimer’s disease (AD). Our high-resolution confocal microscopy study demonstrates an upregulation of p-Syk in microglia of Aβ-overexpressing Tg PS1/APPsw and Tg APPsw mice compared to wild-type littermates. Moreover, BACE-1/sAPPβ-positive dystrophic neurites in the vicinity of β-amyloid deposits also show an increase in p-Syk, implying a contribution of Syk to the degeneration of neurites. In addition, human tau-overexpressing Tg P301S mice exhibit an age and tau-dependent increase in neuronal p-Syk compared to wild-type mice. Interestingly, Syk activation in the CNS is restricted to neurons and microglia excluding astrocytes. In summary, our investigation of three distinct mouse models of AD, supports a functional role of Syk in microglial activation, in the degeneration of neurites around β-amyloid deposits and in the formation of pathological tau species.

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Nanosymposium

109. Glia and Immune Responses in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

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CIRM Grant RT3-07893

Alzheimer's Association BFG-14-317000

NIH Grant AG016573

Title: Using pluripotent stem cells to study microglial function and the genetics of Alzheimer's Disease

Authors: *M. BLURTON-JONES[1], E. ABUD[1], R. RAMIREZ[1], E. MARTINEZ[1], L. HEALY[2], C. NGUYEN[1], A. MADANY[3], K. GYLYS[4], W. POON[1], J. ANTEL[2], M. CARSON[3]; [1]Neurobio. & Behavior, Univ. of California, Irvine, Irvine, CA; [2]McGill Univ., Montreal, QC, Canada; [3]Univ. of California, Riverside, Riverside, CA; [4]UCLA, Westwood, CA

Abstract: Microglia have been implicated in the pathophysiology of Alzheimer's Disease (AD) for decades. Yet only recently has genetic evidence underscored the importance of these cells in the development and progression of AD. While several microglial-associated single nucleotide polymorphisms (SNPs) have been linked to modest 10-15% changes in AD risk, coding mutations in one gene, Triggering receptor expressed on myeloid cells 2 (TREM2), are associated with a far larger 2-4 fold increase in AD risk. As a myeloid-lineage gene, TREM2 is thought to influence the innate immune response to pathogens or injury. However, growing evidence indicates that microglia also play critical roles in brain development, homeostasis, and synaptic plasticity. Thus, mutations in TREM2 likely influence multiple aspects of brain function and AD pathogenesis. To further study the role of TREM2 in AD we developed anapproach to differentiate microglial-like cells from patient-derived induced pluripotent stem cells (iPSCs). Using a paradigm that mimics the developmental origins of microglia, we have produced highly pure populations of cells that exhibit both genetic and functional characteristics that are highly similar to tissue-derived microglia. The resulting cells exhibit characteristic CX3CR1+/CD11b+/CD45-lo FACS signatures and express numerous microglial-enriched

transcripts and proteins including P2yr12, TREM2, OLFML3, and GPR34. Functional assays also demonstrate that iPSC-derived microglia migrate toward an ADP gradient, phagocytose human synaptosomes and beta-amyloid, and respond to LPS stimulation. To begin to examine the influence of TREM2 mutations on human microglia function, microglial-like cells have been generated from patient iPSC lines carrying the R47H TREM2 mutation and ongoing studies will examine the effects of these mutations on microglial function and gene expression.

Disclosures: **M. Blurton-Jones:** None. **E. Abud:** None. **R. Ramirez:** None. **E. Martinez:** None. **L. Healy:** None. **C. Nguyen:** None. **A. Madany:** None. **K. Gylys:** None. **W. Poon:** None. **J. Antel:** None. **M. Carson:** None.

Nanosymposium

109. Glia and Immune Responses in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NS 35144

AG 000538

Title: Role of microglial C5aR1 in the arctic alzheimer's disease mouse model

Authors: ***M. X. HERNANDEZ**[1], M. I. FONSECA[2], S.-H. CHU[2], A. J. TENNER[2];
[1]Pathology and Lab. Med., [2]Mol. Biol. and Biochem., UC-Irvine, Irvine, CA

Abstract: C5aR1, a G-protein coupled receptor for C5a, is primarily expressed on cells of the myeloid lineage, and to a lesser extent on endothelial cells and neurons in brain. Previous work demonstrated C5aR1 antagonist, PMX205, decreased amyloid pathology and suppressed cognitive deficits in Alzheimer Disease (AD) mouse models. However, the molecular mechanism of this protection has not been definitively demonstrated. Historically, it has been difficult to distinguish microglia from infiltrating macrophages, most relying on CD45 levels to make the distinction. Here we have taken advantage of the $CX3CR1^{GFP/GFP}$ and $CCR2^{RFP/RFP}$ reporter mice to distinguish microglia as GFP-positive and macrophages as GFP and RFP positive. To understand the role of microglial C5aR1 in the Arctic AD mouse model, $CX3CR1^{GFP/+}CCR2^{RFP/+}Arctic^{+/-}$ were crossed with C5aR1 knock out mice to generate $CX3CR1^{GFP/+}CCR2^{RFP/+}Arctic^{+/-}C5aR1^{-/-}$ and $CX3CR1^{GFP/+}CCR2^{RFP/+}Arctic^{-/-}C5aR1^{-/-}$ mice. Mice were aged to 2, 5, 7 and 10 months to investigate the transcriptome of the microglia with age and progression of plaque pathology. Immunohistochemical analysis showed amyloid beta

(Aβ) deposition in the Arctic mice consistent with previous results, with no change in pathology seen in the Arctic C5aR1 knockout (KO) mice. Interestingly, by FACS analysis of isolated microglia, the $CCR2^{+}$ macrophage population was 2-6 % of the total $CX3CR1^{+}$ population at all ages and genotypes, with no $CCR2^{+}$ macrophages near the plaques. First, at 5 months there were no DE genes between C5aR1KO and WT, suggesting that C5aR1 has little effect on microglia function in the absence of injury/pathology. Secondly, analysis of the top 500 differentially expressed (DE) genes, as sorted by p-value, from the microglia at 5 months using IPA software suggests most DE genes are decreased in the Arctic and increased in the Arctic C5aR1KO. Furthermore, at 7 months, cellular movement is the most common functional group activated in the Arctic and it is also activated in the Arctic C5aR1KO. At 5 months, cellular assembly and organization and molecular transport functions are disrupted in the Arctic relative to the wildtype and deletion of C5aR1 rescues those functions in the Arctic C5aR1KO relative to Arctic. At 7mo deleting C5aR1 in the Arctic model has less of an effect in those functional categories, suggesting that the alterations seen at 7 months may reflect damage resulting from excessive amyloid accumulation independent of C5aR1.

Disclosures: **M.X. Hernandez:** None. **M.I. Fonseca:** None. **S. Chu:** None. **A.J. Tenner:** None.

Nanosymposium

109. Glia and Immune Responses in Alzheimer's Disease

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Presentation Number: 109.09

Topic: C.02. Alzheimer's Disease and Other Dementias

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American Federation of Aging Research/Ellison Medical Foundation Julie Martin Mid-Career Award in Aging Research (M11472, to T.T.)

Zilkha Neurogenetic Institute startup funds

Title: T cell TGF-beta signaling control of the immune response to cerebral Amyloid-beta deposition

Authors: *M.-V. GUILLOT-SESTIER[1], A. W. VESLING[1], J. RODRIGUEZ, Jr.[1], K. REZAI-ZADEH[2], T. TOWN[1];
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Abstract: Alzheimer's disease (AD) is hallmarked by cerebral amyloid-β (Aβ) deposition, tauopathy, neuronal loss, and chronic neuroinflammation. Presence of circulating Aβ-reactive $CD4^+$ T cells has been reported in AD patients, suggesting that these adaptive immune cells may play a role in disease pathogenesis. However, mechanisms of T cell response(s) to Aβ and implication(s) for AD etiopathology remain unclear. As a major immune regulator within the CNS, transforming growth factor-beta (TGF-β) generally acts as an anti-inflammatory cytokine to tightly control immune responses. TGF-β mRNA levels are elevated in AD patient brains, and we previously showed that blockade of macrophage TGF-β signaling activated innate immunity and licensed Aβ clearance in a mouse model of cerebral amyloidosis. In addition to its role in regulating innate immunity, TGF-β is also a master regulator of T cells. To begin to understand the impact of blocking T cell TGF-β signaling on T cell response(s) to Aβ, we bred the APPswePSEN1dE9 mouse model of cerebral amyloidosis (APP/PS1) with a dominant-negative transgenic mouse that expresses an inhibitory form of TGF-β receptor type II in $CD4^+$ T cells. Strikingly, our data reveal that APP/$PS1^+$CD4-DNR^+ bitransgenic mice present reduction of cerebral amyloid burden and CAA, but have the net negative consequence of early death; likely due to overly exuberant brain inflammation. Indeed, reduced amyloid burden in APP/$PS1^+$CD4-DNR^+ brains occurs with increased $CD4^+$ T-cell numbers and increased Iba1 immunoreactivity. Furthermore, we observed significantly increased numbers of $CD45^+CD3^+$ T cells in parenchyma and blood vessels in cerebral cortex and hippocampus of APP/$PS1^+$CD4-DNR^+ mice. Abundance of $CD45^+CD3^+$ T cells was also augmented in the choroid plexus of bitransgenic mice. Interestingly, β-amyloid plaque-associated $CD3^+$ T cells appeared "ruffled", a morphological feature that is associated with antigen presentation. We assessed recruitment of immune cells to amyloid plaques, T cell-microglia interactions and microglial Aβ phagocytosis using our quantitative three-dimensional *in silico* modeling (q3DISM) technique. Our data show that inhibition of T-cell TGF-β signaling induces brain influx of peripheral T cells, recruitment of microglia and cerebral Aβ clearance. This raises the intriguing possibility that infiltrating T cells may instruct microglia to restrict amyloid burden.

Disclosures: **M. Guillot-Sestier:** None. **A.W. Vesling:** None. **J. Rodriguez:** None. **K. Rezai-Zadeh:** None. **T. Town:** None.

Nanosymposium

109. Glia and Immune Responses in Alzheimer's Disease

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Presentation Number: 109.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIMH R01 MH096093

NIMH R01 MH087660

NIGMS P50 GM085273

NINDS R01 NS062184

Title: The inflammation connection between PTSD, Alzheimer's Disease, and infection.

Authors: *B. MANIFOLD-WHEELER[1], C. M. FLORUTA[2], C. S. MEDINA[3], A. J. ZIMMERMAN[1], F. CHAVEZ[3], R. E. JACOBS[4], E. L. BEARER[1];
[1]Dept. of Pathology, [2]Dept. of Neurosci., [3]Univ. of New Mexico, Albuquerque, NM; [4]The California Inst. of Technol., Pasadena, CA

Abstract: Alzheimer's disease (AD) is the leading cause of dementia world-wide, with 6% of people over the age of 65 and 30% over the age of 85, afflicted by AD, and over $200 billion dollars spent each year towards caring for these individuals, in the United States alone. Given this, further understanding of significant risk factors for AD development is critical. Recent literature has indicated a correlation between individuals who suffer from PTSD and/or traumatic brain injury, with increased risk of AD development. However, there is still a need to decouple the contributions of physical brain injury and the psychosomatic trauma of exposure to life-threatening fear. Psychosomatic effects of PTSD are contributed to by environmental stress through chronic activation of the HPA-Axis, oxidative stress, and cortisol release. Oxidative stress triggers inflammatory signaling and is implicated in the neuronal cell death of AD. In a pilot study of psychosomatic trauma in young children, we analyzed DNA methylation profiles longitudinally using Illumina bead chip technology, and found significant alterations in genes after traumatic events. Genes regulating inflammatory pathways, including IL-6, IL-1, IL-1R, and IL-10, were differentially methylated. We also found 38,000 methylation sites altered by early life adversity in mouse brain. Finally, we observed altered circuitry by manganese enhanced MRI tract tracing in living double transgenic mouse models of AD expressing the human $APP^{swe/ind}$, familial AD mutations. These mice have 3.5-fold higher APP expression and display plaques and p-tau by 6 mo of age, and have disruption of microcircuitry in the hippocampus at 10-15 mo. We conclude that AD pathologies contribute to vascular disruption and likely lead to neurodegeneration in our AD model mouse. Underlying immune dysregulation

mediated by epigenetic changes of inflammatory pathways could exacerbate these effects. These data suggest that early life adversity, a risk factor for PTSD, can also lead to a vicious cycle of epigenetic changes in regulation of inflammation, worsening AD pathology, neurovascular damage, and neurodegeneration.

Disclosures: **B. Manifold-Wheeler:** None. **C.M. Floruta:** None. **C.S. Medina:** None. **A.J. Zimmerman:** None. **F. Chavez:** None. **R.E. Jacobs:** None. **E.L. Bearer:** None.

Nanosymposium

109. Glia and Immune Responses in Alzheimer's Disease

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Alzheimer’s Association Zenith Fellows Award

AFAR/Ellison Medical Foundation Julie Martin Mid-Career Award in Aging Research

BrightFocus Foundation Alzheimer's Disease Research Fellowship Award (A2015309F)

NSF Graduate Research Fellowship Program

Title: STAT3 signaling referees microglial amyloid clearance in Alzheimer's disease.

Authors: *K. R. DOTY[1], M.-V. GUILLOT-SESTIER[1], B. P. LEUNG[2], T. TOWN[1]; [1]Zilkha Neurogenetic Institute, Keck Sch. of Med. of USC, Los Angeles, CA; [2]Dana and David Dornsife Col. of Letters, Arts and Sci., USC, Los Angeles, CA

Abstract: Background: Alzheimer’s disease (AD) is defined by brain build-up of amyloid-β (Aβ) as “senile” plaques. It is becoming widely-recognized that failure in Aβ clearance−rather than overproduction−is the etiologic culprit in sporadic AD. CNS-resident microglia lose their physiologic ability to restrict cerebral Aβ accumulation, and this phenotype switch is poorly understood. We previously showed that deletion of *Il10* in the *APP/PS1* mouse model of cerebral amyloidosis altered the neuroinflammatory milieu and licensed Aβ phagocytosis by activated monocytes, preserving synaptic integrity and cognitive function. STAT3 is the proximal downstream *Il10* signaling molecule. We hypothesize that ablating STAT3 signaling will return microglia to health; modifying immune/inflammatory hub genes to endorse Aβ phagocytosis.

Methods: We interrogated expression of STAT3 in AD brains and in the *APP/PS1* mouse model of cerebral amyloidosis. Further, we developed microglial cell lines deficient in STAT3 and investigated phenotypic changes. Depletion of STAT3 *in vivo* is accomplished with inducible Cre technology and floxed STAT3 alleles in the APP/PS1^{+} transgenic animals. Using quantitative 3D *in silico* modeling (q3DISM) technology, we quantified microglial Aβ phagocytosis in response to STAT3 targeting *in vitro* and *in vivo*.
Results: We show that all elements of the STAT3 signaling pathway are abnormally elevated in AD patients' brains and in the *APP/PS1* mouse model of the disease. Recent network analysis studies in a large late onset AD (LOAD) patient cohort validate this finding. In microglial cells, Aβ does not induce STAT3 signaling directly; however, co-stimulation with IL-10 inhibits Aβ phagocytosis. *Stat3* deficiency 1) promotes microglial Aβ phagocytosis, 2) reverses IL-10 inhibition, and 3) phenocopies enhanced Aβ phagocytosis in *Il10* deficient microglia. We are currently evaluating whether *in vivo* depletion in presence of established plaques similarly activates amyloid phagocytosis.
Conclusions: We conclude that ablating microglial STAT3 signaling endorses Aβ phagocytosis, and propose that STAT3 signaling promotes "frustrated phagocytosis" in aging and in AD. Blocking STAT3 signaling in mononuclear phagocytes may be therapeutically relevant for AD.

Disclosures: K.R. Doty: None. **M. Guillot-Sestier:** None. **B.P. Leung:** None. **T. Town:** None.

Nanosymposium

110. Retina Photoreceptor and Circuitry

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Presentation Number: 110.01

Topic: D.06. Vision

Support: Bert and Ethel Aginsky Research Scholar Award

Title: Shedding light on melanopsin-expressing retinal ganglion cell circuitry

Authors: *Y. C. LIU[1], K. KIM[3], M. ELLISMAN[3], S. PANDA[2];
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Abstract: Intrinsically photosensitive melanopsin-expressing retinal ganglion cells (ipRGC) express the photopigment, melanopsin, which is maximally sensitive to light around 480 nm. Though capable of detecting light and independent of rod and cone photoreceptors, ipRGCs integrate light information generated by direct melanopsin activation and from rods and cones and transmits this information to many regions of the brain that are essential for both image-

forming and non-image-forming visual processes, such as circadian photoentrainment and pupillary light reflex. The large variation in melanopsin expression among the five morphologically-defined ipRGC subtypes has complicated the cyto- and histochemical study of these cells and their intraretinal interactions. The subtypes of bipolar and amacrine cells involved in ipRGC subtype-specific signaling is largely unknown. By combining Cre-dependent AAV vector-mediated expression of miniSOG, a novel correlated light and electron microscopy marker, in all ipRGCs in the mouse retina and serial blockface scanning electron microscopy (SBEM), we are able to identify and reconstruct ipRGCs in large EM volumes. The ultrastructural resolution afforded by SBEM is sufficient for resolving synaptic contacts between ipRGCs and other retinal cells that synapse onto ipRGC dendrites. Amacrine and bipolar cells that form synaptic contacts with ipRGC processes are reconstructed using IMOD and identified based on morphology. Data generated using these methods have revealed previously unknown differences in ipRGC circuitry between ipRGC processes that stratify in the ON- and OFF-sublamina of the inner plexiform layer. Differential inputs to differentially-stratified ipRGCs suggest that ipRGC subtypes may serve specific functions.

Disclosures: **Y.C. Liu:** None. **K. Kim:** None. **M. Ellisman:** None. **S. Panda:** None.

Nanosymposium

110. Retina Photoreceptor and Circuitry

Location: SDCC 24A

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Presentation Number: 110.02

Topic: D.06. Vision

Support: Fyssen foundation

Catharina foundation

Title: Beta arrestins shape melanopsin dependent responses to light.

Authors: ***L. S. MURE**[1,2], M. HATORI[2,3], K. RUDA[3], J. DEMAS[3], S. PANDA[2];
[1]Salk Inst. Lab. of Genet., La Jolla, CA; [2]the Salk Inst., La jolla, CA; [3]St Olaf Col., Northfield, MN

Abstract: Melanopsin is an opsin class of G-protein coupled receptor (GPCR) expressed in a small subset of retinal ganglion cells which are intrinsically photosensitive (ipRGCs). ipRGCs contribute to the brightness perception of vision, while they are indispensable for non-image forming visual responses including light modulation of pupil constriction, circadian rhythm, sleep and mood. However, mechanisms behind unique responses properties of ipRGCs (slow

activation, light integration and delayed deactivation) are still poorly understood.
Here we explored the role of β-arrestins in melanopsin signaling. Arrestins are multifunctional adaptor proteins that, once recruited by an activated GPCR, promote signal termination and receptor internalization. We first showed *in vitro* that melanopsin functionally interact with both β-arrestin 1 and 2 upon light stimulation and subsequent phosphorylation of melanopsin C terminus tail. We then tested the role of the two β-arrestins by measuring ipRGCs electrophysiological responses in either β-arrestin deficient mice retina or model where we over-expressed β-arrestins. Finally, we showed that alterations observed *in vitro* translated *in vivo* by assaying negative phototaxis and pupillary reflex to light.
This study identified the key and distinct roles of β-arrestin 1 and 2 in melanopsin signaling and shows that both are critical for proper adaption of the animal behavior to its environment.

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Nanosymposium

110. Retina Photoreceptor and Circuitry

Location: SDCC 24A

Time: Sunday, November 13, 2016, 8:00 AM - 10:15 AM

Presentation Number: 110.03

Topic: D.06. Vision

Title: Toward complete ganglion cell classification in the mouse retina: cross-validation of electron microscopic anatomy with visual physiology

Authors: *S. MU[1], J. S. KIM[2], K. L. BRIGGMAN[3], H. S. SEUNG[1];
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Abstract: The neural computation of visual perception begins in the retina. The retinal neural circuits receive inputs from the photoreceptors, spread out along interneurons, and converge to retinal ganglion cells (RGCs). The axons of RGCs are the only output of the retina and carry all the visual information from the retina to the rest of the brain. Each type of RGCs is thought to be associated with one microcircuit and to process distinct visual information. Therefore, classifying the types is an important step towards understanding the neural computation in the retina and retina's role in vision.
We anatomically classified roughly 400 RGCs based mainly on dendritic stratification profiles. The RGC dendritic arbors were reconstructed from serial electron microscope (EM) images of a $(0.3\ mm)^2$ patch of the inner plexiform layer of the mouse retina, and include all RGCs with cell bodies within the area. The reconstruction was carried out on EyeWire, a web-based EM

reconstruction pipeline that combines artificial intelligence of deep learning and human intelligence of a community of 'citizen neuroscientists'. This is the first time EM reconstruction was done on a large enough area to potentially sample and identify all RGC types.
For cross-validation of our anatomical classification, we compared with visual responses previously recorded from the same neurons by two-photon calcium imaging. The comparison confirmed that our classification recovered all well-known ganglion cell types including On-Off direction selective ganglion cells (DSGCs), sustained/transient On DSGCs, asymmetric Off DSGC types, sustained/transient and On/Off alpha cells, and W3 cells. We also found orientation selective or direction selective responses in some cell types that were not previously well-characterized or were previously unknown.
In addition to the On-Off DSGCs that are known to receive synaptic inputs from starburst amacrine cells (SACs), we identified two other direction selective types with distinctive stratification profile against the SACs. We predicted their preferred directions by dendritic contact with SACs and the results matched their physiology from calcium imaging. Stratification reliably predicts the transiency of responses for On types of cells, but not for Off types.
In all, our classification includes over 40 types of retinal ganglion cells.

Disclosures: **S. Mu:** None. **J.S. Kim:** None. **K.L. Briggman:** None. **H.S. Seung:** None.

Nanosymposium

110. Retina Photoreceptor and Circuitry

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Topic: D.06. Vision

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Pew Charitable Trust Scholarship in the Biomedical Sciences

Title: Identification and characterization of low-density ganglion cells in the primate retina

Authors: ***C. RHOADES**[1], A. TIKIDJI-HAMBURYAN[2], G. GOETZ[2], N. BRACKBILL[3], N. SHAH[4], A. SHER[5], A. LITKE[5], E. J. CHICHILNISKY[2];
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Abstract: Retinal ganglion cells (RGCs), the output neurons of the retina, send information to the brain in patterns of electrical activity. Previous anatomical work has revealed that roughly 20 types of RGCs exist in the macaque monkey and likely encode different features of the visual scene (Dacey *et al.*, 2003). The functions of only the five highest-density types have been extensively investigated. In order to understand the neural code of the retina, it is necessary to understand the visual features extracted by the remaining low-density RGC types.
Large-scale multi-electrode recordings from *ex vivo* preparations of macaque retina revealed several types of low-density RGCs in addition to the numerically dominant RGC types. Reverse correlation of the spike times with a white noise visual stimulus enabled classification of different cell types and characterization of the spatiotemporal response properties of each cell. The response properties of two types of low-density RGCs were examined further, one exhibiting ON light responses and one exhibiting OFF light responses. As with previously studied RGC types, the two low-density cell types each formed a complete mosaic tiling the recorded region. They had similar temporal response properties to the ON and OFF parasol cells, respectively, but their receptive fields were 2-3 times larger in diameter. These properties, combined with the known dendritic field sizes and overlapping dendritic stratification of parasol cells and smooth cells (Dacey, 2004), suggest that these cell types correspond to the ON and OFF smooth cell types (Petrusca *et al.*, 2007; Crook *et al.*, 2008). Despite animal-to-animal variability, the putative smooth cells were identified in multiple recordings by normalizing their responses to the responses of well-studied RGC types in the same preparation. High-resolution visual stimulation revealed microstructure within the receptive field of the putative smooth cells as well as interdigitation of the irregular structure in the receptive fields of neighboring cells. The receptive field of each cell contained 3 to 5 zones of high sensitivity. The putative smooth cells would be expected to pool inputs from up to several hundred bipolar cells with much smaller summation areas, indicating that each of the observed zones of high sensitivity was made up of multiple bipolar inputs. In response to images of natural scenes with simulated eye movements, the putative smooth cells fired almost exclusively at simulated saccades, suggesting involvement in the signaling of large movements or changes in the visual scene.

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Nanosymposium

110. Retina Photoreceptor and Circuitry

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Title: Developing retinal ganglion cells balance input from bipolar cell types to ensure response homeostasis

Authors: *N.-W. TIEN, D. KERSCHENSTEINER;
Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Developing neurons form precise connections with different presynaptic partners and thus attain specific response properties. Here, we study a well-characterized retinal circuit to determine if postsynaptic neurons establish connections with different presynaptic partners independently, or whether they balance converging inputs to achieve response homeostasis. Retinal ganglion cells that respond with sustained spiking and high contrast sensitivity to light increments (αON- RGCs) receive excitatory input from multiple bipolar cell types dominated by B6 cells. Here, we generate mice in which B6 cells are selectively removed from developing circuits by transgenic expression of diphtheria toxin (*B6-DT* mice). In *B6-DT* mice, αON-RGCs adjust connectivity patterns with other bipolar cells in a cell-type-specific manner. Whereas input from some bipolar cell types is upregulated, input from others remains unchanged. Electrophysiological recordings reveal that spatiotemporal receptive field properties, sustained responses, and contrast sensitivity of αON-RGCs are preserved in *B6-DT* mice, supporting the notion that anatomical plasticity ensures response homeostasis in this circuit.

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Nanosymposium

110. Retina Photoreceptor and Circuitry

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Topic: D.06. Vision

Support: CDMRP W81XWH

Title: Color components under joint recording from the human retina and cortex with a high-density electrode net

Authors: *C. W. TYLER, S. C. NICHOLAS, L. T. LIKOVA;
Smith-Kettlewell Brain Imaging Ctr., Smith-Kettlewell Eye Res. Inst., San Francisco, CA

Abstract: To gain insight into the transmission of information along the visual pathway, we recorded electroretinographic (ERG) and electroencephalographic (EEG) signals simultaneously from a high-density EEG system for the first time. High-quality ERG and EEG responses for full-field stimulation as a function of wavelength and intensity were obtainable with this approach.
Methods. We used an EGI 128-electrode whole-head EEG scalp recording system to study joint ERG/EEG responses from to 2.5 Hz On-Off epochs of full-field stimuli at 480, 540 and 610 nm peak wavelengths, plus their sum (white), over a 2.5 log unit range of intensities from scotopic to photopic levels. Data were obtained for 5 healthy individuals and averaged.
Results. The average data were analyzed by singular value decomposition, under which 6 components accounted for 97% of the variance. There were two ERG components (responses restricted to the electrodes around the eyes), one peaking around 40 ms with a spectral sensitivity distribution matching the melanopic template, the other peaking around 60 ms with a uniform distribution across all color conditions. A small photopic component could also be identified with a focal occipital distribution corresponding to primary visual cortex, peaking at 90 ms. Three further EEG components had a ventral occipital scalp distribution corresponding to the cortical color areas adjacent to retinotopic V4, were each dominated by one color stimulus. The red-dominant component peaked at 100ms, the green-dominant component was primarily negative peaking at 90 ms, and the blue-dominant component peaked at 170 ms. Several of the ERG/EEG components showed sub-additivity of the white response relative to the color contributions, implying inhibitory relationships among the underlying color channels.
Conclusions. The ability to measure simultaneous ERG/EEG from a whole-head electrode net, developed for the first time in this study, allows the tracking of signals through the visual system from the retina to the primary visual cortex and then to higher cortical areas. The timings of the ERG components area are consistent with standard light-adapted ERG measures, though with unusual spectral characteristics, while the cortical color responses are in the typical range for early visual evoked potentials but reflected unexpected specificity to particular stimulus wavelengths.

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Nanosymposium

110. Retina Photoreceptor and Circuitry

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Title: Deep convolutional neural network models of the retinal response to natural scenes

Authors: *L. T. MCINTOSH[1], N. MAHESWARANATHAN[1], A. NAYEBI[1], S. GANGULI[2], S. A. BACCUS[3];
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Abstract: A central challenge in sensory neuroscience involves understanding neural computations and circuit mechanisms underlying responses to ethologically relevant, natural stimuli. However, the ubiquity of cascaded nonlinear processes like synaptic transmission and spiking dynamics in multilayered circuits has presented significant obstacles to the goal of learning accurate computational models of circuit responses to natural stimuli from neural recordings. Instead, simple models of responses to spatiotemporal white noise have formed the conceptual bedrock of our understanding of early sensory function. Surprisingly, few studies have addressed whether or not these simple models generalize to natural stimuli (Heitman et. al. 2016).

To address these challenges, we employ deep convolutional neural networks (CNNs), which demonstrate success at many pattern recognition tasks (LeCun et al. 2015). These models cascade multiple layers of filtering and rectification — exactly the elementary computational building blocks thought to underlie complex functional responses of sensory circuits. Previous work utilized these models to understand responses in IT cortex (Yamins et. al. 2013), but not in early sensory areas where knowledge of neural circuitry can provide important validation for such models.

We find that CNNs are considerably more accurate at capturing retinal responses to held-out natural scenes than linear-nonlinear (LN) models and related models, such as generalized linear models (GLMs). Furthermore, we find CNNs generalize significantly better across classes of stimuli (white noise vs. natural scenes) they were not trained on. Remarkably, analysis of these CNNs reveals internal units selective for visual features on the same small spatial scale as the main excitatory interneurons of the retina, bipolar cells. Moreover, probing the model with reversing gratings, paired flashes, and contrast steps reveals that the CNN learns nonlinear retinal response properties such as frequency doubling and adaptation, even though the CNNs were not trained on such stimuli.

Our work brings models with rich computational capacity to bear on the problem of understanding natural scene responses, and demonstrates the power of CNNs to not only accurately capture sensory circuit responses to natural scenes, but also uncover the circuit's internal structure and function. Moreover, our methods can be readily generalized to other sensory modalities and stimulus ensembles.

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Nanosymposium

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Title: Vision at its sensitivity limit: Linking neural circuit function with behavior

Authors: ***L. SMEDS**[1], D. TAKESHITA[1], T. TURUNEN[2], J. TIIHONEN[2], P. ALA-LAURILA[1,2];
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Abstract: Correlating neural circuit function with behavior is a central goal in neuroscience. This has been difficult because of the complexity of neural circuits and the computations relevant for behavior. Vision at the sensitivity limit offers an outstanding possibility to overcome these challenges. Nevertheless, it has remained unclear to which extent the two fundamentally different retinal outputs – On and Off pathways – define behavior. We measured the sensitivity limit of light detection at the level of mouse rods, retinal ganglion cells (On and Off sustained alpha-like cells), and behavior. Responses of single rods were recorded with suction pipettes and ganglion-cell responses with patch electrodes in flat-mounted dark-adapted retinas. Visually guided behavior was measured in a water maze test using a novel tracking method of mice. By manipulating single-quantum responses via transgenic techniques in a mouse line (OPN) expressing human L-cone pigment in its rods, we show that visually guided behavior at the detection threshold relies on information provided by the retinal On pathway.

Higher-order decision mechanisms cannot integrate information across On and Off retinal outputs even when it would allow higher visual sensitivity. High amplification of single-photon responses in rods and nonlinear signal processing in the On pathway are the key determinants defining behavioral threshold.

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Nanosymposium

110. Retina Photoreceptor and Circuitry

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CNPq Fellowship 233829/2014-8

Title: Multifocal steady-state visual evoked potential-based assessment for visual field defects using ngoggle

Authors: ***Y.-T. WANG**[1], F. MEDEIROS[2], M. NAKANISHI[1,2], Y.-Y. CHIEN[1], C.-S. WEI[1], J. ZAO[3];
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Abstract: Glaucoma is a progressive optic neuropathy that can be associated with irreversible visual field loss. Standard automated perimetry (SAP) is the standard method used for assessment of functional loss in the disease. However, SAP is limited by the subjectivity of patient responses and considerable variability. In addition, SAP testing is generally costly and lacks portability, precluding its use as a screening device in remote locations and also for at-home testing. In previous studies, we have shown the feasibility of applying an objective method for assessment of functional deficits in glaucoma using multifocal steady state visual-evoked

potentials (mfSSVEPs) on a mobile, wearable and wireless AR/VR platform, nGoggle (Cerebra Technologies, Inc., Hsinchu, Taiwan). But, the study only reported the results of full-field SSVEPs for assessing retinopathy, which lacks specificity. In this study, we extend our previous work by applying a novel analytical method to assess mfSSVEPs elicited by visual stimuli flashed with different frequencies at different areas of the visual field, providing imperative specificity.Three glaucoma patients and one healthy subject participated in this pilot study. The visual stimulus consisted of 20 sectors of visual stimuli flickering with different frequencies ranged from 8.0 to 11.8 Hz with a step of 0.2 Hz. The EEG data recorded by nGoggle were band-pass filtered from 5 Hz to 50 Hz. Artifact subspace reconstruction (ASR, EEGLab) method was applied to remove high-amplitude artifacts. The trials with severe artifacts were then manually removed. Canonical correlation analysis (CCA) and Fast Fourier transform (FFT) were then applied to the artifact-reduced EEG to assess visual field loss. The CCA-based spatial filter was used to remove background EEG activities and improve the signal-to-noise ratio of measured mfSSVEP. FFT was then applied to the output of the CCA to measure spectral amplitudes within each sector, which were compared against the SAP sensitivity of the corresponding region.Study results showed that glaucoma eyes had lower mfSSVEP amplitude compared to healthy eyes. In glaucoma patients with unilateral visual field defect, the contralateral eye showed lower mfSSVEP amplitudes compared to controls, suggesting the possibility of early detection of visual field damage by the nGoggle. In conclusion, this pilot study suggests the feasibility of using the nGoggle, a portable and wireless AR/VR platform, to assess visual field loss in glaucoma patients.

Disclosures: **Y. Wang:** None. **F. Medeiros:** None. **M. Nakanishi:** None. **Y. Chien:** None. **C. Wei:** None. **J. Zao:** None.

Nanosymposium

111. Neuroethology of Auditory Communication

Location: SDCC 30B

Time: Sunday, November 13, 2016, 8:00 AM - 9:45 AM

Presentation Number: 111.01

Topic: F.01. Neuroethology

Title: Predicting song structure from social context

Authors: *A. J. CALHOUN[1], P. COEN[3], M. MURTHY[2];
[1]PNI, Princeton Univ., Princeton Junction, NJ; [2]Princeton Univ., Princeton, NJ; [3]UCL, London, United Kingdom

Abstract: Individuals make decisions differently when they are in competition versus when they are alone. In the fruit fly Drosophila, males court females by following and singing to them; song

structure is highly variable and sculpted by sensory feedback from the female (Coen et al. Nature 2014; Coen et al. Neuron 2016). In fact, generalized linear models (GLMs) are effective at predicting the full structure of song from the dynamics of fly movements and interactions. This is because the decision to sing - and what type of song, or with what intensity - is tightly controlled by sensory stimuli (in particular, how fast the female is moving and how far away she is). However, these experiments have so far only examined male behavior in the presence of a single female. How does song patterning change when multiple males are present? And how does the female decide with which male to mate? We quantify the effects of allowing two males to compete for a single female. Wing-clipped males (that cannot sing) lose disproportionately (>85% of the time) to competing wildtype males, even though they continue to chase females during courtship. This suggests that song is integral to the copulation decision. In competitions between two males that can both sing, the individual that sings the most bouts of songs 'wins' the courtship. In order to identify whether song decisions are patterned similarly under conditions that include competition we compute GLMs using an extended parameter set that now includes all single-animal, pairwise, and triplet interactions (we further include nonlinear combinations of parameters in our refined model). Using these models we find that both the sensory cues that drive song patterning and the structure of song change in the presence of a second male. These data suggest that song production decisions in Drosophila are actively modulated by the social context. We will present these data and ongoing work to build a generative model for song patterning that includes long timescale interactions.

Disclosures: A.J. Calhoun: None. **P. Coen:** None. **M. Murthy:** None.

Nanosymposium

111. Neuroethology of Auditory Communication

Location: SDCC 30B

Time: Sunday, November 13, 2016, 8:00 AM - 9:45 AM

Presentation Number: 111.02

Topic: F.01. Neuroethology

Support: Conacyt/Cambridge Trusts

Title: Behavioural dissection of a song-pattern recognition network

Authors: *B. HEDWIG[1], E. J. SARMIENTO-PONCE[2];
[1]Univ. of Cambridge, Cambridge, United Kingdom; [2]Dept. of Zoology, University of Cambridge, United Kingdom

Abstract: Signalling with repetitive sound patterns is essential for mate attraction in many insects and vertebrates. At the receiver side signal processing requires neural recognition

mechanisms tuned to species-specific acoustic patterns. Behavioural studies with systematically varied sound patterns aim to characterise the temporal tuning of the phonotactic behaviour and to reveal the principles of the underlying processing mechanisms. Phonotaxis experiments in crickets, however, are generally based on setting the same pulse duration or interval within chirps for a complete test series. They cannot reveal any dynamic changes in the neural filter properties while a sequence of sound pulses is processed in a delay-line and coincidence detection network. Due to the dynamic changes in neural processing in this detector network that underlies pattern recognition, we hypothesized that manipulating individual sound pulses or intervals in the sequence of chirps will have specific effects on female cricket phonotactic behaviour. Corresponding tests with 3 pulsed chirps reveal very different characteristic female phonotaxis response curves for changes in the duration of the first, second or third sound pulse. Such different responses were not expected from conventional phonotaxis tests and provide further insight into the delay-line and coincidence detection network in the cricket brain. The sound patterns will be important test tools for neurophysiological analysis.

Disclosures: **B. Hedwig:** None. **E.J. Sarmiento-Ponce:** None.

Nanosymposium

111. Neuroethology of Auditory Communication

Location: SDCC 30B

Time: Sunday, November 13, 2016, 8:00 AM - 9:45 AM

Presentation Number: 111.03

Topic: F.01. Neuroethology

Support: JSPS KAKENHI #26240019

JSPS KAKENHI #16H01481

Title: Arousal state-dependent neural activity in the songbird premotor nucleus HVC: an investigation by local field potentials.

Authors: ***S. SHIBA**[1], K. OKANOYA[2];
[1]Life Sci., Grad. Sch. of Arts and Sciences, the Universi, Tokyo, Japan; [2]Life Sci., The Univ. of Tokyo, Tokyo, Japan

Abstract: Songbird is a popular model animal for neuroethological studies on vocal and auditory processing because they learn complex vocalizations. In the songbird brain, the nucleus HVC is considered as a premotor area, mainly generating the timing of each element in vocalization. At the same time, the HVC receives inputs from higher auditory areas, and there are neurons responding to specific auditory stimuli. Some of such HVC neurons are known to change their

response characteristics to specific auditory stimuli according to the state of individual, such as awake or asleep. However, the neural mechanism or the function of this change is poorly understood. To know these in detail, it is needed to investigate not only the characteristics of neural response to stimulus but also that of resting-state spontaneous activity, related with the state of the individual. This research demonstrates the relationship between the change of individual state and the change of the characteristics of neural activity. By continuous and long-time recording of local field potentials (LFP) in the HVC in Java sparrow (*Padda oryzivora*) using an ultra-light wireless transmitter, we investigated the activities of large proportion of the HVC neurons. The frequency component of 30-50 Hz was larger while asleep (nighttime) than while awake (daytime) in the HVC spontaneous activity. The amplitude of LFP was lower when playing back bird's own song during nighttime than during daytime. We discuss these results in light of the function of HVC related with the states, including the anatomical and physiological characteristics of each neuron.

Disclosures: S. Shiba: None. **K. Okanoya:** None.

Nanosymposium

111. Neuroethology of Auditory Communication

Location: SDCC 30B

Time: Sunday, November 13, 2016, 8:00 AM - 9:45 AM

Presentation Number: 111.04

Topic: F.01. Neuroethology

Support: Kakenhi #26240019

ImPACT 16H01498

Title: Auditory selectivity in the songbird nucleus taeniae of the amygdala

Authors: *T. FUJII[1], M. IKEBUCHI[2], K. OKANOYA[1,2];
[1]Dept. of Arts and Sci., The Univ. of Tokyo, Tokyo, Japan; [2]Cognitive and Behavioral Sci. Joint Res. Lab., RIKEN Brain Sci. Inst., Wako, Saitama, Japan

Abstract: Animals must decipher information encoded in vocalizations and take adaptive behaviors in response. Songbirds are an excellent model to search for the neural basis of such process, because they produce and recognize various vocalizations related to complex social factors. The avian nucleus taeniae of the amygdala (TnA) has been regarded as the counterpart of the mammalian medial amygdala from previous neuroanatomical studies, and functional studies on the effect of lesions on sexual and social behaviors in songbird and non-songbird species. Recent studies examining the expression of immediate early genes suggested that neural activity

in TnA can reflect familiarity or quality of songs. Based on these findings, one can speculate that the TnA plays a role in linking perception of vocal stimuli to subsequent relevant behaviors. In considering this possibility, auditory response properties in the TnA is worth being investigated at the level of single-unit activity. Here we explored auditory responses of TnA neurons in Bengalese finches, particularly focusing on stimulus selectivity.Adult male and female birds were anesthetized with urethane and exposed to conspecific and heterospecific songs, conspecific distance calls, as well as white noise. Single-unit activities in the TnA and adjacent Arcopallium were recorded during the stimulus presentation. In total, we recorded neural activity of 115 TnA neurons (84 from 10 males, 31 from 7 females). Analysis of firing rate revealed that 79 out of 115 units were responsive to some stimulus, with 16 of these selective for a specific stimulus or stimuli. The stimuli that elicited significant responses varied among units and there seemed no trends of selectivity among the population. We also examined sex differences in the proportion of auditory responsive and selective units and found that the the percentage of responsive and selective units were higher in males than in females. The Arcopallium neighboring to the TnA also had substantial population of responsive (302 / 417) and selective units (103 / 302). In summary, we showed that neural activity in the songbird TnA is capable of representing attributes of vocal sounds beyond simple acoustical features. Because the proportion of responsive and selective neurons in the TnA was comparable to that in the surrounding Arcopallium, structural and functional correspondence between the mammalian and avian amygdala should be further investigated.

Disclosures: **T. Fujii:** None. **M. Ikebuchi:** None. **K. Okanoya:** None.

Nanosymposium

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Presentation Number: 111.05

Topic: F.01. Neuroethology

Support: Canadian Institute for Advanced Research

NIIH Grant 1RC1GM091556

Title: A distributed neurogenomic response in a songbird to the experience of sound chamber isolation

Authors: J. M. GEORGE, Z. W. BELL, *D. F. CLAYTON;
Queen Mary, Univ. of London, London, United Kingdom

Abstract: Many animal experiments begin by placing a subject inside an observation or testing chamber - a manipulation which itself may alter the state of the animal compared to the true resting or normal condition, especially for animals that live in social groups. In research using songbirds, individuals are typically placed alone overnight inside a sound isolation chamber, so that immediate auditory experience can be controlled and vocalizations easily recorded. What impact might this manipulation itself have on the underlying neurophysiology of auditory processing and vocal communication? Here we applied a combination of techniques (RNAseq, qPCR, in situ hybridization) to assess the effect of overnight isolation on gene expression in the zebra finch forebrain, focusing on the auditory caudomedial pallium ("auditory lobule"). Deep RNA sequencing was first performed on individual auditory lobules from 24 females, half after solo isolation in a standard sound chamber and the others taken directly from a social aviary. Isolation suppressed expression of EGR1 and BDNF, genes linked to neurophysiological activity, learning and neuroplasticity. Yet in the same animals, isolation boosted expression of a urotensinergic neuropeptide (encoded by UTS2B) and the glucocorticoid receptor chaperone, FKBP5. These effects were replicated using quantitative PCR, both in males and also in birds from a separate aviary. In situ hybridisation showed that the effects were not limited to the auditory forebrain nor to the same region or cell type in each case. Rather, multiple brain systems seem to be affected at the transcriptional level by the experience of acute isolation. These results have practical implications for the design and interpretation of common experiments where the "control" condition is a bird place alone overnight in a sound chamber: these birds are in a different neurogenomic state from birds in a more normal social context. A broader implication is that higher perceptual and cognitive processes may be altered by the immediate social or physical environment through dynamic changes in brain gene expression.

Disclosures: **J.M. George:** None. **Z.W. Bell:** None. **D.F. Clayton:** None.

Nanosymposium

111. Neuroethology of Auditory Communication

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Presentation Number: 111.06

Topic: F.01. Neuroethology

Support: SFB 870 B17

NSF-IOS1457108

Title: New insights into extreme synchrony of neuronal firing: neurochemical profile of a vocal central pattern generator

Authors: *E. WEISE[1], A. BASS[2], B. CHAGNAUD[1];
[1]Ludwig-Maximilians-University Munich, Planegg-Martinsried, Germany; [2]Cornell Univ., Ithaca, NY

Abstract: Vocalization is a trait shared among many vertebrates and often essential for reproductive success. In toadfish, a model for vertebrate vocal networks, the basic features of vocalizations are determined by a hindbrain vocal central pattern generator (vCPG) that consists of three anatomically separate nuclei: the vocal pre-pacemaker nucleus (VPP), the vocal pacemaker nucleus (VPN) and the vocal motor nucleus (VMN). The neurochemical profile of this vocal network remains largely unexplored.
Using immunohistochemistry combined with transneuronal labeling (neurobiotin) of the entire vCPG, we investigated the neurotransmitter profile of the vCPG nuclei in two toadfish species, *Allenbatrachus grunniens* and *Opsanu beta*. Consistent with prior studies in other toadfish species, neurobiotin delineated three vCPG nuclei with VMN motor neurons being cholinergic and receiving extensive GABAergic innervation. We now also show that VMN receives a prominent glycinergic input. Both types of inhibitory neurons are within the VPN region, though morphologically distinct from the majority of neurobiotin-labeled VPN neurons. However, a subset of the glycinergic, but not GABAergic, neurons was co-labeled with neurobiotin, suggesting gap junction coupling to VPN and/or VMN neurons. Thus, three inhibitory neuron groups constitute novel vCPG populations within the VPN region: GABAergic neurons, glycinergic neurons, neurobiotin/glycinergic co-labeled neurons. GABAergic and glycinergic input was also detected in VPN and in VPP, though neurons in the VPP region were not co-labeled with neurobiotin. The presence of both GABAergic and glycinergic inputs strengthen the previously demonstrated necessity for inhibitory input to VMN in generating a highly synchronous, temporally stable vCPG output.
Glutamatergic somata were identified in all vCPG nuclei, including VMN motor neurons, though vocal motor axons were not labeled. In agreement with vocal mechanisms in other vertebrates and prior studies of VMN, there was a robust serotonergic input to all vCPG nuclei. Notably, serotonergic innervation was mainly in an area where VMN somata cluster in *A. grunniens*, but lateral to VMN in a region where VMN and VPN dendrites overlap in *O. beta.*
In summary, our comparative data for toadfish identifies glutamate as a major excitatory transmitter in all nodes of the vCPG with GABA and glycine providing prominent inhibitory input that likely supports the highly stable, temporal pattern of vCPG output and natural vocalization. A prominent serotonergic input to all vCPG nodes offers a potential mechanism for modulation of vocal patterning at all levels of the hindbrain vocal network.

Disclosures: E. Weise: None. **A. Bass:** None. **B. Chagnaud:** None.

Nanosymposium

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Title: Function and mechanism of the female song system, mating preferences, and social behaviour in the brown-headed cowbird (Molothrus ater)

Authors: E. L. COLDIN[1], H. DAVIES[1], A. PERKES[2], S. A. MACDOUGALL-SHACKLETON[3], M. F. SCHMIDT[2], *D. J. WHITE[1];
[1]Psychology, Wilfrid Laurier Univ., Waterloo, ON, Canada; [2]Biol., Univ. of Pennsylvania, Philadelphia, PA; [3]Psychology, Univ. of Western Ontario, London, ON, Canada

Abstract: In songbirds, females dictate reproduction and evolution through mate choice, selecting only the highest quality males, based on analysis of their song quality. It has been suggested that the female song control system facilitates these song preferences, though the neurological and behavioural mechanisms by which song preferences lead to reproduction are unknown. The current study examined the role of female song preferences and social behaviour in courtship, mate selection and reproductive success. A subset of females from two flocks of brown-headed cowbirds (*Molothrus ater*) received either lesions to song area HVC, previously shown to disrupt song preferences, or sham lesions. Social and reproductive behaviour was quantified through the following breeding season. Initial analyses indicate that females use a behavioural strategy during courtship and pair bonding, including management of distance, time, and use of positive and negative feedback toward particular males during social interactions. The importance of this behaviour independent of song preference disruption was also demonstrated by altered behavior in non-lesioned females housed with lesioned individuals. Additional analyses will focus on examining disruptions to reproductive success in females exhibiting an altered behavioural strategy, including number, strength and quality of pairings, as well as the survival rate and performance of offspring. These results provide crucial evidence for the function and mechanism of the female song control system and mating preferences, demonstrating the importance of behavioural strategy during courtship and the critical role of females in courtship and reproduction in songbird species.

Disclosures: **E.L. Coldin:** None. **H. Davies:** None. **A. Perkes:** None. **S.A. MacDougall-Shackleton:** None. **M.F. Schmidt:** None. **D.J. White:** None.

Nanosymposium

112. Interactions Between Stress and Immune Function

Location: SDCC 5B

Time: Sunday, November 13, 2016, 8:00 AM - 11:30 AM

Presentation Number: 112.01

Topic: F.04. Stress and the Brain

Support: NIMH IRP to MAH

Title: Effects of microglia depletion on behavior, stress reactivity, and recovery

Authors: ***T. K. WEIGEL**, M. L. LEHMANN, M. HERKENHAM;
Natl. Inst. of Mental Hlth., NIH, Bethesda, MD

Abstract: Microglia are responsive to psychosocial stress, which can cause their proliferation and activation, but the role that they play in responding to this stress is not fully known. Depletion of microglia in adult mice under normal conditions has been shown to have no effect on behavioral tests of anxiety, but previously no studies have examined the effects of microglia depletion on the behavioral outcomes of chronic stress. We used the drug PLX5622, a colony stimulating factor 1 receptor (CSF1R) inhibitor, to deplete microglia in chronically socially stressed mice and study their stress reactivity and recovery. After 14 days of treatment with PLX5622, the number of microglia was decreased to <5% of baseline. We examined the social and sexual behavior of mice administered PLX5622 feed or nondrug feed before and after exposure to chronic social defeat stress. The behaviors of the drug and nondrug groups were not significantly different before stress. After chronic stress, the nondrug group showed a dramatic decrease in social interaction while the PLX5622 group did not show a significant change from their pre-stress scores. This suggests that microglia depletion does not alter baseline social behavior but it protects against the decreased social interaction resulting from chronic social defeat. After the chronic social defeat ended, a group of PLX5622-treated mice was switched to nondrug feed for a two-week recovery period. In this period, microglia repopulated the brain, and at the end, the microglia-repleted mice showed a significant decrease in social interaction. This result shows that microglia reconstitution in the aftermath of stress removes the protection provided by the original microglia depletion. We have observed CCR2+ cells in the brain parenchyma during microglia reconstitution, a sign of peripheral macrophages crossing the blood-brain barrier. Our results suggest that microglia play a role in effecting depressive-like behavioral changes in chronically socially stressed mice and that microglia depletion can protect against these effects during and after chronic stress.

Disclosures: **T.K. Weigel:** None. **M.L. Lehmann:** None. **M. Herkenham:** None.

Nanosymposium

112. Interactions Between Stress and Immune Function

Location: SDCC 5B

Time: Sunday, November 13, 2016, 8:00 AM - 11:30 AM

Presentation Number: 112.02

Topic: F.04. Stress and the Brain

Support: 5R21MH097182-02

Title: Lipopolysaccharide-induced changes in adolescent prefrontal cortex AMPA receptors after early life stress

Authors: P. GANGULY[1], S. SHAH[1], E. COLEY[1], *H. C. BRENHOUSE[2];
[2]Psychology, [1]Northeastern Univ., Boston, MA

Abstract: Early life stress (ELS) exposure in rodents alters the development of prefrontal cortex (PFC) activity, which is largely regulated by inotropic glutamate receptors such as AMPA. Active regulation of AMPA receptor (AMPAR) trafficking to the plasma membrane is fundamentally important for synaptic plasticity and behavior. Interestingly, recent studies have shown that AMPAR trafficking is mediated by neuroimmune signaling, as production of the cytokine tumor necrosis factor-α (TNF-α) causes rapid trafficking of GluR2-lacking AMPARs to the surface membrane. Decreased GluR2 can enhance excitotoxicity, a process that plays a major role in the etiology of several neuropsychiatric diseases. TNF-α levels are also increased after ELS, suggesting a possible neuroimmune mechanism that yields ELS populations more vulnerable to psychiatric disorders. Indeed, several studies have shown that ELS disrupts normal developmental trajectory of PFC AMPAR receptor expression and may also upset glutamate receptor trafficking and mobility.
We hypothesized that ELS yields PFC dysfunction via heightened neuroimmune activity and consequentially altered AMPA composition. We aimed to determine whether ELS in the form of maternal separation would increase vulnerability to a subsequent adolescent immune challenge with lipopolysaccharide (LPS), hence increasing TNF-α mediated rapid exocytosis of GluR2-lacking AMPARs to the plasma membrane. We further determined whether administration of the TNF-α antagonist Ibudilast could successfully prevent ELS and/or LPS induced changes. Male and female rats were either reared under control conditions or were separated from their mother and littermates for 4h/day from postnatal days 2-20. On postnatal day 40, animals were administered LPS and/or Ibudilast. Five hours after LPS, behavior was assessed in an open field and the PFC was extracted for qPCR analysis of TNF-α and western blot analyses on membrane fractions of GluR2 and GluR1.

We observed that LPS decreases GluR2 levels in the PFC, while Ibudilast protects against GluR2 loss in both male and female adolescents. Maternally separated male rats, but not female rats, also showed lower GluR2 levels. LPS exacerbates this loss, while Ibudilast protects against GluR2 depletion. Taken together, these findings suggest that ELS affects neuroimmune signaling in male adolescents, which leads to decreased PFC GluR2 that may reflect increased trafficking of GluR2-lacking AMPAR.

Disclosures: **P. Ganguly:** None. **S. Shah:** None. **E. Coley:** None. **H.C. Brenhouse:** None.

Nanosymposium

112. Interactions Between Stress and Immune Function

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Time: Sunday, November 13, 2016, 8:00 AM - 11:30 AM

Presentation Number: 112.03

Topic: F.04. Stress and the Brain

Support: NIMH Grant 5R21MH097182

Title: Early life stress increases microglia activation in juvenile rats and confers sensitization in microglia to LPS induced immune activation

Authors: ***S. A. GOFF**, V. THOMPSON, P. GANGULY, H. C. BRENHOUSE;
Psychology, Northeastern Univ., Boston, MA

Abstract: Overwhelming evidence suggests that adversity during early life markedly increases vulnerability to a myriad of neuropsychiatric disorders including depression, anxiety, and schizophrenia. Importantly, stress during this time modifies circulating levels of stress hormones, which in turn has downstream effects on neuroimmune function. We hypothesize that, these changes likely negatively impact overall neural development via neuroimmune signaling - particularly within the prefrontal cortex (PFC) - thereby leading to altered pathology associated with neuropsychiatric dysfunction. While the etiological mechanisms are not fully understood, resident microglia are thought to be a common source of increased neuroimmune activity through production of inflammatory molecules (e.g. cytokines, chemokines) in response to disruption in homeostasis. Microglia are capable of provoking long-term changes in brain structure and function, particularly within local microcircuitry. Importantly, they have the ability to become chronically sensitized, or 'primed', to over-activation following insult. Early life stress via maternal separation (MS) is thought to alter microglial reactivity to subsequent immune activation across development. In order to better understand the impact of MS on microglial priming in the developing immune system, rat pups were separated from their dams for 4 hours per day from P2-20. In order to stress immune reactivity following MS, rats were

exposed to lipopolysaccharide (LPS) at distinct developmental time points (P9, P20, or P40), and the concentrations of ramified and amoeboid PFC microglia were quantified to gain insight to activity states. Our findings reveal that by P20, MS rats show a higher baseline level of activated microglia, and by P40 there is evidence of microglial sensitization to LPS immune activation. Taken together, these findings provide compelling evidence for a role of early life adversity in altering microglia function in later life.

Disclosures: S.A. Goff: None. **V. Thompson:** None. **P. Ganguly:** None. **H.C. Brenhouse:** None.

Nanosymposium

112. Interactions Between Stress and Immune Function

Location: SDCC 5B

Time: Sunday, November 13, 2016, 8:00 AM - 11:30 AM

Presentation Number: 112.04

Topic: F.04. Stress and the Brain

Title: Neonatal inflammation induces mechanical allodynia andsex-specific gene expression in brain areas involved in the active modulationof neuropathic and inflammatory pain

Authors: S. YAN, *A. C. KENTNER;
Sch. of Arts and Sci., MCPHS Univ., Boston, MA

Abstract: Exposure to painful procedures and/or stressors during the early neonatal period can reprogram the underlying neurocircuitry involved in neuropathic and inflammatory pain perception. The reprogramming of these systems can result in an enduring elevation in sensitivity towards mechanical and thermal stimuli. Recent evidence suggests that exposure to mild inflammatory mediators during the neonatal period can induce similar pain responses in both adolescent and adult rats. Therefore, we sought to profile changes in the expression of several genes across brain areas involved in the active modulation of neuropathic and inflammatory pain using a well-recognized model of neonatal inflammation. In the present study male and female Sprague-Dawley rats were administered either the inflammatory endotoxin lipopolysaccharide (LPS; 0.05mg/kg, i.p.) or saline (equivolume) on postnatal days (PND) 3 and 5. During adolescence, hind paw mechanical withdrawal thresholds were evaluated using an electronic von Frey anesthesiometer (IITC Life Science, Woodland Hills, CA). Animals challenged neonatally with LPS had increased pain sensitivity on this measure. Preliminary results indicate that neonatal LPS reduced the expression of Oprm1 in the periaqueductal grey and prefrontal cortex of male and female rats whereas Cnr1 was only decreased in the prefrontal cortex of female animals. Our data suggest that the experience of a mild inflammatory sickness induced in early

life has a far reaching impact on adolescent pain responses and underlying gene expression profiles across important brain areas involved in pain processing.

Disclosures: **S. Yan:** None. **A.C. Kentner:** None.

Nanosymposium

112. Interactions Between Stress and Immune Function

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Southern Regional Educational Board (SREB)-State Doctoral Scholars Program Doctoral Award

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Title: The effects of cyclooxygenase-2 inhibition on stress-induced behavioral and synaptic pathologies

Authors: *J. C. GAMBLE-GEORGE[1], R. BALDI[1], L. HALLADAY[4], A. KOCHARIAN[4], N. D. HARTLEY[1], C. G. SILVA[1], H. ROBERTS[1], A. HAYMER[1], L. J. MARNETT[2], A. HOLMES[4], S. PATEL[3];
[1]Dept. of Psychiatry, [2]A.B. Hancock Jr. Mem. Lab. for Cancer Research, Departments of Biochemistry, Chemistry, an, [3]Departments of Psychiatry and Mol. Physiol. and Biophysics and the Vanderbilt Brain Inst., Vanderbilt Univ. Sch. of Med., Nashville, TN; [4]Lab. of Behavioral and Genomic Neurosci., Natl. Inst. on Alcoholism and Alcohol Abuse (NIAAA), Bethesda, MD

Abstract: Cannabinoid receptors have been examined as potential targets to alleviate the negative consequences of anxiety, trauma-related, and stress-related disorders. However, in preclinical animal studies, synthetic cannabinoids can produce adverse motoric and cognitive effects. Thus, pharmacological strategies that augment endocannabinoid levels in the brain, with

the aim of enhancing signaling through cannabinoid receptors, are being investigated for their ability to modulate anxiety and stress responses. Previously, we have demonstrated that either genetic removal of prostaglandin-endoperoxide synthase 2 gene, which codes for the cyclooxygenase-2 (COX-2) enzyme that degrades the endocannabinoids, anandamide and 2-arachidonylglycerol, or pharmacologically inhibiting COX-2 activity with a substrate-selective COX-2 inhibitor (SSCI), LM-4131, can increase brain anandamide levels. These elevations in endocannabinoid levels in the rodent brain resulted in enhanced endocannabinoid signaling through the cannabinoid type 1 receptor and, subsequently, reduced anxiety-like behaviors in mice under basal conditions. Using the novelty-induced hypophagia (NIH) assay, elevated plus maze, and *ex vivo* and *in vivo* electrophysiology, we tested the hypothesis that endocannabinoid augmentation via SSCIs may have the potential to counteract stress-induced anxiety-like behaviors. We have found that the SSCIs, LM-4131 and lumiracoxib, and the selective COX-2 inhibitor, celecoxib, can reduce anxiety-like behaviors in mice subjected to footshock stress. In contrast, these inhibitors had little effect in non-stressed mice. The anxiolytic action of the SSCI, LM-4131, was mediated through the cannabinoid type 1 receptor under non-stressed (control) conditions, but mediated through the small conductance calcium-activated potassium (SK) channels when mice were subjected to footshock stress. Also, we have found that the anxiolytic effects of SSCIs in stressed mice may be due to a decrease in excitatory cell firing in the amygdala. Ongoing studies will further elucidate the receptor mechanisms in terms of brain region specificity that are involved in the anxiolytic effects of SSCIs after stress exposure.

Disclosures: J.C. Gamble-George: None. **R. Baldi:** None. **L. Halladay:** None. **A. Kocharian:** None. **N.D. Hartley:** None. **C.G. Silva:** None. **H. Roberts:** None. **A. Haymer:** None. **L.J. Marnett:** 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; collaborative research contract with Lundbeck Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); co-inventor on a patent entitled “Composition and method of Substrate-Selective COX-2 inhibition”. **A. Holmes:** None. **S. Patel:** 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; collaborative research contract with Lundbeck Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); co-inventor on a patent entitled “Composition and method of Substrate-Selective COX-2 inhibition”.

Nanosymposium

112. Interactions Between Stress and Immune Function

Location: SDCC 5B

Time: Sunday, November 13, 2016, 8:00 AM - 11:30 AM

Presentation Number: 112.06

Topic: F.04. Stress and the Brain

Support: R01MH097243

R01MH093473

T32DE014320

F31MH109234

Title: Extramedullary monopoiesis underlies stress-sensitization and recurring anxiety

Authors: *D. B. MCKIM, J. P. GODBOUT, 43004, J. F. SHERIDAN;
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Abstract: In humans, chronic stress is associated with an increased prevalence of mental health complications including anxiety and depression. Repeated social defeat (RSD) in mice recapitulates key deficits associated with psychosocial stress in humans. We have reported that exposure to sub threshold stress 24 days after RSD caused the recurrence of anxiety that was dependent on monocyte trafficking from the spleen to the brain. We hypothesized that extramedullary production of monocytes in the spleen underlies enhanced monocyte trafficking and recurring anxiety following psychosocial stress. Here we show novel data that RSD causes substantial engraftment of hematopoietic stem progenitor cells (HSPCs) in the spleen. For example, RSD significantly increased the presence of all sub-types of progenitor lineages in the spleen, but profoundly increased lineages associated with: Monocytes (M), Granulocytes (G), Granulocytes-Monocytes (GM) and Multipotent Progenitors (GEMM). Moreover, there was a 30-fold increase in the number of Lin-/Sca1+/cKit+ (LSK) progenitor cells in the spleen after stress that were proliferating (S/G2/M cell cycle phase). Next, we tested if these stem cells in the spleen after stress could re-establish the bone marrow of a BM-ablated mouse. In these experiments, mice were subjected to RSD and splenocytes from CD45.1+ mice were co-transferred with GFP+ bone marrow competitor cells into myoablated CD45.2+ mice. Engraftment of donor stem cells was determined 4 months later. There was significant increase in BM engraftment (28-fold) of CD45.1+ splenocytes derived from RSD mice. If extramedullary hematopoiesis was responsible for enhanced monocyte trafficking from the spleen 24 days after RSD, then progenitor proliferation would have to be maintained for this same time period. Even 24 days after RSD, there was a significant increase in the number of splenic CFU, specifically of the GM-CFU subtype. Furthermore, using a BrdU pulse chase experiment, there was significant

increase in the number of BrdU+/CD11b+ splenic monocytes in the red pulp 24 days after RSD. These data indicate that RSD caused extramedullary hematopoiesis that resulted in long term monocyte proliferation in the spleen that persisted for at least 24 days.

Disclosures: D.B. McKim: None. **J.P. Godbout:** None. **J.F. Sheridan:** None.

Nanosymposium

112. Interactions Between Stress and Immune Function

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Time: Sunday, November 13, 2016, 8:00 AM - 11:30 AM

Presentation Number: 112.07

Topic: F.04. Stress and the Brain

Support: R01-MH093473

Title: Anxiety-like behavior induced by repeated social defeat stress is caused by IL-1b producing monocytes selectively recruited to the brain by resident microglia

Authors: *A. NIRAULA;
Ohio State Univ., Columbus, OH

Abstract: In humans, chronic stress is associated with an increased prevalence of anxiety and depression. Repeated social defeat (RSD) is a murine stress model that recapitulates several immune and behavioral outcomes of chronic stress. We have reported that RSD in mice induces the release of monocytes from the bone marrow that traffic to the brain, augment neuroinflammatory signaling and cause prolonged anxiety. Therefore, our objective was to determine the specific roles of microglia and infiltrating monocytes in the development of anxiety-like behavior following RSD. Here, we show a spatial association between neuronal activation, microglia activation, development of the reactive brain endothelium, and the presence of inflammatory monocytes in the limbic system following stress. Several strategies were used to delineate these events in the context of RSD. For example, the anti-anxiolytic drug clonazepam blocked stress-induced threat appraisal, microglia activation, and the induction of adhesion molecules on the vascular endothelium. Moreover, the anti-inflammatory drug minocycline prevented microglial activation, infiltration of peripheral monocytes to the brain and anxiety. These data highlight the critical role of microglia in monocyte recruitment to the brain during stress. FAC-sorting and mRNA analyses of resident microglia and infiltrating monocytes revealed cell-specific differences, i.e. microglia were the producers of the chemokine CCL2 and the infiltrating monocytes were the primary inducers of interleukin IL-1b. Consistent with these data, stress induced a robust expression of IL-1 receptor on the brain endothelium, which co-localized with sites of monocyte adhesion. Furthermore, depletion of functional IL-1b signal in

Caspase-1KO and monocyte-specific Caspase-1KO mice had no effect on stress-induced threat appraisal, microglial activation or the presence of monocytes in the brain. Importantly, monocyte-specific Caspase-1KO mice failed to develop anxiety, which underscored the role of monocyte-specific IL-1 signaling on stress-induced anxiety. Taken together, microglial activation with chronic stress causes selective neurovascular recruitment of inflammatory monocytes which, in turn, provide a robust IL-1b signal that causes anxiety-like behavior.

Disclosures: A. Niraula: None.

Nanosymposium

112. Interactions Between Stress and Immune Function

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Topic: F.04. Stress and the Brain

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NIH/NIHM Grant R01MH093472

Title: Short and long-term behavioral and neuroinflammatory changes induced by repeated social defeat stress in mice are attenuated by a cannabinoid receptors agonist: implications for PTSD

Authors: *S. F. LISBOA[1], A. NIRAULA[2], D. SHEA[3], L. B. RESSTEL[4], F. S. GUIMARAES[4], J. P. GODBOUT[2], J. F. SHERIDAN[2];
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Abstract: The repeated social defeat (RSD) stress in mice induces peripheral immune system activation, myeloid cell trafficking, microglia activation and prolonged anxietylike behavior. Endocannabinoid system (eCBS) molecules are expressed by immune and neuronal cells, modulating their functions. One purpose of this study was to test if cannabinoid receptors activation in mice would attenuate neuroinflammatory and behavioral effects of RSD. Stress exposure may lead to a persistent stage of low-grade inflammation and alterations in other systems, including the eCBS, which could predispose the individual to the development of psychopathology, such as the PTSD. Social stress might contribute to PTSD and, therefore, the RSD in mice would mimetize some PTSD features, such as alterations in conditioned fear

processing. Therefore, we also tested the hypothesis that RSD changes conditioned fear response 1 week after the end of RSD and that treatment with a cannabinoid agonist during RSD attenuates this effect. For those purposes, C57BL/6 mice have received a nonselective cannabinoid agonist, WIN55,2122 (1 mg/Kg, i.p.), 30 minutes prior to each of the six exposures to SD stress session (2h/each). The mice were tested in the morning following the last SD cycle for anxiety-like behavior and cellular/molecular alterations. Independent groups were left undisturbed and single-housed during 7 days after RSD and then were submitted to the contextual fear conditioning (CFC; 3 electrical foot-shock, 0.75 mA, 2s/each). 24h later, they were returned to the same chamber for evaluation of fear expression/ extinction acquisition (20 min session; no foot-shock presentation). Following additional 24h hours, the extinction recall was evaluated (5 min session) and the frontal cortex (FC) and hippocampus (HIP) were dissected. WIN administration during RSD attenuated anxiety-like behavior, decreased redistribution of immune system cells to the blood, spleen and brain and attenuated microglia activation in the brain. RSD induced-later fear sensitization and impaired extinction recall in the CFC were prevented by WIN administration during RSD. WIN also attenuated the observed increase in IL-1β mRNA in the FC and HIP after evaluation of extinction recall. Our data, therefore, suggest that modulation of cannabinoid system, targeting both neuronal and immunological components of stress, could be potential therapeutic intervention in stress-related disorders where inflammation is observed, such as PTSD. Moreover, they also suggest that PFC and HIP IL-1β signaling would be involved in fear sensitization after stressful events.

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Nanosymposium

112. Interactions Between Stress and Immune Function

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Presentation Number: 112.09

Topic: F.04. Stress and the Brain

Support: NIH Grant MH109165

NIH Grant MH099482

Title: Social stress and the induction of neuronal IL-1 expression in the hippocampus activate microglia and cause the recruitment of leukocytes to the brain and spleen

Authors: ***D. J. DISABATO**[1], D. P. NEMETH[2], X. LIU[2], J. P. GODBOUT[1], N. QUAN[2]; [1]Neurosci., [2]The Ohio State Univ., Columbus, OH

Abstract: Chronic stress is associated with an increased prevalence of mental health complications including anxiety and depression. We have previously reported that repeated social defeat (RSD) stress caused microglial activation, monocyte infiltration into brain, and robust IL-1β signaling. These neuroimmune effects with stress were associated with the induction of prolonged anxiety-like behavior. Thus, we hypothesize that IL-1 signaling in the brain with chronic stress plays a critical role in the regional-dependent recruitment of monocytes. Therefore, the role of central IL-1b signaling was examined by using adenoviral IL-1β (adIL-1β) administration into the ventral hippocampus (VH) in mice concomitantly subjected to a sub-threshold social defeat stress (STS) induced by paired fighting. This viral-mediated induction of IL-1β was primarily localized to hippocampal neurons. Mice were exposed 30 minutes of paired fighting for 6 consecutive days. This was a milder stress because these mice did not develop anxiety and showed no evidence of microglial activation. In addition, viral-mediated expression of IL-1β in the hippocampus alone activated microglia and increased leukocyte recruitment to the brain. This, however, did not increase leukocyte trafficking to the spleen to induce splenomegaly. The combination of the stress and viral-mediated expression of IL-1β in the hippocampus resulted in neuroinflammation and a significant increase in spleen size. Activated microglia and an increase in CD45+ infiltrating leukocytes were evident with a distinct localization within the dentate gyrus and hilus regions of the hippocampus, where IL-1β induction was prominent. Furthermore, this localization neuroinflammation was associated with a reduction in cellular density within the hippocampus. Taken together, these data indicate that neuronal IL-1 expression is a critical step in microglia activation and the subsequent recruitment of monocytes with a chronic stressor.

Disclosures: D.J. Disabato: None. **D.P. Nemeth:** None. **X. Liu:** None. **J.P. Godbout:** None. **N. Quan:** None.

Nanosymposium

112. Interactions Between Stress and Immune Function

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Time: Sunday, November 13, 2016, 8:00 AM - 11:30 AM

Presentation Number: 112.10

Topic: F.04. Stress and the Brain

Support: NIMH IRP

Title: Role of microglia in stress-induced changes in ventromedial prefrontal cortex structure

Authors: *M. L. LEHMANN, H. A. COOPER, T. K. WEIGEL, M. A. HERKENHAM; Natl. Inst. of Mental Hlth., NIH, Bethesda, MD

Abstract: Our lab uses the social defeat (SD) model of psychosocial stress in mice to study stress-related affective behavioral phenotypes and associated histological changes in the brain. We are interested in finding causal changes in neuroanatomy that underlie stress induced-mood disorders. Previously we showed that the ventromedial prefrontal cortex (vmPFC) mediates behavioral responses to SD and we were curious to know how SD alters the mRNA transcription profile within this region. We preformed a microarray on vmPFC samples from homecage (HC) and mice exposed to SD. A highly significant change in a rather small subset of genes was observed. Ingenuity Pathway Analysis of these changes suggested that chronic SD stress caused demyelination, neurite degeneration, and increased inflammation in this region. We hypothesized that alterations in white matter density might be reflected in microglial function in the affected areas. We first visualized SD-induced changes in myelin within the vmPFC using Black-Gold stain that specifically stains myelin. Concordant with the array data, chronic SD reduced myelin fiber density quantified by an automated analysis tool. We next examined the cellular source of Lipocalin-2 (LCN2), osteopontin (SPP1), and beta hemoglobin (HBB), the three most significantly upregulated genes in our array. LCN2 is a stress responsive-gene shown to downregulate spine density and it also attenuates neuroinflammation and deactivates macrophages. Using Fluorescence-based *in situ* hybridization (FISH), we demonstrate that parenchymal microglia, marked by a highly microglia-selective gene *Hexb* and perivascular macrophages, marked by the equally selective *Fn1* probe, are the sole LCN2 mRNA expressing cells within the vmPFC. LCN2 presence in microglia may be indicative of increased motility of these cells further suggesting that inflammatory processes are at play. Supporting this notion, *Spp1*, a marker for reactive astrocytes, showed elevated cell labeling by FISH. HBB, typically a marker for erythrocytes was markedly expressed in SD-exposed brains and may indicate the presence of microhemorrhages. We plan to explore whether enhanced LCN2 and SPP1 are drivers of demyelination or are induced by the presence of myelin debris.

Disclosures: M.L. Lehmann: None. **H.A. Cooper:** None. **T.K. Weigel:** None. **M.A. Herkenham:** None.

Nanosymposium

112. Interactions Between Stress and Immune Function

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Topic: F.04. Stress and the Brain

Support: NIH R01 MH090264

NIH R01 MH104559

NIH P50 AT008661-01

Title: Role of blood-brain barrier permeability and tight junction protein claudin 5 in vulnerability to social stress and major depressive disorder

Authors: *C. MENARD[1], M. L. PFAU[1], V. X. WANG[2], V. KANA[1], G. E. HODES[1], H. ALEYASIN[1], M. E. FLANIGAN[1], A. TAKAHASHI[3], S. A. GOLDEN[1], M. HESHMATI[1], M. CAMPBELL[4], M. MERAD[1], C. Y. TANG[2], S. J. RUSSO[1];
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Abstract: Background: Multiple clinical studies suggest that heightened peripheral inflammation contributes to major depression disorder (MDD) pathogenesis. It has been hypothesized that circulating inflammatory molecules are released following chronic stress, penetrate the blood brain barrier (BBB), and affect neural circuits mediating stress vulnerability and depression. **Methods:** In this study we investigated the effect of chronic social defeat stress (CSDS), a mouse model of depression, on BBB permeability and regulation of tight junction protein claudin 5 (Cldn5). **Results/Discussion:** We found that after 10 days of CSDS, Cldn5 mRNA and protein expression is reduced in the nucleus accumbens (NAc) of stress-susceptible mice when compared to resilient mice and unstressed controls. This was associated with epigenetic changes along the Cldn5 gene promoter in stress-susceptible mice compared to resilient mice. We found a similar decrease of Cldn5 mRNA in the NAc of depressed patients. In mice, chronic down-regulation of Cldn5 expression with an AAV-shRNA was sufficient to induce social avoidance and depression-like behaviors as assessed with sucrose preference and forced swim tests. Magnetic resonance imaging scans revealed higher penetration of a gadolinium-based contrasting agent in stress-related brain regions of defeated animals suggesting reduced BBB integrity. By understanding how chronic stress affects the BBB we may be able to augment current antidepressant treatment or design new therapeutic strategies targeting BBB permeability.

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Nanosymposium

112. Interactions Between Stress and Immune Function

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Japan Society for the Promotion of Science Postdoctoral Fellowship (CA)

Japan Society for the Promotion of Science Postdoctoral Fellowship (TI)

Title: C1 neurons mediate a stress-induced anti-inflammatory reflex in mice

Authors: *P. G. GUYENET[1], C. ABE[2], T. INOUE[3], R. L. STORNETTA[2], D. L. ROSIN[2], M. D. OKUSA[3];
[1]Pharmacol., Univ. Virginia Sch. Med., Charlottesville, VA; [2]Pharmacol.,
[3]Medicine/Nephrology, Univ. of Virginia, Charlottesville, VA

Abstract: C1 neurons (C1), located in the medulla oblongata, facilitate autonomic responses to physical stressors. We describe here a powerful effect of acute psychological stress mediated by C1: protection against renal ischemia-reperfusion injury (IRI). Restraint stress (RS) protected mice from renal IRI inflicted 24 hrs later. This protective effect was absent in *α7nAChR*$^{-/-}$ mice, required glutamate release by C1, was reduced by simultaneous pharmacogenetic inhibition of C1 and eliminated when C1 were selectively destroyed. Optogenetic C1 stimulation protected the kidneys from IRI and increased vagal and sympathetic efferent activity and plasma corticosterone. Injury protection by C1 stimulation required the spleen, was corticosterone-independent, and was eliminated by blocking autonomic ganglia or β_2-adrenergic receptors. In short, acute psychological stress reduces renal IRI by activating the autonomic nervous system via C1. The cholinergic anti-inflammatory pathway likely mediates this protection. Additionally, this study shows that localized brain stimulation can produce anti-inflammatory effects and protects kidneys from IRI.

Disclosures: **P.G. Guyenet:** None. **C. Abe:** None. **T. Inoue:** None. **R.L. Stornetta:** None. **D.L. Rosin:** None. **M.D. Okusa:** None.

Nanosymposium

112. Interactions Between Stress and Immune Function

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Time: Sunday, November 13, 2016, 8:00 AM - 11:30 AM

Presentation Number: 112.13

Topic: C.09. Brain Injury and Trauma

Support: VA Merit Award BX002558-01

Title: IL-1beta signaling in response to stress and modulation by the COMTval158met polymorphism: an *In vivo* study to understand the role of inflammation in PTSD

Authors: J. DESLAURIERS[1,2], X. ZHOU[1], *V. B. RISBROUGH[1,2];
[1]Dept. Psychiatry, Univ. of California San Diego, La Jolla, CA; [2]Veterans Affairs Ctr. of Excellence for Stress and Mental Hlth., La Jolla, CA

Abstract: Background and objectives: Posttraumatic stress disorder (PTSD) affects 8% of the American population while rates are higher in military veterans. The catechol-*O*-methyltransferase (COMT) enzyme is implicated in the catabolism of dopamine and plays a key role in cortical signaling. The COMTval158met polymorphism has been associated with a greater risk of neuropsychiatric disorders, including PTSD. We have previously shown, in a mouse line "humanized" for the COMTval158met polymorphism, that Val/Val carriers, compared to Met/Met carriers exhibited a greater response to a predator stress, which mimicked a severe trauma, demonstrated by higher avoidance behavior. Since alterations in the immune system have been observed in PTSD patients and catecholamines play a key role in regulating immune response, we hypothesized that the COMTval158met polymorphism modulates immune response to stress, playing a key role in the development of avoidance behavior.
Methods/results: Male and female Val/Val or Met/Met carriers were grouped into stress or control groups, with stressed groups being placed in a cage and exposed to a cat for 10 minutes (no physical contact). One week after predator stress, brain tissue and blood (plasma) were collected for cytokines quantification. Male Val/Val carriers had increased CRP plasma levels, regardless of stress, and had elevated CRP brain levels in response to stress. No changes in overall TNF-α or IL-6 signaling was found, but we observed increased IL-1β signaling (as measured by IL-1β/IL-1Ra brain ratios) in stressed males, regardless of genotype. In females, stressed mice showed high CRP plasma levels, regardless of genotype, and Val/Val carriers, independently of stress, showed increased IL-1β/IL-1Ra brain ratios. Also, increased TNF and IL-6 signaling were observed in Met/Met carriers in response to stress. Decreased levels of IL-1Ra explained the elevated IL-1β/IL-1Ra brain ratios observed in both sexes.
Conclusions: These results suggest that the COMTval158met polymorphism modulates the response to an acute trauma, as modeled by the predator stress, through the IL-1β/IL-1Ra signaling pathway. To confirm the role of IL-1β/IL-1Ra pathway in the development of

avoidance behaviors in stressed Val/Val carriers, results of pre-treatment with Anakinra, an interleukin-1 receptor antagonist (IL-1Ra), on avoidance behaviors will also be presented. This two-hit model may be useful to understand the immune mechanisms implicated in the risk for development of PTSD-like symptoms.
CRP, C-reactive protein; IL-1β, interleukin-1β; IL-1Ra, interleukin-1 receptor antagonist; IL-6; interleukin-6; TNF-α, tumor necrosis factor-α

Disclosures: J. Deslauriers: None. **X. Zhou:** None. **V.B. Risbrough:** 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; VA Merit Award BX002558-01. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Johnson & Johnson.

Nanosymposium

112. Interactions Between Stress and Immune Function

Location: SDCC 5B

Time: Sunday, November 13, 2016, 8:00 AM - 11:30 AM

Presentation Number: 112.14

Topic: F.05. Neuroimmunology

Support: NIH Grant MH109165

Title: Cell type specific IL-1R1 mediates distinct functions in neuroinflammation and anxiety

Authors: *X. LIU, D. P. NEMETH, D. B. MCKIM, J. P. GODBOUT, J. F. SHERIDAN, N. QUAN, D. J. DISABATO, L. ZHU;
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Abstract: Interleukin-1 (IL-1) mediates diverse neuropathological effects and behavioral changes in the central nervous system (CNS) through type I IL-1 receptor (IL-1R1). However, cell-type-specific IL-1R1 mediated functions remain to be elucidated and a specific IL-1R1 expressing cell type which mediates IL-1-induced neuroinflammation and anxiety-like behavior has yet to be identified. In the current study, we investigated the responses to IL-1 in multiple mouse lines in which IL-1R1 was selectively expressed in endothelial and hematopoietic cells (Tie2Cre-IL-1R1r/r), myeloid cells (LysMCre-IL-1R1r/r), microglial cells (CX3CR1Cre-IL-1R1r/r), astrocytes (GFAPCre-IL-1R1r/r) or hippocampal neurons (AAV2Cre-IL-1R1r/r). Acute intracerebroventricular (ICV) injections of IL-1 in these lines induced an increase of proinflammatory cytokines IL-1 and TNF as well as anxiety-like behavior in the wild type (WT) and Tie2Cre-IL-1R1r/r mice, but not in other mouse lines. ICV IL-1 injections in the WT mice also induced leukocyte infiltration and microglial activation with Iba-1-labeled elongated

processes and increased branches, which were found in the Tie2Cre-IL-1R1r/r mice and GFAPCre-IL-1R1r/r mice but not in other mouse lines. To determine the role of infiltrating leukocytes in triggering the microglial activation, we depleted leukocytes in the Tie2Cre-IL-1R1r/r mice with vinblastine or cyclophosphamide. After the depletion, microglial activation was still detected in response to IL-1. In the AAV2Cre-IL-1R1r/r mice, ICV IL-1 injections induced unique microglial morphological alterations. These results demonstrated that astrocytic, endothelial and neuronal IL-1R1 mediates distinct functions in response to central IL-1 and specifically, endothelial IL-1R1 can mediate IL-1-induced anxiogenic neuroinflammation.

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Nanosymposium

113. Ingestive Behavior

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Topic: F.10. Food Intake and Energy Balance

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Title: Feeding states regulate excitatory synaptic transmission onto oxytocin neurons in the hypothalamic paraventricular nucleus

Authors: *S. SUYAMA[1], M. KODAIRA-HIRANO[1], Y. UETA[2], M. NAKATA[1], T. YADA[1]; [1]3311-1 Yakushiji, Jichi Med. Univ., Tochigi, Japan; [2]Univ. of Occup. and Envrn. Hlth., Kitakyushu, Japan

Abstract: It has been shown that synaptic plasticity onto the neurons in the arcuate nucleus of hypothalamus is modulated by peripheral hormones reflecting energy state, and that this modulation of synaptic plasticity plays a role in regulation of feeding. Oxytocin (Oxt) neurons in

the paraventricular nucleus (PVN) of hypothalamus have been shown to integrate peripheral and central signals as the 2nd order neurons and thereby induce satiety. However, it remains unclear whether Oxt neurons in PVN show synaptic plasticity in response to metabolic energy states. We investigated the excitatory synaptic transmission onto Oxt neurons under fasted/fed states. The excitatory postsynaptic currents (EPSCs) mediated by AMPA type and NMDA type ionotoropic glutamate receptors were decreased under fasted, compared to ad lib fed, state. AMPA/NMDA ratio of evoked EPSC was significantly different between fasted and fed states. In Oxt neurons, dynein light chain 2 (DYNLL2), a protein implicated in the NMDA receptor trafficking to the postsynapses, was decreased under fasted, compared to fed, state. The present results suggest that fasting decreases excitatory synaptic input on Oxt neurons via down-regulation of DYNLL2 expression and NMDA receptor-mediated synaptic transmission.

Disclosures: S. Suyama: None. **M. Kodaira-Hirano:** None. **Y. Ueta:** None. **M. Nakata:** None. **T. Yada:** None.

Nanosymposium

113. Ingestive Behavior

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Topic: F.10. Food Intake and Energy Balance

Title: TGF-β signaling in PVN neurons regulates body weight and food intake.

Authors: *I. PAPAZOGLOU, Z. CUI, J.-H. LEE, O. GAVRILOVA, S. G. RANE; NIDDK, Bethesda, MD

Abstract: The maintenance of energy homeostasis is fundamental for the survival of every living organism. In mammals, the regulation of food intake and body weight is predominantly controlled by specific neurons within the hypothalamus. Neurons located in the paraventricular nucleus (PVN) integrate diverse metabolic signals and help maintain energy homeostasis. However, the extracellular signaling pathways that regulate the function of PVN neurons are less clear. We previously showed that the transforming growth factor beta (TGF-β) signaling pathway regulates whole body glucose homeostasis via actions on multiple peripheral tissues. Here, we describe the role of TGF-β signaling in PVN neurons responsible for regulation of food intake and body weight. We find high expression of TGF-β receptor 1 (TβR1) and, its main downstream transcription factor, Smad3, in the PVN. The majority PVN localized TβR1 and Smad3 positive cells also express oxytocin (OXT) or vasopressin (AVP), but not corticotropin releasing hormone (CRH). To directly investigate TGF-β's functional role in this neural circuitry, we used a combination of genetically engineered mice (TβR1 flox/flox) and

stereotactic viral injection (AAV-hsyn-GFP-Cre) that allows targeted deletion of TβR1 in PVN neurons. Loss of TβR1 in PVN neurons resulted in significant body weight gain and fat mass increase over time due to an increase in food intake. In addition, TβR1 ablation in the PVN induced hyperinsulinemia suggestive of a breakdown in glucose homeostasis. Interestingly, we observed a substantial morphological change in the PVN of mice lacking TβR1 including a significant increase in proliferation and apoptosis of GFP+ neurons within the PVN that reflected the AAV targeted deletion of TβR1. We hypothesize that these changes in the proliferation and cell death rates within the PVN account for the increased food intake and body weight increase, perhaps due to altered neuronal circuitry that depends on intact TGF-β signaling. To further define the underlying mechanisms, we are (i) conditionally activating TGF-β signaling using a "gain of function" model and (ii) conditionally ablating Smad3 within the PVN. Taken together with our prior findings reporting the importance of TGF-β signaling in peripheral metabolically active tissues, these studies establish the importance of TGF-β signaling in regulating the central mechanisms of energy homeostasis with implications to diabetes and obesity pathogenesis.

Disclosures: I. Papazoglou: None. **Z. Cui:** None. **J. Lee:** None. **O. Gavrilova:** None. **S.G. Rane:** None.

Nanosymposium

113. Ingestive Behavior

Location: SDCC 2

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Presentation Number: 113.03

Topic: F.10. Food Intake and Energy Balance

Support: HHMI

JPB Foundation

Title: A brainstem circuit for controlling feeding-related behaviors

Authors: *A. R. NECTOW[1], B. C. FIELD[2], N. RENIER[3], H. ZHANG[4], Y. LIANG[3], M.-H. HAN[4], J. M. FRIEDMAN[2];
[1]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [2]Lab. of Mol. Genet., [3]The Rockefeller Univ., New York, NY; [4]Mount Sinai Sch. of Med., New York, NY

Abstract: In mammals, a state of negative energy balance leads to the search for and consumption of food through the activity of neurons in the hypothalamus and elsewhere. While numerous cell types within the hypothalamus have been directly implicated in the control of feeding, little is known about the role of extrahypothalamic cell types. One largely unexplored

locus likely responsible for the control of food intake is the dorsal raphe nucleus (DRN). The DRN is a molecularly heterogeneous brainstem structure directly downstream of numerous hypothalamic nuclei implicated in feeding-related behaviors, and pharmacological manipulations within the raphe can lead to increased food intake. Here, we use methods for molecular profiling and brain-wide projection mapping to define distinct populations of GABAergic and glutamatergic neurons within the DRN, and we find that these two populations are capable of modulating feeding-related behaviors. Together, these data establish a role for the dorsal raphe nucleus in controlling feeding behavior and add an important new anatomic site that controls energy balance.

Disclosures: **A.R. Nectow:** None. **B.C. Field:** None. **N. Renier:** None. **H. Zhang:** None. **Y. Liang:** None. **M. Han:** None. **J.M. Friedman:** None.

Nanosymposium

113. Ingestive Behavior

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Topic: F.10. Food Intake and Energy Balance

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NIH Grant R21 AA020593

Title: Prenatal CXCL12 mediates effects of perinatal exposure to dietary fat on neuropeptides and behavior in offspring

Authors: ***K. POON**[1], J. R. BARSON[2], H. SHI[1], G. Q. CHANG[1], S. F. LEIBOWITZ[1]; [1]The Rockefeller Univ., New York, NY; [2]Drexel Univ., Philadelphia, PA

Abstract: Ingestion of a high-fat diet (HFD) during pregnancy causes neurochemical changes in the offspring's hypothalamus that, in turn, stimulate excessive consumption of this diet. Recent studies indicate a role for the immune system in mediating the downstream effects of a HFD on neuronal function, with our recent publication (Poon et al, 2016) suggesting the involvement of the chemokine, CXCL12, in stimulating the neuropeptide, enkephalin (ENK), in the paraventricular nucleus of the hypothalamus (PVN). Combined with evidence showing inflammatory mediators to affect the function and development of neurons, this finding indicates a strong relationship between HFD and this chemokine. The present studies investigated, first, whether prenatal exposure to HFD affects CXCL12 levels in the dam, as well as the CXCL12 chemokine system and ENK in the hypothalamus of offspring while altering behavior and,

second, whether administration of CXCL12 itself during pregnancy has similar effects to HFD. In the first set of experiments, exposure to HFD during gestation (E7-E19) increased circulating levels of CXCL12 in dam and expression of CXCL12 and its receptors CXCR4 and CXCR7 in cultured hypothalamic neurons. It also increased expression of CXCL12, its receptors, and ENK in the PVN of postnatal offspring (days 15 and 30), while having no effect in the arcuate nucleus or perifornical lateral hypothalamus. This was accompanied by an increase in novelty-induced locomotor activity, a decrease in novelty-seeking with a novel object, and a decrease in the amount of time spent in the open arms of an elevated plus maze, suggesting increased anxiety. In the second set of experiments, we found that daily intraperitoneal injections in dam (from E7-E19) of 2 μg or 8 μg CXCL12 elevated circulating CXCL12 to levels that, respectively, were similar to or higher than the levels seen in HFD-exposed dams. Prenatal administration of CXCL12, similar to HFD, significantly increased expression of ENK in PVN, at both postnatal day 15 and 30, and increased novelty-induced locomotor activity, decreased novelty-seeking of a novel object, and reduced time spent in the open arms of the elevated plus maze, indicating increased anxiety. A brief HFD challenge (5 days) in offspring prenatally exposed to CXCL12 or HFD induced similar increases in body weight and caloric intake as compared to chow-exposed offspring. These results show that prenatal HFD exposure stimulates the endogenous CXCL12 system in the hypothalamus and that prenatal CXCL12 exposure induces similar behavioral and neurochemical changes, suggesting that this chemokine may be a downstream mediator of the effects produced by a fat-rich diet.

Disclosures: **K. Poon:** None. **J.R. Barson:** None. **H. Shi:** None. **G.Q. Chang:** None. **S.F. Leibowitz:** None.

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Title: vmat2-mediated neurotransmission in mediating midbrain leptin action on feeding regulation

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Abstract: Neurons that express leptin receptor (LepR) in the midbrain contribute to feeding regulation and the midbrain contains a mixture of GABAergic, glutamatergic and dopaminergic (DA) neurons, the latter also capable of releasing GABA mediated by vesicular monoamine transporter 2 (VMAT2). However, neurotransmitters mediating the midbrain leptin action remain unclear. Here, we demonstrated that, among all brain LepR neurons, only those in the midbrain express VMAT2, a subset of which also express glutamatergic (vesicular glutamate transporter 2) and GABAergic markers (vesicular GABA transporter). We generated mice (KO) with specific deletion of VMAT2 in midbrain LepR neurons and found that, while these mice showed no obvious abnormality on chow diet, they exhibited resistance to high-fat diet (HFD)-induced obesity associated with reduced HFD feeding. When tested on a paradigm with intermittent access to HFD where first 2.5-hr HFD feeding (binge-like hedonic feeding) and 24-hr HFD feeding (homeostatic feeding) were measured, KO mice exhibited more binge-like feeding while less homeostatic feeding. Interestingly, while homeostatic HFD feeding in controls was reduced by leptin treatment, the one in KO mice showed no response. Taken together, these results suggest that VMAT2-mediated dopamine and GABA co-transmission contributes to leptin action on HFD feeding inhibition and surprisingly restrains hedonic feeding.

Disclosures: **Y. Xu:** None. **Y. Lu:** None. **E. Isingrini:** None. **Y. Xu:** None. **B. Giros:** None. **Q. Tong:** None.

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113. Ingestive Behavior

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Topic: F.10. Food Intake and Energy Balance

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Title: Loss of MHC class I protein H2-K1 leads to dysregulated energy balance and obesity

Authors: *N. W. DEKORVER[1], T. CHAUDOIN[2], J. ARIKKATH[3], S. J. BONASERA[2];
[2]Intrnl. Med. Geriatrics, [3]Developmental Neurosci., [1]Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Aging is commonly associated with metabolic dysfunction that can lead to either unintended weight gain or weight loss, which confer risk of cardiovascular disease and frailty, respectively. Understanding how aging impacts the cellular and molecular mechanisms underlying metabolism is crucial for the development of novel therapeutic strategies. We have previously demonstrated aged Balb/C mice (22-24 mo.) show significant weight loss compared to young controls (2-3 mo.), despite increased food intake. This phenotype mimics age-related weight loss in humans. Additionally, we have shown increased expression of immune molecules and pattern recognition receptors in the hypothalamus of aged Balb/C mice. Of note, the classical major histocompatibility complex H2-K1 was increased 1.5-fold in the aged Balb/C hypothalamus compared to young controls. Classical MHCI molecules have been demonstrate to be crucial for the proper synaptic development within the visual cortex and hippocampus, however there is currently no data signifying a functional role for MHCI in the developing hypothalamus. Here, we present data demonstrating that H2-K1 is differentially expressed from P1 to adult in the developing hypothalamus in several key nuclei for regulating feeding behaviors and activity. Additionally, we show that MHCI colocalizes with post synaptic density marker PSD95, but shows little colocalization with presynaptic markers of excitatory or inhibitory pre-synaptic proteins (Vglut2, Vgat). The level of colocalization with PSD95 differs developmentally, decreasing from post natal day 5 to adult. To assess whether specific MHC I molecules play a significant role in regulating behavior we tested mice constitutively lacking H2-K1 (B6.C-H2-Kbm1/ByJ) in a state-of-the-art home cage monitoring system. We found that loss of H2-K1 leads to a profound progressive obesity phenotype starting at 3 months of age. H2-K1 null mice are significantly heavier (36.26 ± 2.08 g, 27.53 ± 1.78 g, $p < 0.00001$), have increased adiposity (28.03 ± 5.35 %, 12.89 ± 1.73 %, $p < 0.00001$), and show significant hepatic steatosis. Interestingly, this increase in weight and adiposity occurs despite marked hypophagia. Further analysis of mouse active states demonstrated that H2-K1 null mice are significantly more sedentary compared to WT controls, spending 10% more time in an inactive state. To our knowledge, this is the first time an MHC I molecule has been shown to be involved in the regulation of energy balance. Further analysis of the role of H2-K1 signaling in hypothalamic development may provide novel therapeutic targets to combat metabolic deficits associated with aging and obesity.

Disclosures: **N.W. Dekorver:** None. **T. Chaudoin:** None. **J. Arikkath:** None. **S.J. Bonasera:** None.

Nanosymposium

114. Neural Mechanisms Underlying Risk/Reward Decision-Making

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Presentation Number: 114.01

Topic: G.02. Motivation

Support: UCLA Division of Life Sciences Recruitment and Retention Fund (A. Izquierdo)

Title: A role for basolateral amygdala in updating expected outcome value in volatile environments

Authors: *A. STOLYAROVA, A. IZQUIERDO;
Brain Res. Institute, Dept. of Psychology, UCLA, Los Angeles, CA

Abstract: In naturalistic settings, outcomes of choices are not singular events of constant value but are instead embedded in reward distributions that fluctuate from one experience to the next. The basolateral amygdala (BLA) and orbitofrontal cortex (OFC) both participate in outcome valuation in such volatile environments but their specific roles in this process are frequently difficult to dissociate. To systematically study the neural mechanisms of value updating in uncertain reward conditions, we developed a novel decision-making task in which outcome values are determined by normally-distributed delays to reward receipt. At baseline, rats (n=8) were required to respond to one of two options on a touchscreen, each identical in mean reward rate (1 sucrose pellet/ 10s) but different in the variance of outcome distributions (high variability, HV vs. low variability, LV). Following the establishment of stable performance, rats experienced reward upshifts (1/ 5s with variance kept constant) and downshifts (1/ 20s) on each option independently and in counterbalanced order, always followed by a return to baseline conditions. Rats distributed their choice behavior uniformly at baseline and significantly changed their preference in response to all shifts, suggesting they were able to infer mean option values despite stochastic fluctuations in outcomes. However, the degree of choice adaptations was asymmetric: HV facilitated response adaptations to upshifts, but rendered animals suboptimal during downshifts; conversely, LV led to decreased reward procurement during upshifts. To assess the OFC-BLA mechanisms of these responses, we trained a separate cohort of animals (n=16) on the initial phase of our task, but restricted their experience to one of the options exclusively (LV or HV). We studied BLA and OFC expression of gephyrin, a reliable proxy for membrane-inserted $GABA_A$ receptors, 24 h after the last training session in these two groups relative to control animals (n=8) that experienced identical environmental conditions and secondary reinforcers, but no primary rewards. We found a variability-dependent gephyrin upregulation in BLA, but not OFC, suggesting a unique role for BLA in variability coding. We then addressed the function of BLA directly via NMDA lesions to this brain region. Consistent with our molecular findings, BLA lesions reduced the variability-induced asymmetry in choice adaptations in response to value shifts and induced an uncertainty-avoidant phenotype. Ongoing experiments are aimed at comparing the contributions of OFC and ventral striatum to that of BLA in updating outcome value in volatile, uncertain reward conditions.

Disclosures: A. Stolyarova: None. **A. Izquierdo:** None.

Nanosymposium

114. Neural Mechanisms Underlying Risk/Reward Decision-Making

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Presentation Number: 114.02

Topic: G.02. Motivation

Support: CIHR MOP-133579

Title: Temporal dynamics of amygdala-ventral striatal interactions mediating risk/reward decision-making

Authors: *D. BERCOVICI, S. B. FLORESCO;
Psychology, Univ. of British Colombia, Vancouver, BC, Canada

Abstract: Assessing costs and benefits associated with different options that vary in terms of reward magnitude and uncertainty is an adaptive behaviour which motivates us to select the optimal course of action. Previous studies using reversible inactivations have shown that communication between the basolateral amygdala (BLA) and the nucleus accumbens (NAc) promotes choice towards larger, riskier rewards. Neural activity in both the BLA and NAc show distinct, phasic changes in firing prior to action initiation and following action outcomes. Yet, how temporally-precise patterns of activity within BLA-NAc circuitry influences choice behavior during decision making is unclear. To address this, we assessed how optogenetic silencing of BLA projection terminals in the NAc altered risk/reward decision-making. Rats that received infusions of AAV-CaMKIIa-eArchT3.0-EYFP in the BLA and implanted with optical fibers in the NAc were well-trained on a probabilistic discounting task, wherein they chose between a small/certain (1 pellet) and a large/risky reward (4 pellets). The odds of obtaining the large reward changed over trial blocks during a daily session, initially set at 50% and then shifting to 12.5%. Silencing tests were administered ~2 months after viral infusion, where we delivered brief (5-7s) pulses of green light (532nm at 30mW) at specific task events; during a "pre-choice" period (5s prior to making a choice) or different "outcome" periods (during receipt large or small rewards, or reward omissions). Silencing BLA inputs to the NAc during "pre-choice" periods reduced choice of the more preferred option during the different blocks (i.e.: rats chose less risky in the 50% block and more risky in the 12.5% block), suggesting that activity in this circuit prior to action selection promotes selection of more preferred rewards. Silencing during reward omissions increased risky choice during the low-probability block and similar manipulations after receipt of small rewards increased risky choice and reduced lose-shift behavior. Collectively these data clarify how temporally-specific patterns of pre-choice and outcome related activity in BLA-NAc circuitry convey different types of information that guide optimal action-selection in situations involving reward uncertainty.

Disclosures: D. Bercovici: None. **S.B. Floresco:** None.

Nanosymposium

114. Neural Mechanisms Underlying Risk/Reward Decision-Making

Location: SDCC 23A

Time: Sunday, November 13, 2016, 8:00 AM - 11:30 AM

Presentation Number: 114.03

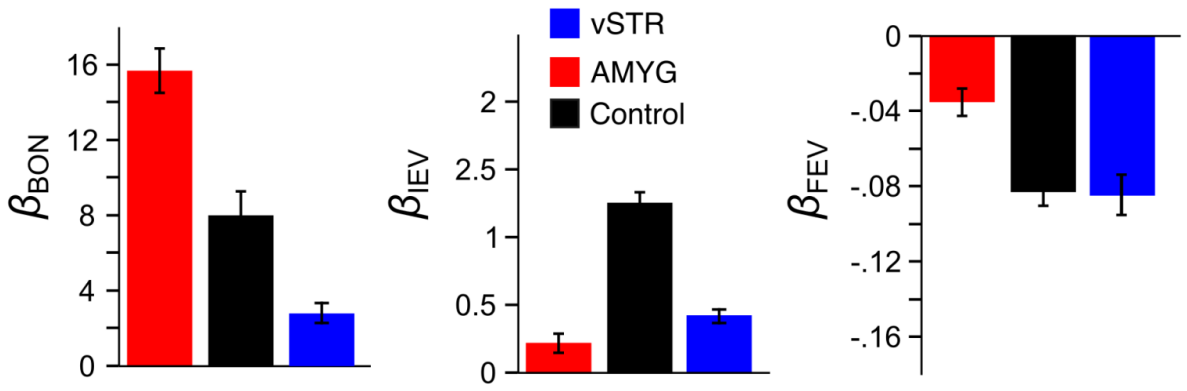
Topic: G.02. Motivation

Support: NIMH DIRP

Title: A subcortical competition to manage the explore-exploit tradeoff

Authors: *V. D. COSTA, R. VICARIO-FELICIANO, K. ROTHENHOEFER, E. A. MURRAY, B. B. AVERBECK;
Lab. of Neuropsychology, NIMH/NIH, Bethesda, MD

Abstract: The tradeoff between exploring novel options and exploiting options is important in environments where choice options are constantly changing. The amygdala and ventral striatum are both known to be involved in appetitive learning and to enable flexible affective responses when contingencies are extinguished or reversed. Less is known about the roles of the amygdala and ventral striatum in managing the explore-exploit tradeoff that underlies decisions between multiple choice options. To understand the contributions of these two regions on exploratory choice behavior we compared the relative impact of bilateral excitotoxic lesions of the amygdala (N = 4) or ventral striatum (N = 3) on choice behavior of rhesus macaques in a three-arm bandit task, relative to a group of unoperated control monkeys (N=8). During the task, the monkeys learned to choose between three, probabilistically rewarded images. Periodically one of the three choices was replaced with a novel image the monkey had not yet associated with reward. A finite state, discrete time, discounted Markov decision process (MDP) model was used to derive value estimates for each of the monkeys' choices in terms of an exploration bonus (BON), immediate (IEV), and future expected value (FEV). These value estimates were then used to predict the monkey's ability to manage the explore exploit tradeoff. We found that the amygdala and striatal lesions had dissociable effects on exploratory behavior. Amygdala lesions caused the animals to explore more than controls, whereas the striatal lesion group explored less than controls. While the amygdala and striatal lesions both decreased the animals' ability to learn immediate cue values, the amygdala group was more impaired relative to the striatal lesion group. These results suggest the amygdala and ventral striatum are both important for reinforcement learning but have opponent roles on exploratory choice behavior.



Disclosures: **V.D. Costa:** None. **R. Vicario-Feliciano:** None. **K. Rothenhoefer:** None. **E.A. Murray:** None. **B.B. Averbeck:** None.

Nanosymposium

114. Neural Mechanisms Underlying Risk/Reward Decision-Making

Location: SDCC 23A

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Presentation Number: 114.04

Topic: G.02. Motivation

Support: 1R01MH098039-01

Title: Integration of reward probability, reward based salience and new information in monkey lateral intraparietal cells

Authors: ***N. DADDAOUA**[1], J. GOTTLIEB[2];
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Abstract: Humans and non-human primates gather visual information by making rapid eye movements (saccades) to selected portions of complex scenes. Converging evidence shows that saccade-related neural responses are sensitive to reward contingencies, consistent with reward-maximizing decision mechanisms. However, much less is known about how oculomotor neurons integrate reward and informational factors. We investigated this question in the monkey lateral intraparietal area (LIP) using a novel paradigm in which a monkey had the opportunity to sample reward predictive cues on the background of different prior expectations about reward probability. Each trial began with a period of central fixation and a first visual cue indicating whether the trial had a 0%, 50% or 100% reward probability. During the delay period following the disappearance of this cue, LIP cells encoded reward probability, maintaining low sustained firing at 0% reward probability, and higher rates that were equivalent after 50% and 100% cues.

At the end of the delay period, the fixation point disappeared, and a second peripheral cue was shown which signaled whether the trial had a 0% or 100% reward probability. Cue 2 fell in one of 4 categories depending on whether it signaled a negative or positive outcome (0% or 100% probability) and whether it provided a redundant or informative signal (confirming the 0% or 100% probability signaled by the first cue or resolving the uncertainty left by a 50% first cue). If cue 2 signaled a negative outcome (0% probability), LIP visual and pre-saccadic responses were greatly enhanced by informativeness: responses were low when the cue merely confirmed prior expectations but were greatly enhanced, and had shorter latencies if the cue provided new information. If cue 2 signaled a positive outcome however, there was a much weaker effect of new information and responses were high for both redundant and informative cues (comparable to those induced by a negative informative cue). The effects of reward and informativeness were independent of the probability or reaction times of saccades to cue 2. In a second experiment cue 2 was initially covered by a mask and revealed its information only if it was fixated, in gaze-contingent fashion. During the fixation period prior to the revealing of cue 2, LIP neurons had higher responses on trials with 50% relative to 100% reward probability, suggesting that they encoded the gains in information expected upon revealing cue 2. We propose that LIP neurons integrate information about prior probability, reward-based salience and new information in a manner consistent with active information seeking strategies.

Disclosures: **N. Daddaoua:** None. **J. Gottlieb:** None.

Nanosymposium

114. Neural Mechanisms Underlying Risk/Reward Decision-Making

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Topic: G.02. Motivation

Support: NSF Grant #1207573

Title: Novelty and uncertainty as separable exploratory drives

Authors: ***J. COCKBURN**, J. P. O'DOHERTY;
Caltech, Pasadena, CA

Abstract: Despite the real-world importance of balancing exploration and exploitation, the computational mechanisms brought to bear on the problem by humans and other animals are poorly understood. Strategies for motivating exploration in computational reinforcement-learning include boosting the value of novel stimuli to encourage sampling in new regions of the environment, or augmenting the value of a given option according to the degree of uncertainty in

the estimate of predicted reward. While there is preliminary evidence of both uncertainty and novelty directed exploration in humans, the nature of the relationship between them is unknown. In the present study we sought to address how these variables relate to each other. We also sought to address the paradox of why uncertainty driven exploration can co-exist alongside the frequently observed contrarian behavioral imperative of uncertainty avoidance.
To this end we tested human participants on a bandit task where these variables were systematically manipulated. We found clear evidence of both novelty and uncertainty driven behavior. Uncertainty and novelty driven exploration were found to evolve differently over time. Approximately half of our sample exhibited uncertainty-seeking early on when there was ample opportunity to exploit what was learned. As participants approached the end of the sampling period all participants became increasingly uncertainty-averse. Conversely, the majority of our participants exhibited novelty-seeking response patterns throughout the session. Moreover, we found a negative correlation between the degree to which participants were influenced by novelty and uncertainty, suggesting an antagonistic relationship between the two exploratory drives. These results support the existence of separable valuation processes associated with novelty and uncertainty as motivations to explore, and provide one possible account for why two competing attitudes toward uncertainty can co-exist in the same individual.

Disclosures: J. Cockburn: None. **J.P. O'Doherty:** None.

Nanosymposium

114. Neural Mechanisms Underlying Risk/Reward Decision-Making

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Title: Neuroanatomy accounts for age-related changes in risk preferences

Authors: *M. A. GRUBB[1,2], P. W. GLIMCHER[1], I. LEVY[3];
[1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]Psychology, Trinity Col., Hartford, CT; [3]Section of Comparative Med. and Dept. of Neurosci., Yale Sch. of Med., New Haven, CT

Abstract: Many decisions involve uncertainty, or “risk”, with regard to potential outcomes. Substantial empirical evidence has demonstrated that human aging is associated with diminished tolerance for risky rewards. *Which neurobiological markers of aging might account for this*

change in preference? Last year we identified a region in right posterior parietal cortex (rPPC) whose gray matter volume (GMV) accounts for individual variation in risk preferences in young adults: decreased rPPC GMV is predictive of increased risk aversion (Gilaie-Dotan, S., *et al. JNeurosci, 2014*). Gray matter loss is part of healthy aging, with parietal regions showing particularly enhanced local declines. Here, we tested the hypothesis that reduced rPPC GMV, rather than age *per se*, may best account for age-related changes in risk preferences.
Risk preferences were assessed using a well-validated, incentive-compatible procedure. 52 participants (18-88 y.o., 30 F) made 60 binary choices between a certain gain of $5 and a lottery whose value and probability of payout was systematically manipulated. We modeled the expected utility (EU) of each option with the functional form: $EU(v,p)=p*V^{\alpha}$, where v = value, p = probability, and α = risk preference parameter. Choice data were fit using maximum likelihood, with the probability of choosing the lottery given by a logistic choice function. $1mm^3$ resolution anatomical images were acquired with MRI. Using voxel-based morphometry, we sampled GMV in the rPPC region-of-interest and confirmed that rPPC GMV decreases with age in our sample ($r = -0.66$, $p < 0.001$).
To assess the relationship between risk preferences and our variables of interest we allowed alpha to vary during the estimation procedure as a linear function of age ($\alpha = \beta1 * age + \beta0$) and rPPC GMV ($\alpha = \beta1 * rPPC\ GMV + \beta0$). We found a significant negative relationship between alpha and age ($z = -2.58$, $p = 0.01$) and a significant positive relationship between alpha and rPPC GMV ($z = 3.51$, $p < 0.001$), replicating previous results. A third model included both age and rPPC GMV ($\alpha = \beta1 * age + \beta2 * rPPC\ GMV + \beta0$); *again we found a significant positive relationship between alpha and rPPC GMV (z = 2.13, p = 0.03). Critically however, when the linear regression was computed in this manner, age no longer had any influence on alpha* ($z = -0.24$, $p = 0.81$), indicating that rPPC GMV, and not age *per se*, modulates risk preferences. Two additional models confirmed that these results are specific to rPPC gray matter decline.
These results provide a basis for understanding the neural mechanisms that mediate risky choice and offer a glimpse into the neurodevelopmental dynamics that impact decision-making under uncertainty in an aging population.

Disclosures: **M.A. Grubb:** None. **P.W. Glimcher:** None. **I. Levy:** None.

Nanosymposium

114. Neural Mechanisms Underlying Risk/Reward Decision-Making

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Title: Dynamic control models for strategic interaction

Authors: *J. M. PEARSON[1], S. N. IQBAL[1], C. B. DRUCKER[2], M. L. PLATT[3];
[1]Duke Inst. for Brain Sci., [2]Neurobio., Duke Univ., Durham, NC; [3]Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: The ecological niches occupied by most organisms, including humans, are both dynamic and uncertain, requiring that actions be taken in real time and modified in response to changing circumstances. However, most studies of dynamic decision-making to date have explored either repeated trials of the same task under slowly changing circumstances (e.g., bandit problems) or interactions between agents that take place in a restricted action space (e.g., prisoner's dilemma). Here, we examine data from repeated trials of a real-time strategic interaction with continuous freedom of movement. We trained monkeys to play a competitive task in which the goal of one (the "shooter") was to move a colored dot (the "ball") from the left to right side of a computer monitor using joystick input. The goal of the second monkey (the "goalie") was to block the dot by moving a vertical line along the right-hand side of the screen to intercept the it. Thus, each player controlled an avatar with at least one continuous degree of freedom, in principle allowing for dynamic coupling between the two in real time.
We analyzed these data using time series methods borrowed from the machine learning and optimal control literatures. We modeled each player's avatar as a state space model with control input dependent on the dynamics of both players' behavior. That is, the kicker's control action was modeled as a filtered sum of his own and the goalie's past trajectories (and vice-versa for the goalie). The model produces a set of filters and time series that can be used for subsequent analysis of neural data in terms of dynamic control signals derived from behavior.

Disclosures: J.M. Pearson: None. **S.N. Iqbal:** None. **C.B. Drucker:** None. **M.L. Platt:** None.

Nanosymposium

114. Neural Mechanisms Underlying Risk/Reward Decision-Making

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Presentation Number: 114.08

Topic: G.02. Motivation

Title: Posterior cingulate cortex integrates information from the environment and internal state to set foraging policy

Authors: *D. L. BARACK[1], M. L. PLATT[2];
[1]Dept of Neurosci., Columbia Univ., New York, NY; [2]Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Foraging is a fundamental capacity of all organisms. Foraging policy—choosing how and where to forage—is influenced by properties of the environment as well as by internal state. We hypothesize that foraging decisions are regulated by a circuit connecting the anterior cingulate cortex (ACC), the locus coeruleus (LC), and the posterior cingulate cortex (PCC). ACC is known to mediate momentary decisions during foraging, with activity rising to a threshold before decisions to leave a patch. The LC, a brainstem noradrenergic center, responds to surprising events and regulates the balance of focus and distractibility. PCC, a canonical node in the default mode network (DMN), is implicated in attention, learning, and decision-making, shows high noradrenergic receptor expression, and is reciprocally connected with the ACC. We theorize that PCC modulation of ACC thresholds during foraging is partly driven by activity of LC efferents terminating in PCC, reflecting the influence of internal state on foraging decisions. In our first foraging experiment, monkeys made a choice to continue harvesting reward or to replenish a resource patch. PCC neurons signaled decisions to move to a new patch many seconds in advance, reflecting both the environment, the travel time to a new patch, as well as the animal's state, as indexed by the variance in departure times. Firing rate dynamics across the population suggest a polling mechanism for making decisions to depart immediately prior to the decision, with changes in these dynamics driven by the state of the environment. In our second foraging experiment, monkeys harvested rewards from six different locations by moving along a path connecting nearest-neighbor locations, a ubiquitous foraging behavior known as traplining. Monkeys' traplining was more reliable when they were still learning the pattern of rewards on a trial, but showed increased behavioral variability thereafter. While traplining, PCC neurons signaled the resolution of uncertainty about the state of the environment, and also tracked the entropy in the patterns of choices. Together, our results support the hypothesis that PCC integrates information about the environment and the animal's internal state to set foraging policy. A wide variety of cognitive tasks can be construed as the allocation of time to searching for distributed resources, including visual search, free recall, completion of sub-goals, voluntary task-switching, study-time allocation, and problem solving. Hence, foraging may be a core cognitive capacity applicable across behavioral domains, and PCC seems poised to regulate the influence of internal and external factors relevant to such searches.

Disclosures: D.L. Barack: None. **M.L. Platt:** None.

Nanosymposium

114. Neural Mechanisms Underlying Risk/Reward Decision-Making

Location: SDCC 23A

Time: Sunday, November 13, 2016, 8:00 AM - 11:30 AM

Presentation Number: 114.09

Topic: G.02. Motivation

Title: Dopaminergic error signals support associative structures used for model based behaviors

Authors: *M. SHARPE[1,2], C. CHANG[1], M. A. LIU[1,4], Y. NIV[2,3], J. L. JONES[1], G. SCHOENBAUM[1,5,6];
[1]Cell. Neurobio., Natl. Inst. on Drug Abuse, Baltimore, MD; [2]Princeton Neurosci. Inst., [3]Dept. of Psychology, Princeton Univ., Princeton, NJ; [4]Dept. of Psychology, Univ. of California at Los Angeles, Los Angeles, CA; [5]Departments of Anat. & Neurobio. and Psychiatry, Univ. of Maryland, Baltimore, MD; [6]Solomon H. Snyder Dept. of Neurosci., The John Hopkins Univ., Baltimore, MD

Abstract: Prediction errors drive learning. Dopamine transients correlate with these errors, yet current theoretical accounts limit dopaminergic errors to endowing cues with a scalar quantity reflecting only the rewarding value of future events. Here, we tested whether these signals might act more broadly to support learning in which cues are linked with subsequent events to form an associative model of the environment. For this, we designed a novel procedure based on the well-documented sensory-preconditioning effect. This traditionally entails first presenting two innocuous cues in close succession. Subsequently, one of these cues is paired with food. As a result of this training, both the food-paired cue and the neutral cue will now elicit an appetitive response. In our modified version of this task, we reduced the likelihood that subjects would form an association between the two innocuous cues to prevent the generalization of appetitive learning to the neutral cue. We then artificially re-introduced a learning signal during this phase by briefly stimulating dopaminergic neurons in the midbrain. Remarkably, this manipulation restored normal associative learning and behavior about the neutral cue. Subsequent tests showed that this learning was driven by a direct association between the neutral cue and the appetitive outcome. These data show that dopamine transients are sufficient to support the construction of complex associative representations beyond the reach of computational models currently used to interpret these signals.

Disclosures: M. Sharpe: None. **C. Chang:** None. **M.A. Liu:** None. **Y. Niv:** None. **J.L. Jones:** None. **G. Schoenbaum:** None.

Nanosymposium

114. Neural Mechanisms Underlying Risk/Reward Decision-Making

Location: SDCC 23A

Time: Sunday, November 13, 2016, 8:00 AM - 11:30 AM

Presentation Number: 114.10

Topic: G.02. Motivation

Support: CIHR MOP-133579

Title: Modulation of probabilistic discounting and reversal learning by dopamine within the medial orbitofrontal cortex

Authors: *N. L. JENNI, S. B. FLORESCO;
Psychology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Weighing the value of a reward against its likelihood of delivery in order to optimize long-term utility is a key aspect of adaptive decision making. Recent evidence suggests that the medial subregion of the orbitofrontal cortex (mOFC) plays a key role in monitoring probabilistic action-outcome associations and biasing decisions related to reward uncertainty, as inactivation of this subregion in rats impairs both risk/reward decision making (assessed with a probabilistic discounting task) and cognitive flexibility involving reward uncertainty (probabilistic reversal learning). The mOFC receives dopaminergic input from midbrain neurons, yet whether dopamine (DA) modulates mOFC function has been unexplored. Here, we assessed how DA D_1 and D_2 receptors in the mOFC may modulate adaptive decision making in face of probabilistic outcomes. One series of experiments assessed risk/reward decision-making using a probabilistic discounting task in which rats were trained to choose between levers delivering small/certain or large/uncertain rewards. The probability of receiving the large reward decreased across 5 blocks of trials over each daily session (100%-6.25%). Another set of studies assessed probabilistic reversal learning. At the start of a session, one lever was designated the “correct” lever and rewarded choice on 80% of the trials, while the incorrect lever rewarded choice on 20% of the trials. After 8 consecutive correct choices, reward contingencies switched, and this pattern continued over 200 trials each daily session. Separate groups of rats, well-trained on either task, received intra-mOFC microinfusions of selective D_1 or D_2 antagonists prior to task performance. Our results indicate that mOFC D_1 receptors play an important role in mitigating sensitivity to non-rewarded actions during risk/reward decision making, as blockade of these receptors reduced risky choice by increasing lose-shift behavior. DA receptors in the mOFC also facilitate identifying the profitability of probabilistic outcomes, as blockade of D_1 receptors significantly impaired probabilistic learning during the initial discrimination of the reversal task. In contrast, blockade of D_2 receptors reduced the number of errors during the initial discrimination. Together, these findings highlight a novel role for DA in the mOFC in guiding behavior under conditions of reward uncertainty. Elucidating how DA within different nodes of

mesocorticolimbic circuitry influences action selection in these situations will expand our understanding of the mechanisms regulating optimal and aberrant decision-making.

Disclosures: **N.L. Jenni:** None. **S.B. Floresco:** None.

Nanosymposium

114. Neural Mechanisms Underlying Risk/Reward Decision-Making

Location: SDCC 23A

Time: Sunday, November 13, 2016, 8:00 AM - 11:30 AM

Presentation Number: 114.11

Topic: G.02. Motivation

Support: Wellcome Trust Grant 095495

Title: The behavioral and neuronal processing of risk and value.

Authors: ***W. R. STAUFFER**[1], A. LAK[2], W. GENEST[3], W. SCHULTZ[3];
[1]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; [2]Univ. Col. London, London, United Kingdom;
[3]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Rewards are instrumental to everyday life; they confer value and elicit motivation. However, reward prediction is a risky business, as risk is inherent in nearly every decision we make. For this reason, risk and value are intimately intertwined. To measure the behavioral and neuronal relationship between risk and value, we trained rhesus monkeys to make choices between risky and safe options. Using choices between two-outcome, equiprobable gambles and safe rewards, we found that monkeys were risk-seeking at low reward magnitudes, and became increasingly risk averse as reward magnitude increased. This reward-magnitude dependent risk preference resulted in convex then concave utility functions. We then introduced more complex, three outcome gambles with positive, negative or symmetric skewness. The monkeys' choices revealed positive skewness seeking. More interestingly, the utility functions that were measured from choices between two outcome gambles and safe rewards were able to adequately predict the preferences of the monkeys for skewed gambles. These results demonstrate the usefulness of economic utility theory to describe risky choice behavior monkeys. We recorded single unit activity from dopamine neurons in the monkey midbrain. Dopamine neurons code a reward prediction error, the difference between received and predicted reward. We found that the dopamine activations scaled with the differences between the utility of received rewards and the predicted expected utilities. Thus, dopamine neurons code a utility prediction error. This utility prediction error response occurred to reward-predicting cues and to rewards themselves. These neuronal results provide a biological link between the satisfactions experienced from rewards and the utility function defined from choices. Finally, we recorded single unit dopamine neurons

while monkeys learned the value of probabilistically delivered rewards. This data shows how reward probability is transformed into a neuronal utility signal during learning. Together, these behavioral and neuronal data demonstrate that the fundamental economic theory that links together risk and value, i.e. expected utility theory, is applicable to monkey choice behavior and that the abstract concept of utility functions have a concrete biological manifestation in the firing rate of single neurons.

Disclosures: **W.R. Stauffer:** None. **A. Lak:** None. **W. Genest:** None. **W. Schultz:** None.

Nanosymposium

114. Neural Mechanisms Underlying Risk/Reward Decision-Making

Location: SDCC 23A

Time: Sunday, November 13, 2016, 8:00 AM - 11:30 AM

Presentation Number: 114.12

Topic: G.02. Motivation

Title: Increase in preference for uncertainty in rats following chronic dopamine $D_{2/3}$ agonist ropinirole treatment for Parkinson's Disease: Potential recruitment of the Akt/GSK3β signalling pathway

Authors: ***M. TREMBLAY**[1], M. M. SILVEIRA[2], S. KAUR[2], J. G. HOSKING[3], W. K. ADAMS[2], C. BAUNEZ[4], C. A. WINSTANLEY[2];
[1]PSYCHOLOGY, [2]Univ. of British Columbia, Vancouver, BC, Canada; [3]Harvard Univ., Cambridge, MA; [4]Aix-Marseille Univ., Marseille, France

Abstract: The aims were to investigate (1) the effect of chronic dopamine $D_{2/3}$ agonist ropinirole on gambling behaviour on a rodent Betting task (rBT) in healthy rats and in a 6-hydroxydopamine (6-OHDA) lesion model of Parkinson's disease (PD), (2) if prior preference for uncertainty predicted response to ropinirole, (3) the intracellular mechanism underlying the effect observed and (4) the ability of a GSK3β inhibitor to block increases in uncertain choices on the rBT following chronic ropinirole. L-DOPA, the first line treatment for PD, has shown over time to produce debilitating side-effects such as dyskinesia. Preferential dopamine $D_{2/3}$ agonists have been used to treat PD, but can lead to a variety of Impulse Control Disorders (ICD) and gambling disorder. The mechanism mitigating dopamine agonist-induced ICDs is unknown. Activation of D_2 receptors predominantly involves the G-protein/cAMP pathway. However, it has been suggested that the Akt/GSK3β pathway can dominate during prolonged stimulation of D_2 receptors leading to elevation in GSK3β. Elevated GSK3β has been involved in addiction, schizophrenia and bipolar disorder, as well as in hyperdopamine-dependant behaviours. We hypothesized that the Akt/GSK3β pathway may be involved in the development of ropinirole-induced ICDs. Healthy and dorsostriatal 6-OHDA lesioned rats performed the rBT prior to

implantation of an osmotic pump delivering either ropinirole (5mg/kg/day) or saline for 28 days. The rBT is a paradigm which captures decision-making under uncertainty. Chronic ropinirole increased choice of the uncertain lever in the rBT in healthy rats and in the rat model of PD regardless of subjective preference for uncertainty. This effect disappeared after washout. D_2 receptor expression was increased and a trend towards elevated GSK3β was observed in the rats who increased their choice of the uncertain option in response to ropinirole. In another cohort of healthy rats, we replicated the effect of ropinirole on the rBT, but failed to reverse ropinirole-induced increase in uncertain choices with the GSK3β inhibitor SB216763. In conclusion, ropinirole's effect on behaviour in the rBT is reliable and appears unrelated to PD pathology. This outcome may be independent of activation of the Akt/GSK3β pathway.

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Nanosymposium

114. Neural Mechanisms Underlying Risk/Reward Decision-Making

Location: SDCC 23A

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Presentation Number: 114.13

Topic: G.02. Motivation

Support: NSF GFRP

Yale University Cellular & Molecular Biology training grant (NIH T32GM007223)

Gruber Science Fellowship

Title: Neural implementation of reinforcement learning models that underlie learning and decision-making in *Drosophila*

Authors: *L. MCCURDY[1,2], M. N. NITABACH[1,3];
[1]Cell. and Mol. Physiol., [2]Interdepartmental Neurosci. Program, [3]Genet., Yale Univ., New Haven, CT

Abstract: Animals are constantly learning about their environment and using that knowledge to inform decisions regarding how best to maximize reward and minimize punishment. Reinforcement learning algorithms are designed to solve similar types of optimization problems, and thus have been a useful framework for understanding how vertebrates learn and make decisions at a behavioral and neural level. Specifically, dopaminergic neurons in the vertebrate ventral tegmental area implement a temporal-difference algorithm during appetitive conditioning, by calculating the difference between expected and actual reward (i.e. the

prediction error) on a trial-by-trial basis. However, given the complexity and heterogeneity of the dopaminergic system in the vertebrate brain, a precise understanding of how and which dopaminergic neurons perform the computations that give rise to such behaviors is less clear. We took advantage of the well-characterized neural circuitry underlying aversive olfactory learning in *Drosophila* to investigate how an animal chooses between two aversive stimuli to minimize punishment.
To do so, we conditioned *Drosophila* to associate two odors with two sequences of electric shock, and assayed their odor choice behavior as a readout for which electric shock sequence they found to be more aversive. We used the temporal-difference algorithm to predict what the value (i.e. the aversiveness of the punishment) associated with each odor was, and thus which odor *Drosophila* would find to be more aversive**.** Our data suggest that *Drosophila* are indeed capable of deciding between two different electric shock punishments, in a manner that can be explained and predicted by the algorithm.
Next, we will determine if and how this algorithm is implemented at a neuronal level. We will express a genetically-encoded calcium indicator and record neural activity in specific subsets of the PPL1 cluster of dopaminergic neurons while the fly learns different odor-shock associations, to determine how and where the prediction error signal is encoded.
These experiments will shed light on how the *Drosophila* nervous system computes and encodes value during olfactory conditioning, suggesting further similarities between invertebrate and vertebrate biology and computation that underlie learning. This provides further evidence that Drosophila is a well-suited model organism for probing the neural circuitry and computations that underlie learning and decision-making in animals.

Disclosures: L. McCurdy: None. **M.N. Nitabach:** None.

Nanosymposium

114. Neural Mechanisms Underlying Risk/Reward Decision-Making

Location: SDCC 23A

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Presentation Number: 114.14

Topic: G.02. Motivation

Support: Martina Stern Memorial Fund

Title: Reward rule learning in *Drosophila*

Authors: *R. YANG, R. HE, U. STERN;
Duke Univ., Durham, NC

Abstract: The ability to maximize rewards is advantageous for animal survival. While *Drosophila* can be classically conditioned to associate specific sensory cues with rewards, it is unclear whether they can learn to maximize reward collection through trials and errors. Here we show that *Drosophila* readily learn an artificially implemented dynamic reward rule. We first identified a group of neurons whose optogenetic activation was profoundly attractive to flies and thus actively pursued by them. We next developed a high-throughput closed-loop system that can deliver "optical reward" to multiple individual flies if their behaviors conformed to a specific place-action rule we implemented. Impressively, flies started to garner rewards within minutes and readily adjusted behaviors when the reward rule changed. Dissection of the underlying circuit revealed that learning the reward rule critically depended a specific class of dopaminergic (DA) neurons and the mushroom body (MB), a known odor-reward association center in the fly brain. Our results suggest that *Drosophila* have "general-purpose" intelligence for solving simple reinforcement-learning tasks, enabled by a powerful internal reward system reminiscent of the vertebrate VTA-DA neurons. Moreover, our results suggest that MB is a general learning center where complex sensory cues (e.g., place) may be associated with rewards.

Disclosures: R. Yang: None. **R. He:** None. **U. Stern:** None.

Nanosymposium

115. Attentional Networks

Location: SDCC 1B

Time: Sunday, November 13, 2016, 8:00 AM - 11:15 AM

Presentation Number: 115.01

Topic: H.02. Human Cognition and Behavior

Support: Swiss SNSF grant 320030-159708

Title: Left hemisphere provides compensatory mechanisms in neglect: fMRI evidence from prismatic adaptation intervention

Authors: *S. CLARKE[1], C. BINDSCHAEDLER[2], E. FORNARI[3], S. CROTTAZ-HERBETTE[2];
[2]Service de Neuropsychologie et de Neuroréhabilitation, [3]CIBM, Neuroradiology, [1]CHUV, Lausanne, Switzerland

Abstract: Prismatic adaptation has been repeatedly reported to alleviate neglect symptoms; in normal subjects it was shown to enhance the representation of the left visual space within the left inferior parietal cortex. Our study aimed to determine in humans whether similar compensatory mechanisms underlie the beneficial effect of prismatic adaptation in neglect. Fifteen patients with right hemispheric lesions and 11 age-matched controls underwent a prismatic adaptation

session which was preceded and followed by fMRI acquisitions using a visual detection task. In patients the prismatic adaptation session improved the accuracy of target detection in the left and central space and enhanced the representation of this visual space within the left hemisphere in parts of the temporal convexity, inferior parietal lobule and prefrontal cortex. Across patients, the increase in neural activation within the temporal regions correlated with performance improvements in this visual space. In control subjects prismatic adaptation enhanced the representation of the left visual space within the left inferior parietal lobule and decreased it within the left temporal cortex. Thus, a brief exposure to prismatic adaptation enhances, both in patients and in control subjects, the competence of the left hemisphere for the left space, but in patients the regions extended beyond the inferior parietal lobule to the temporal convexity. These results suggest that the left hemisphere provides compensatory mechanisms in neglect by assuming the representation of the whole space within the ventral attentional system. The rapidity of the change suggests that the underlying mechanism relies on uncovering of pre-existing synaptic connections.

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Nanosymposium

115. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

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FRM (SPF20140129218)

McDonnell Scholar Award

Natural Sciences and Engineering Research Council of Canada (NSERC)

Title: Paired-pulse parietal-motor stimulation can act as a neuromodulator when combined with prism adaptation

Authors: ***S. SCHINTU**[1,2,3], E. MARTÍN-ARÉVALO[2,3], M. VESIA[4], Y. ROSSETTI[2,3,5], R. SALEMME[2,3,5], L. PISELLA[2,3], A. FARNÉ[2,3,5], K. T. REILLY[2,3];
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Action & Cognition Team (ImpAct), Lyon Neurosci. Res. Ctr. (CRNL), Lyon, France; [3]Univ. of Lyon 1, Lyon, France; [4]Toronto Western Hospital, Univ. Hlth. Network, Krembil Res. Inst., Toronto, ON, Canada; [5]Neuro-immersion & Mouvement et Handicap, Hospices Civils de Lyon, Lyon, France

Abstract: Prism adaptation (PA) is an apparently simple and quick way of modulating visuomotor correspondences. It consists of adaptation to wedge prisms, usually mounted on goggles that deviate vision. Rightward prism adaptation ameliorates spatial neglect symptoms while leftward prism adaptation (LPA) induces neglect-like biases in healthy individuals. Critically, while there are similarities in the direction of the attentional bias observed in neglect patients and in healthy individuals after LPA, to date there is no evidence for similar physiological changes associated with these attentional biases. Similarly to PA, inhibitory repetitive transcranial magnetic stimulation (rTMS) on the right posterior parietal cortex (PPC) induces neglect-like behavior, whereas on the left PPC, it ameliorates neglect symptoms and normalizes hyper-excitability of left hemisphere parietal-motor (PPC-M1) connectivity. Based on this analogy, we hypothesized that LPA increases PPC-M1 excitability in the left hemisphere and decrease it in the right. To shed some light on the mechanisms underlying LPA's effects on cognition, we investigated this hypothesis in two groups of healthy individuals, measuring PPC-M1 excitability in the right and left hemispheres. We employed a dual-site paired-pulse TMS protocol (ppTMS) that quantifies the influence of PPC over M1 and thus provides an index of the strength of parieto-frontal functional connectivity within each hemisphere. Following LPA we found a left-hemisphere increase and a right-hemisphere decrease in the amplitude of motor evoked potentials elicited by paired as well as single pulses on M1. While this could indicate that LPA biases interhemispheric connectivity, it contradicts previous evidence that M1-only MEPs are unchanged after LPA. In a control study, we investigated the input-output curves in both hemispheres as a measure of corticospinal excitability (CSE). The results of this second experiment showed that the input-output curves were not affected by LPA *per se*. The differential change in CSE in the left and right hemispheres we observed, plus the absence of the well-document right-shift in line bisection judgments, leads us to suggest that LPA interacted with our parietal and motor cortex stimulation. We conclude that LPA combined with ppTMS on PPC-M1 differentially alters the excitability of the left and right M1. In addition, ppTMS on PPC-M1 might itself act as a neuromodulator, when combined with LPA.

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Nanosymposium

115. Attentional Networks

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Presentation Number: 115.03

Topic: H.02. Human Cognition and Behavior

Support: BMBF Grant 01GQ1401

Title: Probabilistic beliefs are updated in the right temporoparietal junction

Authors: *P. MENGOTTI[1], P. L. DOMBERT[1], G. R. FINK[1,2], S. VOSSEL[1];
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Abstract: The right temporoparietal junction (rTPJ) is involved in various cognitive domains. In the field of attention, the rTPJ is thought to initiate attentional reorienting. Here, we provide evidence for a more general and overarching role of rTPJ for "contextual updating", i.e., for the adjustment of internal models after new observations. We combined online TMS with computational modelling to test the causal involvement of rTPJ in updating probabilistic beliefs. In a within-subject design, fifteen participants completed four runs of the experimental task, distributed over two days. In each run, they performed a modified version of a location-cueing paradigm, where false information about the percentage of cue validity (%CV) was provided in half of the experimental blocks to prompt updating of prior expectations. On the basis of response speed (RS; inverse reaction time) trial-by-trial learning of the cue-target contingencies, and therefore belief updating, was assessed with a Rescorla-Wagner (RW) model in which the learning rate determines the impact of the prediction error on the belief update. Online double-pulse TMS was applied 300 ms (TMS300) or 50 ms (TMS50) after target appearance, to investigate the chronometry of rTPJ involvement. Online double-pulse TMS over rTPJ 300 ms after target appearance selectively decreased participants' updating of false prior beliefs concerning %CV, as reflected in a decreased RW-learning rate. Specifically, an ANOVA on learning rate values in different stimulation (sham, TMS300, TMS50) and prior (true, false) conditions revealed a significant interaction between the two factors. Bonferroni corrected post-hoc t-tests of this interaction highlighted a significant difference between TMS300 and sham only in blocks with false prior. Moreover, both sham and TMS50 showed higher learning rates in blocks with false prior, compared with true ones, and this difference disappeared in TMS300. Decreased updating in TMS300 also impacted on participants' explicit beliefs, causing them to overestimate %CV, as shown by the difference in %CV ratings between TMS300 and sham. These results offer a unifying interpretation of rTPJ activity for cognitive domains requiring the formation and updating of probabilistic beliefs about external or internal inputs.

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Nanosymposium

115. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

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KU Leuven OT/12/097

Title: Dissociation between spatial shifting and attentional selection in superior parietal cortex: An electrocorticography study

Authors: *R. R. VANDENBERGHE[1], E. GHUMARE[2], L. SEYNAEVE[4], P. DUPONT[2], W. VAN PAESSCHEN[4], T. THEYS[5], M. SCHROOTEN[3];
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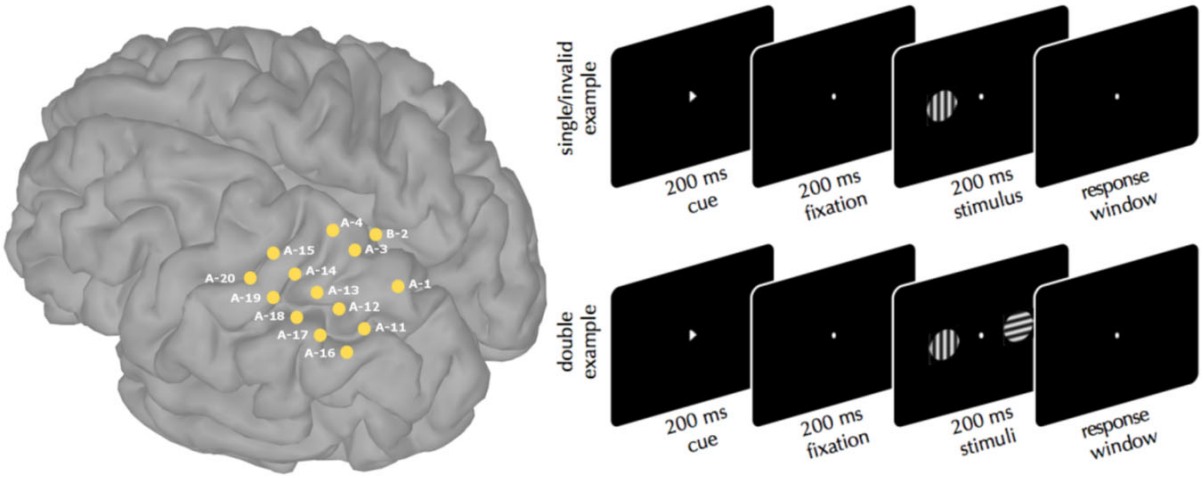
Abstract: Introduction: Spatial-attentional shifting and selection between competing stimuli are two of the most fundamental attentional processes. Methods: A cortical grid in a patient who underwent presurgical evaluation for medically intractable epilepsy covered both banks of the middle and anterior segment of the left intraparietal sulcus (IPS) and the lateral and medial wall of the left superior parietal lobule (SPL) (Fig 1). Recordings were performed during a hybrid spatial cueing paradigm identical to that previously applied in behavioral experiments in patients (Gillebert et al., 2011) and in healthy controls using task-related fMRI. Event-related potentials (ERP) were analyzed as well as event-related spectral perturbations (ERSP). Weighted phase lag index (wPLI) was used to assess directional connectivity. Results: An invalidly cued target evoked a robust ERP in SPL at 180 ms following target onset compared to a validly cued target. The effect lasted until 450 ms following grating onset. The effect of invalidity was significantly larger in SPL than in IPS, confirming the early involvement of SPL in spatial shifting. Along the upper and lower bank of the anterior IPS the presence of a competing distracter evoked an ERP which started around 300-400 ms post grating onset and lasted beyond 800 ms. This effect was significantly stronger in IPS than in SPL. The effect of stimulus competition was distributed in a broad frequency band between 10 and 40 Hz. Based on wPLI, the functional connection during the cue and the delay phase was directed from IPS to SPL and from anterior to posterior IPS until approximately 350 ms after grating onset when it returned to baseline levels. Following an invalidly cued target, the direction of the connection inverted at approximately 250 ms following grating onset for approximately 150 ms, going from SPL to IPS. Conclusion: Electrophysiologically, spatial shifting and selection between competing stimuli elicit temporally, spatially and spectrally dissociable effects within superior parietal cortex.



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Nanosymposium

115. Attentional Networks

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Human Brain Project (HBP)

Title: Task based functional homologies of human superior parietal lobe in monkey

Authors: ***N. S. CASPARI**[1], R. VANDENBERGHE[2,3], W. VANDUFFEL[1,4,5];
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Abstract: We continuously shift our attention between items in the visual environment, which can be based on either task relevance (top-down) or sudden saliency of an unexpected stimulus (bottom-up). As this is a fundamental process for guiding goal-directed behavior, it is to be expected that this property is evolutionary conserved in primates. In humans top-down directed spatial attention shifts most strongly activate the medial superior parietal lobe (mSPL) (Vandenberghe et al. 2001, Yantis et al. 2002). In order to identify a potential monkey homologue of this mSPL region, we scanned (at 3 T) monkeys (N=3) and humans (N=31) performing the same covert selective spatial attention task. We used an event-related fMRI design whereby periods of shifts and sustained attention were interleaved (Caspari et al., 2015; Molenberghs et al., 2007). Two pairs of shapes were presented on the horizontal meridian (9.25 deg), each containing a relevant and irrelevant shape. Subjects fixated centrally and responded manually when the relevant stimulus dimmed. An event consisted of the replacement of the current stimulus pair by the next. In 1/3 of the trials, this change elicited a covert spatial shift in attention as the relevant stimulus was replaced by an irrelevant one. Monkeys were scanned (1.25 mm isotropic) using implanted phased-array Rx coils and MION, and humans (2.75 x 2.75 x 3.5 mm) using BOLD. First, we topologically compared shift-selectivity across species using standard GLM analyses. Next, we performed a novel data-driven Inter Species Beta Correlation (ISBC) analysis using 10 dissociable task conditions modeled by the GLM. These included shift (L/R), sustained attention (L/R), dimming (L/R and relevant/irrelevant) and null events (L/R). The GLM-contrast showed highly similar shift responses across species in the medial superior and inferior parietal lobe, with mSPL as strongest shift-selective region in humans. Monkeys recruited more profoundly frontal regions during shifting compared to humans. The ISBC method revealed a subset of parietal and frontal voxels in monkeys correlating with activity averaged over the shift-selective human mSPL-cluster. Medial parietal cortical areas V6/V6A in monkey correlated best with the shift-selective local maxima within human mSPL (based on the GLM contrast), compared to the cyto-architectonic SPL subdivisions SPL5 and SPL7 from Caspers et al. 2007. Finally, functional connectivity of human shift-selective mSPL at rest resembled known anatomical connections of V6/V6A in monkey, indicating that both species recruit evolutionary largely conserved anatomical regions for spatial attention shifting within medial SPL.

Disclosures: **N.S. Caspari:** None. **R. Vandenberghe:** None. **W. Vanduffel:** None.

Nanosymposium

115. Attentional Networks

Location: SDCC 1B

Time: Sunday, November 13, 2016, 8:00 AM - 11:15 AM

Presentation Number: 115.06

Topic: H.02. Human Cognition and Behavior

Support: the Youth 1000 Talent Program of China, LZ15H180001

Title: rTMS modulates network-wise brain activity during rest and sustained attention

Authors: *W. PENG, S. XUE, D. LI, Y. GUO, Q. GE, J. ZHANG, Z. WANG;
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Abstract: Transcranial magnetic stimulation (TMS) is a noninvasive and reversible neuromodulatory tool that has shown great potentials in treating various brain diseases. But its neuronal mechanism still remains elusive. Using fMRI, researchers have found rTMS effects in various distributed brain regions with or without direction connections to the rTMS site, which strongly suggests a network-wise neuromodulatory mechanism of rTMS. To test this hypothesis, we acquired fMRI data before and after applying 20 Hz rTMS or the corresponding SHAM stimulation from 34 young healthy subjects (17 in each group). rTMS was applied to the left dorsolateral prefrontal cortex (DLPFC). The order of applying or not applying rTMS (or SHAM) was counterbalanced and the second time of experiment was conducted two days later. rTMS stimuli contained 20 pulses per second (20 Hz) for 2.5 sec, with an inter-train interval of 28 sec. The pulse magnitude was adjusted to be 90% of the resting motor threshold. Because DLPFC is part of the default mode network (DMN) and the attention network, we acquired fMRI during rest and during subjects performing the classical sustained attention task. DMN at rest was assessed with the posterior cingulate cortex (PCC)-seeded functional connectivity (FC) analysis. Attention activation was identified with general linear model. rTMS effects were then assessed using a 2x2 ANOVA. The statistical significance was defined with a voxel-wise $p<0.005$ and a cluster-wise $p<0.05$ (corrected for multiple comparison using Monte Carlo permutations). As compared to SHAM, high frequency rTMS increased brain activity during rest and attention task. Resting brain activity increase was only found in DMN. By contrast, task activation increase was found mainly in the limbic system and motor system in the ipsilateral side of brain. Representing the first rTMS+resting state fMRI and task fMRI study, our data showed a rest/task dissociated network-wise modulation effect of rTMS: the network affected during rest involved the TMS site but resided outside of the TMS site during task performance. Increased activity in limbic and sensory system during sustained attention after rTMS may indicate enhanced vigilance and automatic processing, two functions involved in those brain regions.

Disclosures: **W. Peng:** None. **S. Xue:** None. **D. Li:** None. **Y. Guo:** None. **Q. Ge:** None. **J. Zhang:** None. **Z. Wang:** None.

Nanosymposium

115. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

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NIH Grant F32 NS090757

Title: Neural substrates for modulating task-adaptive functional connectivity patterns

Authors: *K. HWANG[1], J. M. SHINE[2], A. JAGADEESH[1], M. D'ESPOSITO[1];
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Abstract: Theories of cognitive control hypothesize that the prefrontal cortex (PFC) guides the optimal routing of information flow along neural pathways (Miller & Cohen, 2001). However, there are few empirical data that directly demonstrate PFC influences information exchange between brain regions. In the current study, we investigated the neural substrates that modulate dynamic functional connectivity (dFC) between visual areas during visual attention. We recruited 28 healthy adult subjects for an fMRI study, and 5 subjects participated in a follow-up TMS-fMRI study. Sequences of superimposed images of faces and buildings were presented centrally to participants, who were required to detect occasional repetitions of a target category (face or building), while ignoring repetitions in the opposite distractor category. Preprocessed fMRI data were analyzed using a dFC method (Shine et al., 2015) to estimate how functional connectivity between visual areas changes across time under different attention conditions. We simulated data with the same constraints as the behavioral task to determine the optimal time windowing lengths for detecting true task-related changes in dFC. Applying the optimal window lengths (15-30 s) to task data, we found that dFC increased between early (V1/V2) and higher-level visual areas (fusiform face area [FFA] or parahippocampal place area [PPA]) for attention targets, and decreased for distractors. By regressing the strength of dFC across the whole brain, we found that increases in dFC during target attention was associated with increased activity in the right inferior frontal junction (IFJ). In contrast, decreases in dFC during the distractor condition was negatively associated with left middle frontal gyrus (MFG) activity. Replicating previous studies, we found that attention enhanced the amplitude of evoked responses for attention targets while reduced amplitudes for distractors in FFA/PPA. However, IFJ and MFG did not exhibit task-related changes in functional connectivity with FFA/PPA, and were only associated with changes in dFC patterns between V1/V2 and FFA/PPA. Instead, bilateral superior precentral sulcus (PCS) and intraparietal sulcus exhibited task-related changes in functional connectivity with FFA/PPA, suggesting dissociable roles for PCS versus IFJ and

MFG. Theta-burst TMS stimulation to MFG and PCS modulated task-related changes in dFC and evoked-response amplitudes. Our results provide causal evidence indicating that PCS, IFJ, and MFG exert distinct top-down biasing signals to modulate stimulus-driven evoked responses, enhance or inhibit task-adaptive functional connectivity patterns.

Disclosures: **K. Hwang:** None. **J.M. Shine:** None. **A. Jagadeesh:** None. **M. D'Esposito:** None.

Nanosymposium

115. Attentional Networks

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Presentation Number: 115.08

Topic: H.02. Human Cognition and Behavior

Title: Differential effects on network dynamics following stimulation of cortical nodes within default and frontoparietal networks

Authors: ***J. M. SHINE**[1], J. PARVIZI[2];
[1]Psychology, [2]Stanford Univ., Stanford, CA

Abstract: The emergence of cognition and attention is critically dependent on interactions between large-scale networks of the human brain (1). A critical axis on which these network interact is in the balance between exogenous and endogenous attention (2). Previous work has shown that activity within frontoparietal and default networks is anti-correlated in the resting state (3), suggesting an antagonistic relationship that is mediated by a cingulo-opercular 'salience' network (4). In this study, we tested this hypothesis by stimulating and recording from pairs of electrodes in subjects that were undergoing electrophysiological mapping prior to surgery to remove epileptic foci. Subjects were implanted with depth electrodes. Discounting electrode pairs associated with pathological epileptic activity and those that were placed within white matter, we stimulated pairs of electrodes (0.5 Hz stimulation; 8-10 mA; biphasic) that belonged to one of three large-scale networks: frontoparietal, salience or default mode networks. The network identity of each electrode pair was defined using an iterative method that translates a subject's individual resting state network connectivity parcellation (2s TR; 4.0mm^3 voxels) according to a pre-defined group-level map (5). Cortical-evoked potentials were then recorded from a larger group of electrodes, each of which was identified as belonging to one of the three stimulated networks. Single 0.5-ms current pulses were delivered every 500ms and the broadband potential was then measured in the 100ms bin after stimulation (discounting the first 10ms due to stimulation artifacts). By comparing the post-stimulus window in each pair of electrodes to the 95th percentile of activity in non-stimulated data, we were able to determine how frequently the stimulation of each network led to evoked activity in each other network, thus

allowing us to decipher patterns of potential information flow around the macroscopic network of the brain. Our results demonstrate that stimulation of frontoparietal and salience networks causes significant evoked activity in each of the other networks, however the stimulation of the default mode network was not associated with post-stimulus activity in the salience network. This suggests a targeted organization of information flow around the structural connectome that has important implications for models of attentional and cognitive function.

Disclosures: J.M. Shine: None. **J. Parvizi:** None.

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Title: Frontal and occipital brain regions interaction in spatial attentional competition: selection overrides inhibition

Authors: *J. A. FRANCO-RODRIGUEZ[1], T. V. ROMÁN-LÓPEZ[1], M. MÉNDEZ-DÍAZ[2], O. PROSPÉRO-GARCÍA[2], A. E. RUIZ-CONTRERAS[1];
[1]Lab. de Neurogenómica Cognitiva, Facultad De Psicología, UNAM, Ciudad de Mexico, Mexico; [2]Gpo. Neurociencias: Lab. Canabinoides. Depto. Fisiología, Fac. Medicina, Cd. Mexico, Mexico

Abstract: Attentional competition is the interplay of top-down and bottom-up influences in the current goal-directed behaviour. There is evidence of enhancement and suppression of brain activity associated with selection of relevant information and inhibition of the irrelevant one, respectively. However, little information is available about the temporal dynamics of visual attentional competition and the brain regions involved. The aim of this investigation was to explore, by means of event-related potentials, visual attentional competition when both selection and inhibition are necessary for the current goal, and to observe the temporal interplay between frontal and occipital regions. Thirty nine young participants solved an attentional competition task were they had to respond to one relevant feature (colour or orientation) on a set of eight rectangles in a visual display. There were four possible display arrangements: homogeneous

(irrelevant features), non-target (presence of one stimulus with the irrelevant feature), target (presence of one stimulus with the relevant feature) or target+non-target (presence of one stimulus with the relevant feature and one with the irrelevant feature). Our results show that the effects of enhancement of the processing of the relevant stimulus are present when it is alone or accompanied by an irrelevant stimulus. When the irrelevant stimulus was presented alone, at 275ms stimulus onset, higher amplitude was observed when the relevant stimulus was present than when the irrelevant stimulus alone was present (positive polarity in frontal region; negative polarity in occipital region). Also, we found a temporal dynamics which involves an early effect (at 100ms) in the frontal region, middle effect (at 175ms) on the occipital region and, finally (at 275), goes back to frontal, presumably reflecting different attentional stages in the resolution of the task: prefrontal regions directing which feature is relevant for the goal directed behaviour, occipital region discriminating these features and probably, prefrontal regions taking action for giving a response.

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Nanosymposium

115. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

Support: NIH R21 MH096239

Title: Network dynamics of control over enhancement versus suppression in sustained attention

Authors: ***A. LENARTOWICZ**[1], S. LU[1], E. LAU[1], G. V. SIMPSON[2], M. S. COHEN[1]; [1]Univ. of California Los Angeles, Los Angeles, CA; [2]ThinkNow Inc., San Francisco, CA

Abstract: Attention control is the ability to control the focus of attention, to enhance signals of interest and to suppress distractions. Enhancement and suppression arise from modulation of neural responses in sensory cortex, with presumed sources of modulation in associative cortex, namely prefrontal and parietal structures. We tested this model within the context of ongoing, sustained attention. Using a combination of functional MRI and EEG data, collected separately and concurrently in an audio-visual sustained attention paradigm, we measured the functional connectivity of control for enhancement of target signals versus suppression of distracting signals. We report two novel findings that are not accounted for by existing models. (1) The neural pathways of enhancement and suppression in sustained attention were separable, with a

prevalent source of suppression arising from supplementary motor cortex rather than prefrontal or parietal cortex. Furthermore, enhancement was not reliably associated with functional connections from associative cortex. (2) We observed reconfiguration of sensory cortex connectivity with respect to task general networks (akin to resting state networks) that were associated with task performance. We conclude that a considerable source of control in sustained attention, and thus of modulation of sensory activity, may lie in network interactions exclusive of bias signals arising from prefrontal and associative structures.

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Nanosymposium

115. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant EY022229

NSF Graduate Research Fellowship DGE-1247312

Title: Visuospatial representations within cerebellar node of the dorsal attention network

Authors: *J. A. BRISSENDEN[1], D. E. OSHER[1], E. J. LEVIN[1], M. A. HALKO[2], D. C. SOMERS[1];
[1]Psychological and Brain Sci., Boston Univ., Boston, MA; [2]Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract: Background: The cerebellum is typically considered to be a motor structure. However, growing evidence reveals cerebellar responses to a wide variety of cognitive tasks (e.g., Allen et al., 1997; Stoodley et al., 2012; Brissenden et al., in press). Furthermore, cortical association networks possess functional connectivity with non-overlapping zones of cerebellar cortex (Buckner et al., 2011). We recently demonstrated that visual working memory (VWM) and visual attention tasks preferentially recruit cerebellar regions that are intrinsically coupled with the cortical dorsal attention network (DAN) (Brissenden et al., in press). Here, we examine whether cerebellar DAN BOLD activity contains information about the pattern of visuospatial attentional deployment in a VWM task.
Methods: Subjects performed a lateralized VWM change detection task in an fMRI scanner. Visual stimulation was matched between visual hemifields (6 bars presented in each hemifield).

At the beginning of each condition block, subjects were cued to covertly attend to either the left or right side of the display. Subjects were instructed to remember the orientation of a subset of bars within the attended hemifield. Target bars were distinguished from distractors by color. Cerebellar regions-of-interest (ROIs) were defined by intrinsic functional connectivity with cortical network seeds (Yeo et al., 2011). To determine whether specific cerebellar regions contain information about the pattern of attentional deployment, we trained linear support vector machine classifiers (nested leave-one-run-out cross-validation; 1000 permutations to establish significance) to decode the attended hemifield within each of our ROIs. Eye position was monitored during scanning to ensure subjects held fixation throughout the task. We also performed support vector regression to determine whether any cerebellar ROI contained information about eye movements.
Results: Cerebellar DAN and ventral attention network (VAN) ROIs were able to classify the direction of attentional deployment (DAN: $p = 0.0012$ uncorrected, 0.012 corrected; VAN: $p = 0.021$ uncorrected, 0.105 corrected). We were unable to decode the attended hemifield in every other cerebellar network ROI. Further analyses revealed no relationship between eye movements predicted from cerebellar BOLD activity and actual eye movements. There was no effect of attended hemifield on behavioral measures of performance ($p > 0.99$).
Conclusions: These findings provide further evidence for cerebellar contributions to VWM and attentional processes and imply a potential role for the cerebellar DAN in spatial orienting of attention.

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Nanosymposium

115. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

Support: NIH R01-EY022229

NIH T90-DA032484

Title: Functional connectivity predicts individual differences in sensory-biased caudolateral prefrontal cortex response to attention and working memory.

Authors: *S. M. TOBYNE[1], D. E. OSHER[2], S. W. MICHALKA[3], A. L. NOYCE[2], D. C. SOMERS[2];

[1]Grad. Program for Neurosci., [2]Psychological and Brain Sci., [3]Biomed. Engin., Boston Univ., Boston, MA

Abstract: Introduction: In our recent work (Michalka et al., Neuron, 2015), we identified four bilateral interleaved regions in caudolateral prefrontal cortex (clPFC) that exhibit a bias for audition or vision during attention and memory tasks. These regions form differentiable networks based upon their intrinsic functional connectivity (iFC) to posterior sensory biased cortex. Here we have used iFC to predict task activation, and thus modality bias, by modeling the differential connectivity of these clPFC regions. We show that we can reliably predict modality biased task activation and identify clPFC regions in novel subjects.
Methods: Two separate fMRI experiments were used in this work. A sustained auditory-visual attention (SAVA) task required subjects to covertly attend to and identify targets within a cued stream amongst 4 simultaneously presented streams (2 visual, 2 auditory) of numbers and letters. A different group performed an auditory-visual working memory (AVWM) task, requiring subjects to separately perform a 2-back paradigm on either male/female faces or dog/cat sounds. In each experiment clPFC regions were identified by contrasting auditory and visual conditions. Resting state fMRI was also acquired. For each vertex of a defined clPFC search space, *t* values were extracted from each subject from each experiment. iFC between each vertex of the search space and all nodes of a 17 network cortical parcellation (Yeo et al., 2011) was also calculated. SAVA iFC data alone was used to train a linear model to predict task task activation in the SAVA task, and accuracy was assessed using leave-one-subject-out cross validation. AVWM data was used as a test set to assess model performance when applied to a unique task with different imaging parameters and novel subjects.
Results: Mean correlation between actual and predicted responses for the training set was 0.597 for right and 0.524 for left search spaces (both $p<0.0001$). Group analysis revealed that individuals were significantly better predicted by their own connectivity patterns than by other subjects (both $p<0.0001$). Furthermore, predicted data were significantly more correlated with actual data than averaged task data (lh: $p=0.029$; rh: $p<0.0001$). Remarkably, mean correlation for the AVWM test group predicted with SAVA data was high: 0.448 for the right and 0.456 for the left search spaces.
Conclusion: These results indicate that individual differences in location and activation strength of recently discovered sensory biased prefrontal cortex can be reliably predicted by whole brain patterns of iFC and that these predictions generalize to novel subjects and experiments that activate these regions.

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Nanosymposium

115. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

Title: Relationship between pre-stimulus individual alpha oscillations and reaction times in visual search

Authors: *A. PASTUSZAK, S. HANSLMAYR, K. L. SHAPIRO;
Univ. of Birmingham, Birmingham, United Kingdom

Abstract: Previous research indicates that anticipatory modulation of alpha oscillation is related to performance on attentional tasks (Sauseng et al., 2005). The present experiment investigated how unmodulated individual alpha frequency (IAF), recorded prior to the stimulus onset, is related to reaction times (RTs) of target detection in visual search (Triesman, et al. 1980). Alpha power increases have been previously associated with inhibition of visual stimuli (Dijk, et al., 2008; Händel, et al., 2011). Therefore, two correlation directions were possible between RTs and IAF power. A positive correlation, with low alpha power related to fast RTs, cf. research wherein increased alpha power was shown to be beneficial for single stimulus detection (Mathewson, et al., 2011). Alternatively a negative correlation, where high IAF power would be related to fast RTs, indicating that alpha frequency power reflects distractor inhibition (Bonneford & Jensen, 2013).
We recorded the EEG signal while participants performed a visual search task, where they were asked to detect the target among a set of distractors. We correlated the RTs for all the trials with occipital IAF power, across 300 ms window preceding the visual search onset. Our results show a significant negative correlation spanning approximately 120 ms, from 160 ms to 40 ms pre-stimulus onset. This indicates that the pre-stimulus IAF power is inversely related to the RTs of detecting the target, with higher pre-stimulus alpha power being associated with faster RTs and vice versa**.** This is in line with research implicating alpha oscillations in distractor inhibition (Payne, et al., 2013; Jensen & Mazaheri, 2010). We assume that with no prior information as to when or where the target will occur it may be beneficial to keep the narrow width of the attentional filter, which would require higher IAF power, to prepare for optimal filtering out of the upcoming distractors. Interestingly, this result is only evident in the smaller set size condition (16 objects presented on the display), but not in the higher set size condition of visual search (24 objects), which may be due to the prolonged times of task completion. We conclude, that endogenous alpha rhythm is related to attentional processing, specifically to inhibition of distractors when the task requires detecting relevant information.

Disclosures: **A. Pastuszak:** A. Employment/Salary (full or part-time): University of Birmingham. **S. Hanslmayr:** A. Employment/Salary (full or part-time): nversity of Birmingham. **K.L. Shapiro:** A. Employment/Salary (full or part-time): University of Birmingham.

Nanosymposium

116. Techniques in Electrophysiological Recording and Stimulation

Location: SDCC 7B

Time: Sunday, November 13, 2016, 8:00 AM - 10:00 AM

Presentation Number: 116.01

Topic: I.04. Physiological Methods

Title: Simultaneous optimization of spike detection thresholds and cluster sorting

Authors: *P. N. STEINMETZ;
Nakamoto Brain Res. Inst., Tempe, AZ

Abstract: Separating the activity of single neurons in extracellular microwire recordings is a key step in understanding how the firing of neurons is correlated with experimental conditions. Such separation is easier to achieve when the experimental subject is well grounded and electrodes are repositionable. It is considerably harder to achieve in recordings from human epilepsy patients which use electrodes fixed to the skull and where the patient is not well grounded. Both factors create electrically noisy recordings with a low signal to noise ratio (~2-3).
Prior studies of spike sorting (detection followed by clustering) have generally focused on either detection or clustering separately. In the context of human extracellular microwire recordings, Wild et al. (2012) recently compared the effects of varying parameters of the clustering algorithm and showed substantial variability in the optimal parameters depending on both noise level, the shape of action potential waveform, and the number of nearby neurons. These studies used a set of fixed waveforms for comparison which thereby excludes effects of differences in thresholds used to detect action potential waveforms. Here I introduce a method, based on calculation of the adjusted mutual information (AMI) between two spike sorting outputs, which can be used to compare the results of the entire process of spike sorting, including filtering, detection, and clustering.
When used to compare the results of sorting simulated neural firing into single neuron activity, this method shows that the threshold used to detect events has a significant impact on the ability to accurately separate single neuron activity; indeed, raising the threshold for detection can often cause a decrease in the AMI between the known (simulated) and sorted activity, even when the threshold remains below the peak value of the simulated waveform. Variations in other parameters of the sorting algorithm, such as the features used, also cause significant variations in the AMI and indicate variations in the quality of spike sorting achieved.

This method based on AMI is general and can be used to compare outputs of entire spike sorting algorithms even when they differ in the types of filtering applied, methods of spike detection, and methods of grouping waveform events into clusters of putative single neuron activity.

Disclosures: P.N. Steinmetz: None.

Nanosymposium

116. Techniques in Electrophysiological Recording and Stimulation

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Topic: I.04. Physiological Methods

Support: This work was supported by the German Research Foundation – Priority Program SPP1665

Title: Towards an implantable integrated neural recording system for neonatal mice.

Authors: *A. BAHR, L. ABU SALEH, D. SCHROEDER, W. H. KRAUTSCHNEIDER; Inst. of Nano- and Med. Electronics, Hamburg Univ. of Technol., Hamburg, Germany

Abstract: The recording of neural signals from the brain of neonatal mice is of interest for the understanding of the functionality of the brain and its development processes. Treatments for certain forms of neural diseases like epilepsy are investigated with the help of neural recordings from mice [Marguet, Nature Medicine 21, 1436-1444, 2015]. For a systematic investigation of these processes, long term neural recordings of neonatal mice are needed. Neonatal mice have a weight of only 3 - 5 grams; commercially available recording systems are much too heavy and large for this application. To overcome this, an implantable neural recording system is proposed that can be placed inside a neonatal mouse (back, neck and head) and record Low Field Potentials (LFP) and Action Potentials (AP) from the brain. The system is connected via a connector at the back of the mouse to a data acquisition system. The miniaturized system consists of a Neuronexus silicone probe, a custom designed Application Specific Integrated Circuit (ASIC) and a connector (Omnetics). The analog signals are recorded, digitized in close proximity to the brain and transmitted via a digital interface. The components are placed on flexible substrate which provides a better handling for the surgeon. The integrated circuit has a size of only 1.5 x 1.5 mm². It is wire-bonded onto the substrate, the analog inputs are bonded directly to the electrode to reduce the wiring and minimize the exposure to noise for the sensitive analogue signals. The ASIC is designed in a 130 nm CMOS technology, comprises 16 analog channels, preamplifiers, multiplexer, a post amplifier with switchable gain and a 10 bit SAR ADC. The analog signals are digitized with 20 kSample/s/channel and transmitted via an SPI

Interface [Bahr, BIOSTEC, V1 263-269, Italy, 2016]. The ASIC was successfully implemented and used in a neural recording from a mouse (Bahr, BMT, 2016). A version with increased bandwidth (0.1 Hz to 10 kHz) has been designed. A prototype is constructed using wire-bonding, the ASIC is bonded onto the PCB, the electrode is connected via an Omnetics connector. The size of the ASIC is small enough to fit into the long term neural acquisition system. This shows the suitability of the proposed design. The results show that the custom designed ASIC is suitable for the proposed system. The used advanced 130 nm CMOS technology is suitable for mixed signal and analog design implementation for biomedical signal acquisition. The technology is power efficient and it enables the required size reduction for the implantable system. With these results it could be shown that the proposed neural recording system can be realized for long term acquisition from neonatal mice.

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Nanosymposium

116. Techniques in Electrophysiological Recording and Stimulation

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Presentation Number: 116.03

Topic: I.04. Physiological Methods

Support: NIH-R01MH092926

Title: Direct current stimulation modulates LTP and LTD: activity-dependence and dendritic effects

Authors: *G. KRONBERG[1], M. BRIDI[2], T. ABEL[2], L. C. PARRA[1];
[1]Biomed. Engin., The City Col. of New York, New York, NY; [2]Biol., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Transcranial direct current stimulation (tDCS) has been reported to improve various forms of learning in humans. Stimulation is often applied during training, producing lasting enhancements that are specific to the learned task. These learning effects are thought be mediated by altered synaptic plasticity. However, the effects of DCS during the induction of endogenous synaptic plasticity remain largely unexplored. To model endogenous plasticity we induced long-term potentiation (LTP) and depression (LTD) at Schaffer collateral synapses in CA1 of rat hippocampal slices. When induction was paired with concurrent DCS, the resulting plasticity was biased towards potentiation, such that LTP was enhanced and LTD was reduced. Remarkably, both anodal and cathodal stimulation can produce these effects, depending on the

dendritic location of plasticity induction. DCS did not affect synapses that were weakly active or when NMDA receptors were blocked, suggesting a sensitivity for active synapses that are already undergoing endogenous plasticity. These results highlight the role of DCS as a modulator, rather than inducer of synaptic plasticity, as well as the complex dependence of DCS effects on the spatial and temporal properties of endogenous synaptic activity.

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Nanosymposium

116. Techniques in Electrophysiological Recording and Stimulation

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Presentation Number: 116.04

Topic: I.04. Physiological Methods

Title: Neurophysiological investigation of resting state functional connectivity in the rat striatum--concurrent multi-channel electrophysiological recording and fMRI

Authors: S. JAIME[1], H. GU[2], E. A. STEIN[2], J. E. CAVAZOS[1], Y. YANG[2], *H. LU[2];
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Abstract: Spontaneous fluctuations in fMRI BOLD signal appear to index a fundamental feature of brain physiology. These fluctuations exhibit distinct spatial and temporal patterns, forming large-scale brain networks. Converging data suggest that alterations in brain networks are implicated in a number of neuropsychiatric diseases. However, the neurophysiological basis of BOLD fluctuations remains poorly understood, underscoring a critical need for well controlled animal models to investigate this phenomenon.
The rat striatum is a key structure involved in adaptive learning. It is the major projection target of midbrain dopamine neurons. In the present study, we developed a concurrent fMRI-electrophysiological recording technique and performed chronic repetitive recordings with microelectrode arrays (16-channel) covering the striatum from its dorsal lateral to the medial ventral domains. By agonizing AMPA receptors within the ventral tegmental area (VTA) micro-circuitry, we systematically modulated striatal dopamine release and neuronal activity. Experiments were performed on 10 rats anesthetized with low dose of isoflurane+dexmedetomidine. Artifacts resulting from MR gradient pulses were corrected using an in-house method. We found strong phase-amplitude coupling (PAC) in the LFP signal, with the phase of LFP at 1-4 Hz significantly modulating the amplitude of higher frequency LFPs (8-50 Hz and >70 Hz). The PAC effect was significantly reduced after VTA AMPA microinjection (1 μl, 100 mM). The LFP signal was further filtered into classical frequency bands (σ:1-4; θ: 5-8;

α:9-14, β:15-30; γ:30-50; and hγ: >70 Hz). The band-limited power time course was calculated, down-sampled and correlated with simultaneously recorded BOLD signal. Results revealed that σ band LFP power was negatively correlated with BOLD fluctuations; while β and γ band LFP power was positively correlated with BOLD fluctuations, with minimal LFP-BOLD correlation in the θ and α band. VTA AMPA microinjection significantly modulated σ band LFP-BOLD correlation in the ventral and middle portion of the striatum; a similar modulation was seen in the BOLD functional connectivity. However, the effect of AMPA modulation on LFP-BOLD correlation in the β and γ bands were seen in voxels outside of the striatum and did not overlap with the effect seen in BOLD functional connectivity.
Our data demonstrate unambiguously the pivotal role of low frequency field potentials underlying spontaneous BOLD fluctuations. We propose that PAC serves as the integral neural mechanism that modulates β and γ bands LFP-BOLD correlation in resting state fMRI.
Acknowledgement: Supported by NIDA-IRP and UTHSCSA.

Disclosures: **S. Jaime:** None. **H. Gu:** None. **E.A. Stein:** None. **J.E. Cavazos:** None. **Y. Yang:** None. **H. Lu:** None.

Nanosymposium

116. Techniques in Electrophysiological Recording and Stimulation

Location: SDCC 7B

Time: Sunday, November 13, 2016, 8:00 AM - 10:00 AM

Presentation Number: 116.05

Topic: I.04. Physiological Methods

Support: NIH RO1 RO1MH101218

ARO W911NF-12-1-0594

Title: Targeted intracellular recordings from deep-layer cortical neurons *In vivo* using nano-pipettes

Authors: ***K. JAYANT**[1], M. WENZEL[2], J. P. HAMM[1], Y. BANDO[2], O. SAHIN[2], K. L. SHEPARD[3], R. M. YUSTE[2];
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Abstract: Targeted intracellular electrophysiology *in vivo*, from identified cells across the cortical column can shed light on a) how single neurons integrate the thousands of synaptic inputs they receive, and b) elucidate the underlying signal processing motifs between different cell types. Yet, targeted intracellular recordings from pre-labeled neurons *in vivo* have traditionally been restricted to superficial cortical layers, specifically layer 2/3. This is in part due

to the poor visualization of target cells and the recording patch pipette in deep scattering tissue. Further, targeted whole cell patch-clamping of cells in the deep cortex requires either excessive laser power or dye perfusion to assist in visualization and guidance. Here, we introduce microprism-assisted targeted intracellular recordings in the deep cortex, using quantum-dot (QD) coated nano-pipettes (tip diameters 15nm - 30nm). QD's have a high two-photon absorption cross-section and are less prone to bleaching which makes them ideal labels for deep-layer *in vivo* studies. The use of nano-pipettes facilitate recordings of action potentials and sub-threshold potentials over extended durations, without intracellular washout and re-usability in the same experimental run, even in the same cell. Signals attenuated due to the high impedance nature of the electrode-membrane interface are easily deconvolved offline to reveal full recovery. Using this approach we demonstrate targeted intracellular recordings from layer V pyramidal cells and parvalbumin positive interneurons, up to a depth of 800-900μm. Moreover, intracellular measurements from deep-layer pyramidal cells were combined with simultaneous two-photon Ca^{2+} imaging of the targeted cell and neighboring neurons through the microprism. Our method allows for precise visual guidance of electrodes to labeled neurons in the deep cortex (limited only by vertical prism dimension) with minimal incident laser power paving the way for high-throughput targeted *in vivo* electrophysiology.
Funding support: NIH RO1 R01MH101218 and ARO W911NF-12-1-0594 (MURI).

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Nanosymposium

116. Techniques in Electrophysiological Recording and Stimulation

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NIH Director's Pioneer Award 1DP1NS087724

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MIT Neurotechnology Fund

Title: Noninvasive deep brain stimulation via delivery of temporally interfering electric fields

Authors: ***N. GROSSMAN**[1,2,9,3], D. BONO[4], S. KODANDARAMAIAH[2,10], A. RUDENKO[5,11], A. CASSARA[12], E. NEUFELD[12], S. A. ANTERAPER[3], A. TAKAHASHI[3], N. KUSTER[12], L.-

H. TSAI[5,13], A. PASCUAL-LEONE[9], E. S. BOYDEN[2,3,6,7,8];
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Abstract: Electrical brain stimulation is a key technique in research and clinical neuroscience studies, and also is in increasingly widespread use from a therapeutic standpoint. However, to date all methods of electrical stimulation of the brain either require surgery to implant an electrode at a defined site, or involve the application of non-focal electric fields to large fractions of the brain. We here report a strategy for sculpting the amplitude of electric fields so as to enable focal, yet noninvasive, electrical neural stimulation. By delivering multiple electric fields to the brain at slightly different frequencies that are themselves too high to recruit effective neural firing, but for which the difference frequency is low enough to drive neural activity, we can cause neurons to be electrically activated at a focus without driving neighboring or overlying regions. We call this method temporal interference (TI) stimulation, since the interference of multiple electric fields is what enables the focality, since only the region for which the amplitude of the envelope at the difference frequency is high will experience neurally relevant frequencies of electric field. We modeled the concept using finite element methods in order to develop principles of how to sculpt fields in 3-D in the brain. We validated that neurons in the living mouse brain could follow the difference frequency electric field, but would not be entrained by the high frequency fields themselves. We further validated, in living mice, using c-fos labeling, that we could stimulate a deep region (e.g., hippocampus) without any stimulation of overlying cortex, via TI stimulation with a difference frequency of 10 Hz; in contrast, simply stimulating with 10 Hz transcranial alternating current stimulation resulted in significant cortical c-fos labeling in regions overlying the activated hippocampus. Finally, we explored the steerability of TI stimulation in the human brain as reflected by fMRI imaging of BOLD signals as we varied the sites and patterns of electric fields applied to specific electrodes on the scalp. TI stimulation may represent a new method of brain stimulation using familiar and well-tested electric fields, but able to achieve focal stimulation without the need for neurosurgery.

Disclosures: **N. Grossman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); N.G is a cofounder of a medical device company. N.G. is inventor on patent applications describing methods and devices for noninvasive brain stimulation. **D. Bono:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); D.B. is inventor on patent applications describing methods and devices for noninvasive brain stimulation.. **S. Kodandaramaiah:** None. **A. Rudenko:** None. **A. Cassara:** None. **E. Neufeld:** None. **S.A. Anteraper:** None. **A. Takahashi:** None. **N. Kuster:** None. **L. Tsai:** None. **A. Pascual-Leone:** None. **E.S. Boyden:** E. Ownership Interest (stock, stock

options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); E.S.B. is inventor on patent applications describing methods and devices for brain stimulation.

Nanosymposium

116. Techniques in Electrophysiological Recording and Stimulation

Location: SDCC 7B

Time: Sunday, November 13, 2016, 8:00 AM - 10:00 AM

Presentation Number: 116.07

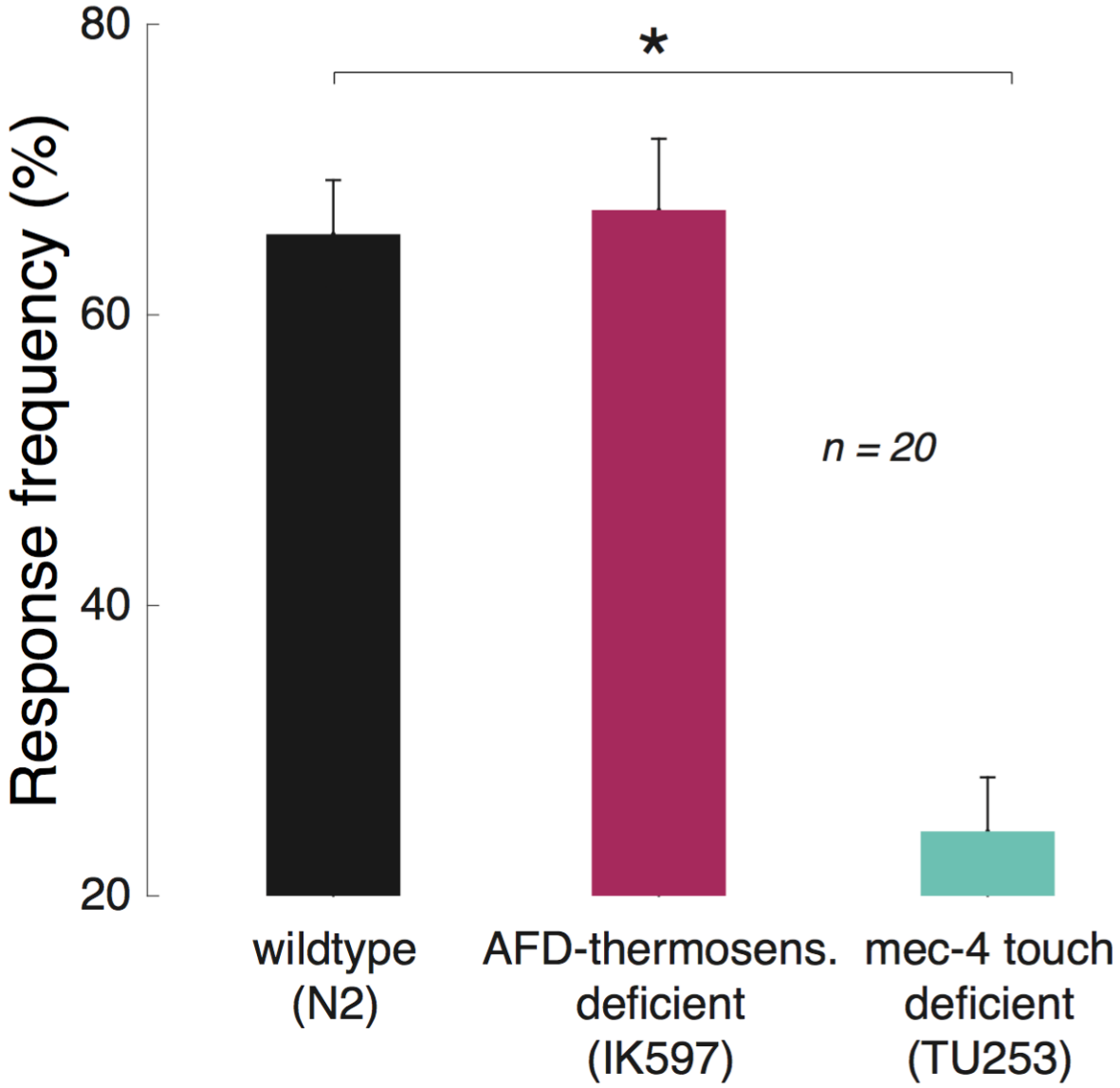
Topic: I.04. Physiological Methods

Support: Big Ideas, Neurocircuit, Stanford Neurosciences Institute

Title: Biophysical and cellular basis of ultrasonic neuromodulation investigated using *C. elegans*

Authors: *J. KUBANEK[1], S. BACCUS[2], M. GOODMAN[3];
[1]Stanford Univ. Sch. of Med., Stanford, CA; [2]Neurobio., [3]Cell. and Mol. Physiol., Stanford Univ., Stanford, CA

Abstract: Transcranial ultrasound stimulates neurons in humans and other vertebrates. Because ultrasound can be focused and propagates deep into the brain, it has the potential to non-invasively stimulate specific brain regions in living animals and humans.
However, little is currently understood about the biophysical mechanism by which ultrasound stimulates neurons. Even less is known about the molecules that might render some neurons more sensitive than others. In principle, ultrasonic stimulation can produce heat or mechanical force and either of these physical stimuli could modulate neural activity.
We, and others (Ibsen et al., Nat Commun 2015, doi:10.1038/ncomms9264) are working to fill this critical knowledge gap by harnessing the power of the genetic toolset developed in C. elegans nematodes. We will present evidence that focusing ultrasound on wild type animals without additional manipulations such as the addition of microbubbles evokes robust reversals and that reversal probability increases with the power applied. This response appears to depend on the six gentle touch receptor neurons, since mutants that lack these sensory neurons have impaired behavioral responses to ultrasound. Loss of a DEG/ENaC sodium channel critical for normal touch sensation likewise decreases sensitivity to ultrasound. Meanwhile, mutants lacking a trio of thermosensitive receptors responsible for the worm's ability to detect tiny thermal fluctuations behaved more like wild type animals.
Collectively, these results suggest that in regard to neuronal excitability, ultrasound-derived mechanical forces are more salient than heating, and that mechanosensitive cation channels can sensitize neurons to ultrasound.



Disclosures: **J. Kubanek:** None. **S. Baccus:** None. **M. Goodman:** None.

Nanosymposium

116. Techniques in Electrophysiological Recording and Stimulation

Location: SDCC 7B

Time: Sunday, November 13, 2016, 8:00 AM - 10:00 AM

Presentation Number: 116.08

Topic: I.04. Physiological Methods

Support: PolyU grants 1-YW0Q and 1-ZVGK

Title: Control of neuron activity by sonogenetics

Authors: *Z. QIU[1], J. GUO[2], L. YANG[1], Y. YANG[1], Y. HUANG[1], S. KALA[1], H. CHAN[2], L. SUN[1];
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Abstract: Ultrasonic brain stimulation, the use of ultrasonic waves to manipulate the activity of specific brain region, has been demonstrated to noninvasively alter neuron activity in animals and humans. Capable of non-invasive transmission trough skull with fine focal size, ultrasonic brain stimulation is an encouraging means and a good alternative to existing stimulating strategies (e.g. deep brain stimulation, optogenetics, transcranial magnetic stimulation, and transcranial direct current stimulation), with the advantages of non-invasiveness, fine spatial control, and deeper tissue penetration. However, the mechanism underlying remains investigation. It has been hypothesized that that ultrasound can gate mechano-sensitive ion channels directly and thus it is able to develop sonogenetics which is analogous to optogenetics. To test this hypothesis, the GFP tagged mechanosensitive ion channels Piezo 1 which are shown to be gated by mechanical stimuli, are expressed in 293T and primary cortical neurons. The well characterized mechanosensitive ion channels MscL (~6 mN/m for 50% open probability) are expressed as well serving as calibrator. Ultrasound (0.5, 1, 2, 4, 8 and 20MHz, <5W/cm^2) with defined acoustic field is delivered to the cells for stimulation. Whole cell patch-clamp, calcium imaging are utilized to evaluate the cell responses.
Our results show that Piezo 1 and MscL can be expressed in neurons. These channels can be gated by ultrasound and induce inward current and calcium influx depending on ultrasound intensity both in 293T and primary cortical neurons. Calcium imaging showed that the calcium spiking rates of Piezo 1 and MscL expressed neurons upon ultrasound stimulation are higher than the wild type neurons. The effects were reversible with repeated stimulation. In Piezo 1 expressed neurons, the effects can be partially blocked by GsMTx-4 which is known as a Piezo 1 blocker. The effects of different parameters on the stimulation have also been determined. These results show that ultrasound is able to modulate neuronal activity by gating mechanosensitive ion channels. It demonstrates reliable control of neuron activity with sonogenetics.

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Nanosymposium

198. Adult Neurogenesis During Aging of the Neural Stem Cell Niche

Location: SDCC 25A

Time: Sunday, November 13, 2016, 1:00 PM - 3:30 PM

Presentation Number: 198.01

Topic: A.04. Transplantation and Regeneration

Support: Swiss National Science Foundation

EMBO Young Investigator Program

European Research Council

Human Frontiers Science Program Long-term Fellowship

EMBO Post-doctoral fellowship

Title: A role for nuclear envelope proteins in mammalian neural stem cell aging

Authors: *D. L. MOORE[1], G. A. PILZ[1], M. K. BIN IMTIAZ[1], Y. BARRAL[2], S. JESSBERGER[1];
[1]Univ. of Zurich, Brain Res. Inst., Zürich, Switzerland; [2]Inst. of Biochem., ETH Zurich, Zurich, Switzerland

Abstract: Adult neurogenesis in the brain occurs throughout life, decreasing with age. We have found that neural stem cells (NSCs) *in vitro* and in embryonic brain slices *in situ* generate a lateral diffusion barrier in the endoplasmic reticulum membrane during mitosis. This diffusion barrier weakens with age, accompanied by a disruption of an asymmetric segregation of damaged proteins between daughter cells. In both embryonic and young adult brain NSC divisions, damaged proteins are asymmetrically inherited by the non-stem daughter cell, whereas in the old adult brain, damage is more symmetrically distributed between progeny, further supporting a weakening of the diffusion barrier with age. Overexpression of the lamin A mutant protein Progerin phenocopies the weakened diffusion barrier, the symmetric segregation of cargoes, and the reduction in proliferation as seen in old NSCs. Interestingly, multiple lamin-binding proteins of the nuclear envelope (NE) are regulated with age, and knockdown of these proteins dramatically affects NSC proliferation rate. These results suggest a novel role for NE proteins in NSC aging, and further identify potential therapeutic targets in the age-dependent decrease in adult neurogenesis.

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Nanosymposium

198. Adult Neurogenesis During Aging of the Neural Stem Cell Niche

Location: SDCC 25A

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Presentation Number: 198.02

Topic: A.04. Transplantation and Regeneration

Support: NIA Grant R01AG041861-01

Title: Peaks and valleys in cell behavior and gene expression during aging of the neural stem cell niche

Authors: *M. APOSTOLOPOULOU[1], T. KIEHL[1], M. WINTER[2], E. CARDENAS[2], S. GODERIE[1], Y. WANG[1], A. COHEN[2], S. TEMPLE[1];
[1]Neural Stem Cell Inst., Rensselaer, NY; [2]Drexel Univ., Philadelphia, PA

Abstract: Neurogenesis in the subventricular zone (SVZ) decreases markedly with aging, thought to occur by a unidirectional decline. However, after conducting an unbiased analysis of SVZ transcriptome at 2, 6, 18 and 22 months, we found that in addition to trends following age, numerous genes had surprising maximal or minimal expression at 18 months, including progenitor and cell cycle-related genes. In vivo, transit amplifying Type C cell number and proliferation also exhibit a nadir at 18 months. We then followed the proliferation and differentiation of isolated SVZ cells in vitro. Lineage analysis of 944 clones showed that age-related declines in neurogenesis were recapitulated. Moreover, even after isolation from the niche, Type C cells show U-shaped cell proliferation rates with age. Our findings indicate that age-related changes in the SVZ are not only monotonic but can exhibit peaks and valleys, and that programmed changes in progenitors are key drivers of neurogenic aging.

Disclosures: **M. Apostolopoulou:** A. Employment/Salary (full or part-time): Rensselaer Polytechnic Institute, Neural Stem Cell Institute. 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; Department of Defense, National Institute of Aging. **T. Kiehl:** A. Employment/Salary (full or part-time): Neural Stem Cell Institute. 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; National Institute of Aging. **M. Winter:** A. Employment/Salary (full or part-time): Drexel University. 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; National Institute of Aging. **E. Cardenas:** A. Employment/Salary (full or part-time): Drexel University. B. Contracted Research/Research Grant (principal investigator for

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Nanosymposium

198. Adult Neurogenesis During Aging of the Neural Stem Cell Niche

Location: SDCC 25A

Time: Sunday, November 13, 2016, 1:00 PM - 3:30 PM

Presentation Number: 198.03

Topic: A.04. Transplantation and Regeneration

Support: US National Institutes of Health Biobehavioral Research Awards for Innovative New Scientists (BRAINS) 1-R01MH104175 (AS)

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Title: Modulating competition dynamics in the dentate gyrus to rejuvenate memory circuits in adulthood and aging

Authors: *K. M. MCAVOY[1,3,4], K. N. SCOBIE[5], S. BERGER[6], C. RUSSO[1], P. DECHARATANACHART[1], N. GUO[1], H. VEGA-RAMIREZ[1], S. MIAKE-LYE[1], M. WHALEN[2], M. NELSON[7], M. BERGAMI[8], R. HEN[5], D. BARTSCH[6], B. BERNINGER[9], A. SAHAY[1,3,4];
[1]Ctr. for Regenerative Med., [2]Neurosci. Ctr., Massachusetts Gen. Hosp., Boston, MA; [3]Harvard Stem Cell Inst., Harvard Univ., Cambridge, MA; [4]Psychiatry, Harvard Med. Sch., Boston, MA; [5]neuroscience, Columbia University, NY, USA, New york, NY; [6]Zentralinstitut für Seelische Gesundheit, Heidelberg Univ., Mannheim, Germany; [7]Echelon Biosci., Salt Lake City, UT; [8]Cologne Excellence Cluster on Cell. Stress Responses in Aging-Associated Dis. (CECAD), Univ. Hosp. of Cologne, Cologne, Germany; [9]Focus Program Translational Neurosci., Johannes Gutenberg Univ., Mainz, Germany

Abstract: Although the effects of neural activity on adult hippocampal neurogenesis are well recognized, the circuit mechanisms governing neural stem cell (NSC) activation and integration of adult-born dentate granule cells (DGCs) in the dentate gyrus (DG) are poorly understood. Adult-born DGCs are thought to compete with mature DGCs for cortical inputs to integrate and survive. To ascertain how changes in inputs of mature DGC affects NSCs and neuronal competition, we engineered a genetic system to reversibly eliminate a subset of dendritic spines in mature DGCs. Overexpression of a transcriptional negative regulator of dendritic spines, Kruppel-like factor 9 (Klf9), in mature DGCs eliminated a subset of their dendritic spines and rapidly enhanced NSC activation and integration of adult-born DGCs without affecting olfactory bulb neurogenesis. Restoration of Klf9 levels in mature DGCs reversed the competitive advantage of adult-born DGCs and NSC activation while modifying the DG with an expanded cohort of age-matched adult-born DGCs. Importantly, an independent strategy targeting *Rac1* to eliminate spines in mature DGCs also increased the number of adult-born DGCs. Retroviral based rabies dependent mono-synaptic retrograde tracing revealed that enhanced integration of adult-born DGCs transiently reorganized local hilar afferent connectivity. Enhancing integration of adult-born DGCs by modulation of competition in adulthood and during aging promoted contextual discrimination and reversal learning. Mice with more adult-born DGCs exhibited enhanced global remapping, a population based coding mechanism that supports pattern separation, when exposed to two similar, but not distinct, contexts, in dorsal and ventral DG. Pharmacological occlusion of the enhancement in neurogenesis in these mice reversed global remapping improvements, thereby casually linking levels of adult hippocampal neurogenesis with efficiency of global remapping in the DG. Together, these observations begin to instantiate circuit mechanisms underlying the integration and functions of adult-born DGCs, while demonstrating how these mechanisms maybe harnessed to enhance adult hippocampal neurogenesis, population based coding in DG, and memory precision in adulthood and during aging.

Disclosures: K.M. McAvoy: None. **K.N. Scobie:** None. **S. Berger:** None. **C. Russo:** None. **P. Decharatanachart:** None. **N. Guo:** None. **H. Vega-Ramirez:** None. **S. Miake-Lye:** None. **M. Whalen:** None. **M. Nelson:** None. **M. Bergami:** None. **R. Hen:** None. **D. Bartsch:** None. **B. Berninger:** None. **A. Sahay:** None.

Nanosymposium

198. Adult Neurogenesis During Aging of the Neural Stem Cell Niche

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Time: Sunday, November 13, 2016, 1:00 PM - 3:30 PM

Presentation Number: 198.04

Topic: A.04. Transplantation and Regeneration

Support: NIH R01-AG041861

Ellison Medical Foundation AG-SS-2655-11

Title: The role of the choroid plexus in neurogenesis in the aging subventricular zone

Authors: *C. S. BJORNSSON[1], K. O'KEEFE[2], M. APOSTOLOPOULOU[1], Y. WANG[1], T. R. KIEHL[1], S. TEMPLE[1];
[1]Neural Stem Cell Inst., Rensselaer, NY; [2]Biol., SUNY Albany, Albany, NY

Abstract: Neurogenesis persists into adulthood in specialized niche environments. One of these, the subventricular zone (SVZ), is situated between vascular and cerebrospinal fluid (CSF) compartments, each contributing extrinsic factors that regulate neural stem cell (NSC) dynamics. The choroid plexuses (ChP) are a rich source of secreted growth factors and cytokines known to affect adult neurogenesis; the lateral ventricle ChPs are immediate neighbors of the SVZ. The ChP undergo a dramatic reduction in CSF production and protein synthesis with age. Neurogenesis similarly diminishes with age, leading to fewer new olfactory bulb interneurons and deficits in fine olfactory discrimination. Multiplex-immunolabeled SVZ whole mounts imaged using confocal microscopy capture population and 3-D architectural changes across the entire aged SVZ niche.
We are interested in how ChP-derived factors impact and contribute to age-related changes in niche architecture and neurogenic output. Other groups have shown previously that intraventricular delivery of growth factors known to be secreted by the ChP, including EGF and FGF2, can partially revitalize neurogenesis *in vivo* in aged mice. Time lapse imaging of SVZ cells seeded at clonal densities and cocultured with explanted ChPs demonstrate a significant increase in NPC proliferation and motility, expanding the pool of activated Type B progenitor cells with a reduction in neuroblast production. Heterochronic and isochronic combinations of ChP explants and SVZ cells suggest this effect is enhanced when young ChP is cocultured with old SVZ cells when compared to old ChP paired with old SVZ. Conditioned media analysis identified elevated levels of several factors that may contribute to these effects. Transcriptome analysis of paired ChP and SVZ samples has revealed a number of interesting age-associated changes in ChP-secreted factors that may affect SVZ NPCs directly, as well as others that may act through alterations in their niche environment. Efforts to characterize the influence of candidate factors on aged SVZ neurogenesis using a high-throughput compound screening assay are currently underway.

Disclosures: **C.S. Bjornsson:** None. **K. O'Keefe:** None. **M. Apostolopoulou:** None. **Y. Wang:** None. **T.R. Kiehl:** None. **S. Temple:** None.

Nanosymposium

198. Adult Neurogenesis During Aging of the Neural Stem Cell Niche

Location: SDCC 25A

Time: Sunday, November 13, 2016, 1:00 PM - 3:30 PM

Presentation Number: 198.05

Topic: A.04. Transplantation and Regeneration

Support: NIH grant NS080913

Title: Origins of adult neurogenesis decline

Authors: ***M. A. BONAGUIDI**[1], A. IBRAYEVA[1], E. PU[1], T. KRIEGER[2], R. STADEL[3], D. BERG[3], G.-L. MING[3], B. SIMONS[2], H. SONG[3];
[1]Stem Cell Biol. & Regenerative Med., USC, Los Angeles, CA; [2]Cambridge Univ., Cambridge, United Kingdom; [3]Johns Hopkins Univ., Baltimore, MD

Abstract: Neuron production in the adult hippocampus drops during aging of unclear cellular sources. We present on neural stem cell diversity, homeostasis, plasticity and underlying mechanisms that collectively contribute to physiological neurogenesis changes.

Disclosures: **M.A. Bonaguidi:** None. **A. Ibrayeva:** None. **E. Pu:** None. **T. Krieger:** None. **R. Stadel:** None. **D. Berg:** None. **G. Ming:** None. **B. Simons:** None. **H. Song:** None.

Nanosymposium

198. Adult Neurogenesis During Aging of the Neural Stem Cell Niche

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Time: Sunday, November 13, 2016, 1:00 PM - 3:30 PM

Presentation Number: 198.06

Topic: A.04. Transplantation and Regeneration

Support: CIHR MOP86600

Title: Neural stem cell proliferation is suppressed by aberrant niche fatty acid metabolism in an animal model of Alzheimer's disease

Authors: *K. J. FERNANDES[1], L. K. HAMILTON[2], M. DUFRESNE[3], S. E. JOPPÉ[3], S. PETRYSZYN, G1V 4G2[4], A. AUMONT[3], F. CALON[4], F. BARNABÉ-HEIDER[5], A. FURTOS[3], M. PARENT[4], P. CHAURAND[3];
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Abstract: It is well established that adult neurogenesis declines in the adult and aging brain, and neurogenic dysfunction is likewise a common feature in a number of neurodegenerative diseases, including Alzheimer's disease (AD). Here, we asked whether disturbances in lipid metabolism, which has been increasingly implicated in neural stem cell (NSC) regulation under physiological conditions, may be involved in age- and AD-associated declines in adult neurogenesis. We show that deposits of Oil Red O-positive neutral lipids accumulate within the aging subventricular zone (SVZ) NSC niche, and that this process is strongly enhanced in the 3xTg model of AD. These increasing lipid accumulations are already detectable by 2-3 months of age in 3xTg-AD mice, coincident with declining SVZ neurogenesis and prior to the reported onset of cognitive deficits in learning and memory. Using electron microscopy, these lipid accumulations were identified as membrane-bound lipid droplets, intracellular stores of neutral lipids. Notably, large lipid droplets were specific to ependymal cells, the principle support cells of the SVZ niche. Imaging Mass Spectrometry (IMS) revealed a panel of 12 oleic acid (OA)-rich triglycerides that were up to 30-fold more abundant in the SVZ of 3xTg-AD mice compared to strain controls. In vitro and in vivo studies were then performed to explore the potential impact of elevated OA on NSC activity. 3xTg-AD SVZ explants and explant-conditioned medium both suppressed growth of wild-type neurospheres, indicating the presence of inhibitory paracrine signals within the 3xTg-AD SVZ. Moreover, exogenous OA i) directly suppressed NSC proliferation *in vitro*, and ii) inhibited AraC-induced activation of SVZ NSCs *in vivo*. These experiments are consistent with an inhibitory paracrine effect of accumulating ependymal OA on NSCs. Microarray analyses revealed substantial changes in lipid metabolism-related gene expression within the 3xTg-AD SVZ. Among the genetic alterations observed was up-regulation of stearoyl-CoA-desaturase (SCD1), the rate-limiting enzyme in production of OA and its downstream unsaturated fatty acids. By pharmacologically inhibiting SCD1 activity, levels of OA-rich triglycerides were reduced in 3xTg-AD mice, and this resulted in a rescue of NSC activity in both the SVZ and dentate gyrus neurogenic niches. These studies collectively support a model in which AD-induced perturbation of niche fatty acid metabolism suppresses NSC proliferation and results in an accelerated aging-associated decline in adult neurogenesis.

Disclosures: K.J. Fernandes: None. **L.K. Hamilton:** None. **M. Dufresne:** None. **S.E. Joppé:** None. **S. Petryszyn:** None. **A. Aumont:** None. **F. Calon:** None. **F. Barnabé-Heider:** None. **A. Furtos:** None. **M. Parent:** None. **P. Chaurand:** None.

Nanosymposium

198. Adult Neurogenesis During Aging of the Neural Stem Cell Niche

Location: SDCC 25A

Time: Sunday, November 13, 2016, 1:00 PM - 3:30 PM

Presentation Number: 198.07

Topic: A.04. Transplantation and Regeneration

Support: Arizona Biomedical Research Commission (ADHS14-082982)

McKnight Brain Research Foundation

The University of Arizona Intramural Funds

Title: Targeting the Nrf2 pathway to improve neural stem cell function with age

Authors: *L. MADHAVAN[1], M. J. CORENBLUM[1], S. RAY[2], M. LONG[3], B. HARDER[3], D. D. ZHANG[3], C. A. BARNES[4];
[1]Neurol., [2]Undergraduate Biol. Res. Program, [3]Pharmacol. and Toxicology, [4]Psychology and Neurosci., Univ. of Arizona, Tucson, AZ

Abstract: Our recent studies have systematically examined the function of subventricular (SVZ) zone neural stem and progenitor cells (NSPCs) during aging. This work indicates that although NSPC function continuously declines with advancing age, there is a critical time period during middle-age when a prominent reduction in NSPC survival and regeneration, and associated behavioral (fine olfactory discrimination) function occurs. We also find that this specific temporal pattern of NSPC deterioration is correlates with the decreasing expression of the redox-sensitive transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2), and its target gene glutamate-cysteine ligase modifier subunit (GCLM), in the NSPCs. Decreasing or increasing Nrf2 expression, via short interfering RNAs or overexpression assays, in young and old NSPCs, respectively, was sufficient to significantly deter or improve the survival and proliferation of these cells. Furthermore, analysis of young Nrf2 knock-out (Nrf2 -/-) mice revealed the proliferative capacity of the NSPCs and their ability to produce new neurons, as well as their fine olfactory discrimination ability, was also notably compromised in these animals as compared to age-matched wild type control animals. Given these data, we generated recombinant adeno-associated viral (rAAV) vectors carrying Nrf2 and administered them into the SVZs of aging rats. Preliminary results indicate that animals receiving rAAV-Nrf2-eGFP have improved fine olfactory discrimination ability in comparison to animals receiving control rAAV-eGFP vectors. We are also performing additional behavioral and histological analyses to further address the effects of increased Nrf2 on NSPC function. These data support Nrf2 pathway modulation as a way to improve aged NSPC function, and have important implications with respect to developing stem cell-based strategies to support healthy aging and to treat age-related neurological disorders.

Disclosures: **L. Madhavan:** None. **M.J. Corenblum:** None. **S. Ray:** None. **M. Long:** None. **B. Harder:** None. **D.D. Zhang:** None. **C.A. Barnes:** None.

Nanosymposium

198. Adult Neurogenesis During Aging of the Neural Stem Cell Niche

Location: SDCC 25A

Time: Sunday, November 13, 2016, 1:00 PM - 3:30 PM

Presentation Number: 198.08

Topic: A.04. Transplantation and Regeneration

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William and Ella Owens Medical Research Foundation

Title: Niche microglia contribute to neural stem cell aging

Authors: ***E. KOKOVAY**[1], R. SOLANO-FONSEA[2], J. O'CONNOR[3], A. CARDONA[4], S. MAHESULA[2,4];
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Abstract: The ventricular-subventricular zone (V-SVZ) is the largest neural stem cell (NSC) reservoir in the mammalian forebrain. NSCs generate neurons and glia throughout life and are mediators of plasticity and repair in the CNS. However, NSC proliferation and neurogenesis is sharply reduced at mid-age through unknown mechanisms. Our studies establish microglia, the resident immune cells in the brain, as integral V-SVZ niche cells that are closely associated with NSCs, germinal pinwheels and the microvasculature. During aging, microglia undergo substantial positional changes within the niche, losing their close association to the vasculature while becoming increasingly associated with the ependyma and germinal pinwheels. We observed an early and chronic activation of V-SVZ microglia that was not reciprocated outside of germinal niche. Microglia activation resulted in increased inflammatory mediators within the NSC compartment. A substantial increase of monocyte infiltration was observed within the aged V-SVZ niche, suggesting the peripheral immune system may also mediate V-SVZ inflammation during aging. Induction of sustained inflammation in young mice recapitulated microglia activation and reduced proliferation observed in the aging V-SVZ. In vitro studies revealed secreted factors from activated microglia reduced proliferation and neuron production compared to secreted factors from resting microglia. Furthermore, minocycline treatment in aged mice reduced microglia activation, niche inflammation and partially restores proliferation in the aged niche. Our results suggest that age-associated chronic inflammation contributes to declines in

NSC function within the aging neurogenic niche and microglia may sustain or negatively affect neurogenesis depending on age.

Disclosures: **E. Kokovay:** None. **R. Solano-Fonsea:** None. **J. O'Connor:** None. **A. Cardona:** None. **S. Mahesula:** None.

Nanosymposium

198. Adult Neurogenesis During Aging of the Neural Stem Cell Niche

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Topic: A.04. Transplantation and Regeneration

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American Federation for Aging Research

Glenn Foundation for Medical Research

Title: Elucidation of transcriptional networks that preserve adult neural stem cells

Authors: ***A. WEBB**, S. Y. KIM, S. DHAKAL;
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Abstract: Adult neural stem cells (NSCs) are the source of new neurons in the adult mammalian brain, and are a promising source of regenerative therapies for neurodegenerative disease. However, the functionality of NSCs in the mammalian brain is reduced with age. The brain contains both actively dividing stem cells and very slowly dividing NSCs, termed quiescent NSCs. These quiescent NSCs are the source of proliferative NSCs, are actively maintained, and have the potential to be reactivated and form new neurons in response to external stimuli. While the quiescent NSCs are ultimately likely to be the critical reserve population of NSCs in the adult brain, the precise mechanisms by which these cells are maintained and how they are activated remain unknown. Our goal is to determine how quiescent NSCs are directly regulated at the transcriptional level. To do so, we took advantage of an vitro model of NSC quiescence which allows us to combine functional and genomics approaches that reveal the critical mechanisms governing quiescent NSC function. Our published and preliminary data implicate the longevity-associated transcription factor FOXO3 as a key regulator of quiescent stem cells in the adult. FOXO3 is a central regulator of both aging and stem cells, and mice lacking FOXO3 have an age-related depletion of NSCs. However, the precise molecular mechanisms underlying FOXO3's role in preserving NSCs in the adult brain remain unknown. Using ChIP-seq, we identified neural stem cell-specific FOXO3 targets, and found that they tend to be specialized for

the nervous system, and include important regulators of cellular homeostasis during aging, including proteostasis and metabolic targets. To begin to understand how FOXO3 regulates an NSC-specific program of gene expression, we have investigated the upstream signals that regulate FOXO function in these cells. Intriguingly, we found that signaling through Bone Morphogenetic Proteins (BMPs) induces FOXO1 and FOXO3 in quiescent neural stem cells. BMPs have been established as key regulators of NSC quiescence during aging, but the downstream mechanisms are not known. Our data suggest that BMPs may regulate FOXO1 and FOXO3 expression to control programs of gene expression that increase stem cell quiescence and preservation with age. Together, these experiments provide new insight into how the conserved pro-longevity FOXO transcription factors function in NSCs to regulate cellular homeostasis and longevity.

Disclosures: **A. Webb:** None. **S.Y. Kim:** None. **S. Dhakal:** None.

Nanosymposium

198. Adult Neurogenesis During Aging of the Neural Stem Cell Niche

Location: SDCC 25A

Time: Sunday, November 13, 2016, 1:00 PM - 3:30 PM

Presentation Number: 198.10

Topic: A.01. Neurogenesis and Gliogenesis

Support: TWAS-CAS FELLOWSHIP

Title: Kisspeptin-10 treatment generated specific GnRH neuronal expression from the rhesus monkey derived Lyon NSCs.

Authors: ***T. ANWAR**[1], W. ZHENGBO[2];
[1]IMBB, The Univ. of Lahore, Lahore Gpo, Pakistan; [2]Kunming Inst. of Zoology, Kunming, China

Abstract: Embryonic stem cells have enormous potential for basic and clinical research into novel cell-based therapies, but the key obstacle is that stem cell differentiation is dependent upon specific cell signaling, which remains poorly understood. Here, we tested whether different dosages of KP-10 led to specific neuronal differentiation of rhesus macaque derived tau GFP-lyon ES cells. We found that KP-10 exhibited an anti-proliferative effect on the cells, leading to differentiation and morphological changes consistent with neuronal stem cell development. More importantly, kisspeptin signaling led the cells to differentiate into GnRH-neuronal types, consistent with previously observed connections between kisspeptin signaling and GnRH neurons in several congenital disorders, such as idiopathic hypogonadotropic hypogonadism (IHH). Formation and development of the GnRH neurons following the application of Kisspeptin

peptides (KP-10) to the Lyon ES cell is a novel and potentially significant finding in a number of areas.

Disclosures: **T. Anwar:** None. **W. Zhengbo:** None.

Nanosymposium

199. Tau: Biochemistry

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Presentation Number: 199.01

Topic: C.02. Alzheimer's Disease and Other Dementias

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Mayo Foundation

Title: Loss of endothelial nitric oxide synthase promotes p25 generation and tau phosphorylation in a murine model of Alzheimer's disease

Authors: ***S. A. AUSTIN**[1], Z. S. KATUSIC[2];
[1]Departments of Anesthesiol. and Mol. Pharmacol. and Exp.Therap., [2]Anesthesiol. and Mol. Pharmacol. and Exptl. Therapeut., Mayo Clin., Rochester, MN

Abstract: Alzheimer's disease (AD) has an unknown etiology; however, cardiovascular risk factors are associated with a higher incidence of AD. A defining feature of endothelial dysfunction induced by cardiovascular risk factors is reduced bioavailable endothelial nitric oxide (NO). We previously demonstrated endothelial NO acts as an important signaling molecule in neuronal tissue. We sought to determine the relationship between the loss of endothelial nitric oxide synthase (eNOS) and tau phosphorylation in neuronal tissue. We utilized eNOS knockout ($^{-/-}$) mice as well as an AD mouse model, APP/PS1 that lacked eNOS (APP/PS1/eNOS$^{-/-}$) to examine expression of tau kinases and tau phosphorylation. Brain tissue from eNOS$^{-/-}$ mice had statistically higher ratios of p25/p35, indicative of increased cyclin-dependent kinase (Cdk) 5 activity as compared to wild type ($n=8$, $P<0.05$). However, tau phosphorylation was unchanged in eNOS$^{-/-}$ mice ($n=8$, $P>0.05$). Next, we determined the role of NO in tau pathology in APP/PS1/eNOS$^{-/-}$. These mice had significantly higher levels of p25 and

a higher p25/p35 ratio as compared to both wild type and APP/PS1 mice (n=12-14, P<0.05). Indeed, Cdk5 activity was significantly higher in brain tissue of APP/PS1/eNOS$^{-/-}$ mice (n=4; P<0.05). Importantly, APP/PS1/eNOS$^{-/-}$ mice also had significantly increased tau phosphorylation (n=4-6, P<0.05). No other changes in amyloid pathology, antioxidant pathways, or neuroinflammation were observed in APP/PS1/eNOS$^{-/-}$ mice as compared to APP/PS1 mice. Our data suggests that loss of endothelial NO plays an important role in the generation of p25 and resulting tau phosphorylation in neuronal tissue. These findings provide important new insights into the molecular mechanisms linking endothelial dysfunction with the pathogenesis of AD.

Disclosures: **S.A. Austin:** None. **Z.S. Katusic:** None.

Nanosymposium

199. Tau: Biochemistry

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Presentation Number: 199.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 1DP3DK094292

NIH 1R24082841

Program fro Neurology Research and Discovery

Title: AMPK activation decreases APP protein level and tau phosphorylation through PI3-K and JNK mediated pathways

Authors: ***B. KIM**, C. BACKUS, E. L. FELDMAN;
Neurol., Univ. of Michigan, Ann Arbor, MI

Abstract: AMP-activated protein kinase (AMPK) is an evolutionarily conserved fuel-sensing enzyme and a key player in regulating energy metabolism. AMPK is activated during energy shortages and suppressed in energy surplus by sensing cellular AMP/ATP ratios. AMPK functions to restore cellular energy metabolism by suppressing energy consuming anabolic pathways and stimulating catabolic energy producing pathways. In Alzheimer's disease (AD), the role of AMPK is controversial, with the reports suggesting both beneficial and detrimental effects on the progression of AD. It is reported that AMPK activation reduces Aβ production in rat cortical neurons, and that leptin and resveratrol reduce Aβ levels and tau phosphorylation through AMPK activation. In contrast, AMPK can directly phosphorylate tau and increased AMPK activation is observed in tangle and pre-tangle bearing neurons in AD.

In this report we examined the effect of AMPK on amyloid precursor protein (APP) and tau in cortical neurons. Treatment of the human cortical stem cell line (HK-532) and rat primary embryonic cortical neurons with the AMPK activator, AICAR, induced the stimulation of downstream signaling pathways measured by the increased phosphorylation of Akt, GSK-3β and JNK, and the decreased phosphorylation of mTOR and ERK. GSK-3β and JNK phosphorylation was maximum at 2 h and returned to basal level after 8 h treatment. Phosphorylation of Akt was maintained up to 8 h. Dephosphorylation of mTOR and ERK were consistent for at least 24 h. Interestingly AICAR treatment decreased amyloid precursor protein (APP) protein levels along with the dephosphorylation of tau at Ser199/202, Ser396 and Thr231. The effect of AICAR treatment on APP protein levels and tau phosphorylation was blocked by inhibitors of PI3-K and JNK. Our results suggest that AMPK activation alleviates AD pathology through PI3-K- and JNK-mediated pathways. This work was supported by the National Institutes of Health (1DP3DK094292, 1R24082841 to E.L.F.), and the Program for Neurology Research and Discovery (www.pnrd.umich.edu).

Disclosures: B. Kim: None. **C. Backus:** None. **E.L. Feldman:** None.

Nanosymposium

199. Tau: Biochemistry

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Presentation Number: 199.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: German center for neurodegenerative diseases

Max-Planck society

Tau consortium

Title: Sorting and missorting of endogenous Tau in neurons studied in microfluidic devices

Authors: *V. BALAJI;
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Abstract: Missorting of Tau represents one of the early signs of neurodegeneration in Alzheimer disease. Tau protein is ubiquitously expressed across all compartments of neurons at early stages of development whereas it gets axonally sorted during maturation. The trigger for the sorting and missorting of tau protein is still a matter of debate. In our study, we investigated the sorting mechanisms of endogenous Tau in cultured primary neurons using microfluidic devices where cell compartments can be observed separately. We found that blocking protein degradation

pathways on the neuritic side of the microfluidic devices with proteasomal or autophagy inhibitors dramatically increased the missorting of Tau in dendrites on the neuritic side, suggesting that degradation of Tau in dendrites is a major determinant for the physiological axonal distribution of Tau. Notably, such missorted dendritic Tau showed a different phosphorylation pattern from axonal Tau, as it was phosphorylated mainly in the repeat domain (antibody 12E8), but not in the proline-rich domains flanking the repeats (e.g. PHF1 and AT8 sites). By contrast, the axonal Tau was phosphorylated at all these sites. Inhibition of local protein synthesis almost completely reversed the missorting of Tau induced by inhibition of protein degradation, indicating that the missorted dendritic Tau is locally synthesized. In support of this view, Tau mRNA was detected not only in cell bodies and axons, but in dendrites as well. Taken together, our results indicate that the protein degradation systems play an important role in the polarized distribution of tau in neurons.

Disclosures: V. Balaji: None.

Nanosymposium

199. Tau: Biochemistry

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Presentation Number: 199.04

Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Sustained activation of CaMKII caused by depletion of mitochondria from the axon enhances tau toxicity

Authors: *K. ANDO[1], A. MARUKO-OTAKE[2], M. HAYASHISHITA[1], M. OKA[1], Y. OHTAKE[2], M. SEKIYA[3], T. SAITO[1], S.-I. HISANAGA[1], K. M. IIJIMA[3];
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Abstract: Tau is a microtubule-associated protein that is expressed in neurons and localizes predominantly in the axons. Abnormal deposition of tau is a common pathological feature of a number of neurodegenerative diseases and thought to play critical roles in their pathogenesis, however, how tau gains toxicity is not fully understood. Reduction in the function and number of mitochondria at the presynaptic terminals has been associated with Alzheimer's disease and other tauopathies. Using a *Drosophila* model of human tau toxicity, we previously reported that loss of axonal mitochondria by knockdown of milton, which is essential for axonal transport of mitochondria, enhances tau-induced axon degeneration. Here we report that sustained activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) may play a role in the enhancement of tau toxicity caused by depletion of mitochondria from the axon. The levels of the autophosphorylated form of CaMKII, which has Ca^{2+}-independent, persistent activity, were increased by milton knockdown. Overexpression of CaMKII was sufficient to cause neurodegeneration in an age-dependent manner, and a mutant form of CaMKII carrying pseudo-phosphorylation at the autophosphorylation site caused more prominent neurodegeneration. CaMKII overexpression synergistically enhanced neurodegeneration caused by tau, and knockdown of CaMKII suppressed tau-induced enhanced by depletion of axonal mitochondria. Tau phosphorylation at an Alzheimer's disease-related site Ser262 is known to play a critical role in the enhancement of tau toxicity caused by depletion of axonal mitochondria. However, we found that CaMKII did not increase the levels of tau phosphorylated at Ser262, suggesting that elevated CaMKII activity maybe yet another mechanism by which depletion of axonal mitochondria enhances tau toxicity. These results suggest that disruption of calcium homeostasis caused by mislocation of mitochondria and elevated levels of Ca^{2+}-independent form of CaMKII contribute to tau toxicity in disease pathogenesis.

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Nanosymposium

199. Tau: Biochemistry

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Topic: C.02. Alzheimer's Disease and Other Dementias

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The JPB Foundation

Title: *In vivo* monitoring of pathogenic tau aggregation and spreading

Authors: *S. DEVOS, Z. FAN, R. E. BENNETT, S. TAKEDA, B. T. HYMAN; Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Tau is a microtubule-associated protein that is capable of aggregating into oligomers and intraneuronal neurofibrillary tangles (NFTs) in primary tauopathies, including Alzheimer's Disease (AD). To better understand how tau aggregates spread and induce aggregation of naïve tau, several models of tau seeding have been developed, ranging from HEK cells stably expressing the repeat domain of tau tagged to CFP and YFP (FRET as a proxy for aggregation), injection of tau fibrils into mice, and generation of unique tau clones from a range of tauopathy patient brain lysate. What is lacking, however, is a tool that provides sensitive detection of tau aggregation and spreading in animal models in real-time, enabling the longitudinal visualization and isolation of tau aggregation and potentially subsequent toxicity. To generate such an innovative tool, we have combined the ultrasensitive FRET-based tau aggregation reporter with the reliable and flexible transgene delivery by AAVs. The tau repeat domain (Tau^{RD}) carrying a FTD mutation (P301L) rapidly aggregates when exposed to misfolded tau; when tagged with CFP and YFP, the triggered aggregation of Tau^{RD} leads to FRET activity, enabling the sensitive detection of minor amounts of tau. In order to improve this tau aggregate sensor to make it suitable for *in vivo* studies, we engineered a self-cleaving 2A peptide between the Tau^{RD} FRET pair. The resulting Tau^{RD}(CFP)/2A/Tau^{RD}(YFP) construct (termed TauFRET2) expresses Tau^{RD}-CFP and Tau^{RD}-YFP in a stoichiometric ratio and eliminates the previously necessary co-transduction of two separate viruses. The AAV_TauFRET2 virus is highly efficacious at expressing Tau^{RD}-CFP/Tau^{RD}-YFP, both in primary neurons as well as *in vivo.* We are now able to monitor AAV_TauFRET2 expressing neurons longitudinally through cranial windows using two-photon microscopy, as well as isolate AAV_TauFRET2 neurons from the adult brain via cell sorting. AAV-TauFRET2 expressing wildtype neurons can develop FRET positive tau aggregates when triggered with exogenously applied lysate and even more interesting, when tauopathy neurons – either *in vitro* P301S primary neurons or *in vivo* Tg4510 neurons – express AAV_TauFRET2, spontaneous aggregates can develop, suggesting that endogenously present aggregates of tau are capable of recruiting and misfolding monomeric tau in our dynamic TauFRET2 system. With the success of our initial AAV_TauFRET2 characterization work, we can begin to longitudinally monitor and assess the role of tau aggregation and spread on neuronal integrity in adult brains.

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Nanosymposium

199. Tau: Biochemistry

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: MSUT2 is a determinant of neuronal vulnerability to pathological tau

Authors: *B. C. KRAEMER[1,2], J. WHEELER[1], P. MCMILLAN[2], T. STROVAS[1,3]; [1]GRECC, Veterans Affairs Puget Sound Hlth. Care Syst., Seattle, WA; [2]Medicine, Psychiatry, and Pathology, Univ. of Washington, Seattle, WA; [3]Pathology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: To study tauopathy disorders in a genetically tractable model system, we developed a transgenic *C. elegans* model for pathological tau by expressing human tau pan-neuronally. This animal model recapitulates several hallmarks of human tauopathies including altered behavior, accumulation of detergent insoluble phosphorylated tau protein, and neurodegeneration. To identify genes required for tau mediated neurodegeneration, we conducted a classical genetic screen for mutations suppressing pathological tau phenotypes in *C. elegans*. We ultimately cloned the *sut-2* gene, mutations in which alleviate tauopathy related phenotypes in *C. elegans*. *sut-2* encodes a distinct sub-type of CCCH zinc finger protein conserved across animal phyla. *C. elegans* SUT-2 shares significant identity with the mammalian SUT-2 (MSUT2) homolog in both humans and mice. To validate the role of MSUT2 in tau pathology we have studied the consequences of genetically reducing or eliminating MSUT2 on existing mouse and cellular models of tauopathy. Knockout of MSUT2 in the PS19 mouse model of tauopathy ameliorates tau related neurodegenerative changes including decreased accumulation of abnormal tau, reduced neuronal loss, and reduced cognitive dysfunction. In Alzheimer's disease MSUT2 levels predict age at disease onset and correlate with pathological protein deposition. The molecular mechanism by which MSUT2 controls tauopathy phenotypes is poorly understood, but under intensive investigation. The CCCH type zinc finger domains of SUT-2 and MSUT2 have been implicated in RNA binding. Human MSUT2 CCCH domains bind to poly adenosine stretches in mRNA as well as the nuclear polyA binding protein PABPN1. MSUT2 and PABPN1 have been previously reported to have reciprocal effects on polyA tail length (PMID 4671764). We have shown depletion of MSUT2 ameliorates tau oligomer and aggregate formation in a human cellular model of tau aggregation while depletion of PABPN1 has the reciprocal effect driving increased tau oligomerization or aggregation. Loss of MSUT2 is epistatic to loss of PABPN1 as concurrent depletion of both does not increase tau aggregation. Furthermore, decreasing polyA

tail length exacerbates tauopathy in human cells. Taken together these findings suggest MSUT2 modulates tau toxicity through binding to and/or regulation of polyA tails. Knocking out MSUT2 in mice protects against pathological tau supporting further translational studies of human MSUT2 as a candidate target for therapeutic intervention in diseases with tau pathology.

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Nanosymposium

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Bright Focus Foundation Grant A20144385

Veteran Affairs Puget Sound

Title: Loss of bas-1 suppresses tau-induced toxicity in tau transgenic *Caenorhabditis elegans*

Authors: *R. L. KOW[1,2], J. M. WHEELER[4], B. KRAEMER[1,4,2,3];
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Abstract: The accumulation of microtubule-associated protein tau into toxic aggregates characterizes multiple neurodegenerative diseases, classified as tauopathies, including Alzheimer's disease and related dementia disorders. We have used a *Caenorhabditis elegans* model of tau toxicity to identify genes that modulate human tau. Expression of human tau in *C. elegans* causes significant defects in motor function, progressive neuron loss, and shortened lifespans. Previous work from our lab demonstrated that loss of *dop-2* and *dop-3,* the two D2-like dopamine receptors in *C. elegans*, significantly improved tau-induced phenotypes in tau transgenic *C. elegans*. To better understand how dopamine signaling pathways modulate tau toxicity, we generated *C. elegans* strains that expressed human tau and loss of function mutations in over 40 dopamine-related genes. We screened these strains for enhancement or suppression of tau-induced locomotor defects and identified *bas-1*, the *C. elegans* homolog of aromatic amino acid decarboxylase (AADC), as a suppressor of tau-induced toxicity. Loss of function in *bas-1* ameliorates tau-induced motor dysfunction and neuronal loss. We are continuing to investigate

the mechanism(s) by which loss of *bas-1* leads to suppression of tau toxicity. Understanding how modulation of dopamine signaling mediates tau toxicity could lead to identification of new therapeutic targets for tauopathies.

Disclosures: **R.L. Kow:** None. **J.M. Wheeler:** None. **B. Kraemer:** None.

Nanosymposium

199. Tau: Biochemistry

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Cure PSP

Ed & Ethel Moore Alzheimer's disease Research Program (Florida Health)

BrightFocus Foundation

Title: A tau-dependent polyamine stress response elicits cognitive impairment and exacerbates neuropathology

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Abstract: In a non-diseased brain, tau stabilizes microtubules; however, in Alzheimer’s disease (AD) and tauopathies, tau becomes hyperphosphorylated, aggregates, and results in neuronal death. Our group recently uncovered a unique interaction between polyamines and tau fate. Polyamines exert an array of physiological effects that support neuronal function and cognitive processing. The direct link between polyamines and cognition is postulated to involve the putative polyamine-binding site on N-Methyl-D-Aspartate (NMDA) receptors. Specific stimuli (physical or emotional), can elicit a polyamine stress response (PSR), resulting in altered central polyamine homeostasis. Furthermore, evidence suggests that while the elevations in polyamines following a short-term stressor are beneficial, persistent stress and subsequent PSR activation may become maladaptive and lead to polyamine dysregulation. Polyamine dysregulation occurs

in numerous disease states, and may contribute to neuropathology and cognitive impairment. We found significant dysregulation of the polyamine pathway suggestive of a PSR in animal models of tauopathy. Furthermore, viral-mediated overexpression of C-terminally truncated tau (recombinant adeno-associated virus serotype 9 (rAAV9) C-Tau D421) also elicited polyamine dysregulation and cognitive impairment. Interestingly, the tau neuropathology in both models elicits a unique signature in the polyamine pathway that may produce a specific tau-dependent PSR. Conversely, we show that targeting the PSR, via arginase 1 (Arg1) overexpression, decreases tau neuropathology in rTg4510 (Hunt et al., 2015) and PS19 tau transgenic mice. Lastly, we also show that polyamines inhibit tau aggregation and oligomerization at physiological concentrations in vitro, while acetylated polyamines fail to mimic this effect on tau. Taken together, these data demonstrate that tau neuropathology impacts the polyamine pathway. We hypothesize that this interaction creates a bi-directional tau-mediated PSR, which in turn exacerbates tau release, aggregation, polyamine dysfunction and cognitive impairment. Furthermore, we hypothesize that the tau mediated PSR impacts NMDA receptor-dependent learning and memory. Electrophysiology and in vivo microdialysis will confirm acute changes in the tau-PSR. Overall, our data suggest that polyamines serve as endogenous inhibitors/stabilizers of tau and that tauopathies promote a unique PSR, which endorses further tau pathology. Importantly, this pathway could be therapeutically targeted to reduce neuropathology and cognitive impairment in AD and tauopathies.

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Nanosymposium

199. Tau: Biochemistry

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Title: Tau antibody derivatives as diagnostic imaging ligands for tauopathies

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Abstract: Several β-sheet dyes are being developed as PET ligands for targeting pathological tau aggregates. In contrast to β-sheet binders, antibody-derived ligands should provide greater specificity for detecting tau lesions, and can be tailored to detect various pathological tau epitopes. Such detailed information may then lead to more efficacious clearance of the tau aggregates, for example with tau immunotherapy targeting these same epitopes.
We have previously reported that a tau imaging probe based on a single chain variable antibody fragment (scFv235) provides a greater in vivo brain signal after peripheral injection than the parent antibody (6B2) although the affinity of the former is much lower (Krishnaswamy S et al, J Neurosci, Dec 10, 2014). This is likely due to its smaller size (25 kDa) compared to the antibody (150 kDa) resulting in improved brain and neuronal permeability. To confirm this in the same animals and to determine if the Fab portion (50 kDa) of the antibody may provide an ideal compromise between size and affinity, tauopathy mice were serially injected with all three probes. The latter two have higher affinity for tau and longer half-life than scFv. Hence, such comparison provides valuable information on how size, affinity and clearance of the probe affects in vivo detection of tau lesions that will aid in further development of this approach for clinical use. The probes were tagged with a near-infrared fluorescent marker to allow detection of brain signal after i.v. injection (50 μg) in intact anesthetized tauopathy mice (JNPL3, htau, htau/PS1; n=7) using the In Vivo Imaging System (IVIS). The strongest average peak IVIS brain signal was obtained after scFv235 injection, both in JNPL3 mice (1.9E+10) and the other models (3.5E+9), compared to 6B2 (1.3E+10 vs. 2.8E+9) and its Fab (8.2E+9 vs. 2.0E+9). All the mice received the probes in the same order, starting and ending with the 6B2 antibody, allowing for probe clearance between injections. The 6B2 signal did not appear to change over this period. Interestingly, the signal from the 6B2 and its Fab are comparable although the whole antibody is three times the size of its Fab. We are verifying that its digestion into Fab did not affect its affinity. Stronger signal in the JNPL3 mice compared to the other models is as expected since they have more tau lesions. We are currently comparing the in vivo signal with tau pathology in the individual mice to better assess the predictive validity of each probe. In conclusion, scFv appears to be more sensitive for detecting tau pathology in vivo than its parent antibody or its Fab fragment although the latter two have substantially higher affinity for tau.

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Nanosymposium

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Title: Dynamics of intracellular interaction of a tau antibody and human pathological tau in a human neuron-like model.

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Abstract: Our laboratory has pioneered targeting pathological tau proteins using active and passive immunotherapies in models of Alzheimer's disease and other tauopathies. Previously, we double differentiated naïve SH-SY5Y cells with retinoic acid and BDNF to create a more neuronal-physiological system. This model showed increased tau levels, decreased tau antibody (Ab) internalization, and greater efficacy of tau Abs to clear pathological tau, compared to non-differentiated cells.
Time lapse live imaging is a very sensitive approach, which allows monitoring in real time the internalization, interaction, and dynamics of both tau Ab and pathological tau within endocytic cellular compartments. The neuron-like cells were pre-treated for 16 h with 50 µg/mL of paired helical filament enriched fluorescently-tagged tau (PHF) and subsequently incubated for 2.5 h with 20 µg/mL CypHer5E-tagged 4E6 tau Ab, which was generated against a P-S396,404 tau immunogen. Alternatively, the cells were co-treated with tagged PHF tau and 4E6 for up to 2.5 h. CypHer5E is a pH sensitive dye, which only fluoresces within acidic compartments, like the endosome-lysosome (E-L) system. Tau Ab signal increased over time in the pre-treated cells, and plateaued at 2 h, while the PHF signal was constant during the 2.5 h experiment. Intracellular co-localization increased over time and was confirmed by intensity correlation analysis (r^2=0-0.23). The co-treated cells showed an increase of both the 4E6 and PHF signals, which plateaued at 1.5 h. Co-localization was robust only within the neurites/axons, in contrast to the pre-treated cells that mainly showed 4E6-PHF binding within the soma. In these co-treated

cells, comparable intracellular interaction was observed between 4E6 and PHF throughout the experiment (r^2=0.55-0.64).
Our laboratory has shown in various models that several tau Abs interact with pathological tau both extra- and intracellularly. In this study we focused on their fast intracellular interaction within the E-L system, where its subcellular location depends on the experimental design. In the pre-treated cells, PHF gradually finds its way into the soma, and the Ab binds to it there. In the co-treated cells, PHF and the Ab are taken up at the same time, possibly as complexes, and are primarily seen to interact within the neurites/axons on their way to the soma, while the majority of the complexes are neutralized in the extracellular space. This approach may clarify the dynamics and mechanisms of intracellular Ab-mediated clearance of pathological tau, and aid in identifying clinical Ab candidates, which can be assessed in their humanized form as the culture model is of a human origin.

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Nanosymposium

199. Tau: Biochemistry

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Tau Consortium

Title: Mutant human Tau (ΔK280) causes an axonopathy which can be treated with adenosine A_1 antagonist Rolofylline.

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Abstract: The protein Tau, known for its role in microtubule stabilization, accumulates in a subset of neurodegenerative diseases called Tauopathies. In these diseases, Tau is mislocalized,

post-translationally modified and folded into a pathological conformation. In turn, this leads to aggregation of Tau and the formation of neurofibrillary tangles. Many post-translational modifications of Tau have been reported but the mode of toxic action of Tau remains enigmatic. Here we use two regulatable transgenic mouse lines in parallel in order to disentangle pathological changes in Tau from epiphenomena. Mice expressing near-endogenous levels of aggregate-prone Tau (pro-aggregant, ΔK280) develop Tau pathology and cognitive decline rapidly, whereas mice expressing anti-aggregant Tau (ΔK280-PP) transgenic mice develop almost no pathology. To study Tau toxicity and for testing compounds we generated organotypic hippocampal slices of both transgenic lines. Both pro- and anti-aggregant Tau transgenic slices show Tau missorting and (hyper)phosphorylation (at epitopes 12E8, AT8, PHF1, etc.). Only pro-aggregant Tau accumulates in axons as spindle shaped grains, reminiscent of argyrophilic grains found in human brains. Strikingly, only the Tau-containing grains in pro-aggregant Tau transgenics are stained with the MC-1 antibody which recognizes a pathological conformation of Tau. Pro-aggregant Tau causes loss of spines and a reduction in neuronal ATP but is not targeted for degradation nor is it packed into aggresomes. Instead, it causes astrocytic and neuronal hypo-activity with matching pre-synaptic impairment. In order to boost pre-synaptic functioning and neuronal activity we treated the slices from pro-aggregant mice with the adenosine A_1 antagonist Rolofylline (KW-3902). Indeed, this restores pre-synaptic functioning and normalizes neuronal activity *in vitro*. Next, we determined if Rolofylline could also restore cognitive function in pro-aggregant Tau transgenic mice. When fed orally for two weeks, Rolofylline restores spatial memory and basic synaptic transmission of the pro-aggregant Tau transgenic mice without causing adverse effects in controls. By comparison, in humans, neuronal hypo-activity is frequently observed during or preceding neurodegeneration. We speculate that Rolofylline, initially developed as a diuretic, could be a promising drug to increase neuronal activity which might delay the onset or progression of neurodegeneration in tauopathies.

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Nanosymposium

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Title: Extracellular tau oligomers induced redistribution and aggregation of endogenous neuronal tau coupled to axonal transport dysfunction

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Abstract: Tau is a neuron-specific, microtubule-associated protein enriched in axons, where its functions include direct binding and stabilization of microtubules, and regulation of axonal transport. CNS tau comprises six isoforms produced by the alternative splicing of a single tau gene, MAPT, with these isoforms characterized by the presence of zero, one or two N-terminal inserts, and three or four C-terminal microtubule binding repeats. Neuronal inclusions composed of hyperphosphorylated tau are a major histopathological feature of a series of neurodegenerative disorders known collectively as tauopathies, of which Alzheimer's disease (AD) is the most prominent. While the clinical and histological presentation of these disorders is heterogeneous, the majority share the following hallmarks: loss of the normal axonal distribution of tau; accumulation of insoluble, fibrillar tau aggregates in neurites and perikarya; synaptic dysfunction; and eventual neuron death. Familial mutations identified in the MAPT gene point to a causative role for tau in these disorders, with changes in the isoform ratio or aggregation propensity of tau causing fully penetrant neurodegenerative diseases. In this study, we demonstrate that externally applied oligomeric tau has two profound effects on neuron physiology: loss of the normal polarized distribution of tau within the cell and perturbation of fast axonal transport. Using a quantitative assay to measure tau aggregation in cultured neurons, we demonstrate that tau oligomers are much more effective than tau monomers or fibrils at inducing endogenous tau redistribution within axons, that the extent of this disruption varies according to tau isoform, and that oligomers made from mixtures of all six CNS tau isoforms are much more potent than oligomers made from individual isoforms. Furthermore, tau oligomers induced mislocalization of tau from its normal, primarily axonal distribution into the somatodendritic compartment, raising the possibility that the oligomers cause breakdown of the axon initial segment. Tau plays an integral role in the regulation of cargo transport along axonal microtubules, and we also show that intracellular tau aggregation induced by extracellular tau oligomers is accompanied by alterations in the velocity, run time and run length of axonal membrane-bounded organelles. These collective results suggest that extracellular tau oligomers trigger seminal steps in the pathogenesis of AD and non-Alzheimer's tauopathies, including tau redistribution and dysregulation of axonal transport, and point to mechanisms by which loss of physiological tau distribution leads to neuronal dysfunction.

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Nanosymposium

199. Tau: Biochemistry

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Byrd Alzheimer's Institute BRD 712

Title: Metabolic changes over the course of aging in a mouse model of tau deposition

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder mainly characterized by cognitive deficits and neuropathological changes but also associated with non-cognitive symptomatology, commonly referred to as behavioral and psychological signs and symptoms of dementia (BPSD). Weight loss and disturbances in food intake that often precedes clinical diagnosis have been extensively reported in AD patient as well as increase in spontaneous activity, wandering and impulsive behavior. These symptoms have been shown in mice model of amyloid deposition but very few studies have been done on tau deposition model, although BSPD are also found in tauopathies like fronto temporal dementia. We have made the recurrent observation that transgenic tau mice seemed to eat more and yet weight less. To further scrutinize these observations, body weight, locomotor activity and metabolic rate were assessed in 2-, 7- and 12-month-old tau rtg4510 mice and wild-type littermates in a test paradigm continuously recording cage activity over a period of 6 days. We observed a significant age related decrease of body weight in tau mice compared to non-transgenic littermates which was not associated with a decrease in food intake but rather due to hyperactivity. Indeed no body weight or activity differences were seen at 2 months but a decrease in body weight at 7 mo and 12 mo was correlated with a hyperactive phenotype. We observed a switch in both food intake and resting metabolism between the asymptomatic phase when mice start to accumulate tau but without pathology tangles (2 mo) and the symptomatic phase where tau pathology is associated with tangles, brain atrophy and cognitive impairments (7 mo). The very late stage (12 mo) seem to resume how metabolism starts to fall apart and triggers survival mechanisms. These findings raise new questions about the role of tau in body weight loss and metabolic deregulation related

to AD pathology especially regarding the hypothesis in which hypermetabolism is responsible for weight loss in AD patients.

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Nanosymposium

199. Tau: Biochemistry

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Immunohistochemical characterisation of novel tau antibodies in transgenic animals

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Abstract: Amyloid plaques and neurofibrillary tangles are key pathological events associated with Alzheimer's disease (AD). To date, considerable efforts have identified a variety of potential novel therapeutics focussed on removing and/or preventing amyloid deposition by either inhibiting the β site amyloid precursor protein cleaving enzyme (BACE) or targeted anti-amyloid antibodies. More recently, directed discovery efforts have focussed on preventing the deposition and spreading of neurofibrillary tangles which are comprised of hyperphosphorylated tau. Here we report on the immunohistochemistry characterisation of multiple antibodies raised against different epitopes of tau, including phospho- and non-phospho epitopes. The focus was to delineate the immunoreactivity signature of these antibodies in tau transgenic mouse models to gain an insight into the CNS distribution of tau. Brains from different mouse models, including wild type, MAPT knockout and Tau transgenic mouse (+/- injection of Tau fibrils), were collected, fixed and paraffin embedded. Immunohistochemistry was performed using the tau antibodies of interest, followed by imaging. Special attention was paid to the time between sacrifice of the animals and fixation to minimalize post-mortem dephosphorylation. Tau1 (non-phosphorylated tau), mTau2 (phosphorylation-independent mouse tau), HT7 (human tau), AT100 and AT8 (aggregated tau) were included in the study as reference antibodies. Non-phosphorylated tau is known to be present mainly in axons and not in neuronal cell bodies of wild type mice. In transgenic models, Tau1 immunoreactivity is observed both in axons and the somatodendritic compartment. Phosphorylated tau on the other hand is a major component of aggregates present in the brains of P301S and P301L animals and is virtually absent from normal neurons. However, by the use of high-affinity phosphorylation-dependent antibodies and by

minimising post-mortem delay, phosphorylated tau could be detected in wild type brains. In contrast to dephosphorylated tau, phosphorylated tau was mainly observed in neuronal somata and dendrites. Until recently, it was generally accepted that phosphorylated tau is scarce in healthy neurons. However, this study clearly identifies the presence of phosphorylated tau in the somatodendritic compartment of neurons under physiological circumstances. This opens up the idea that accumulation of hyperphosphorylated tau is not necessarily triggered by increased kinase or decreased phosphatase activity, but could start from the existing pool of phosphorylated tau.

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Nanosymposium

200. Alzheimer's Synaptic Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DFG

Title: The synaptic adhesion molecules APP/APLPs and the APP/APLPs-interacting FE65 proteins share common synaptic functions

Authors: *S. KINS[1], P. STRECKER[1], S. SCHILLING[1], S. LUDEWIG[2], A. MEHR[3], M. KORTE[2], J. STEPHAN[1], M. RUST[4], S. GUÉNETTE[5], U. MÜLLER[3], S. EGGERT[1]; [1]Univ. of Kaiserslautern, Kaiserslautern, Germany; [2]Zoological Institute, Div. of Cell. Neurobio., TU Braunschweig, Braunschweig, Germany; [3]Inst. for Pharm. and Mol. Biotechnology, Univ. of Heidelberg, Heidelberg, Germany; [4]Inst. of Physiological Chemistry, Univ. of Marburg, Marburg, Germany; [5]MassGeneral Inst. for Neurodegenerative Disease, Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA

Abstract: The Amyloid Precursor Protein (APP) has an essential function at the central and peripheral synapse, including hippocampal LTP and neuromuscular junction (NMJ) formation. Those activities involve trans-cellular dimerization of full length APP and interaction of intracellular APP binding partners. Here we addressed the question if the APP-like protein 1 (APLP1), which also trans-dimerizes, exhibits similar functions as APP and tested whether the main intracellular APP-interaction partner FE65 might be involved in APP/APLPs synaptic function. We observed that aged APLP1-knockout (KO) mice showed in contrast to APP- and APLP2-KO mice no alterations in synaptic plasticity (LTP, PPF), but impaired basal

transmission and a reduced frequency of mEPSCs, most likely caused by reduced spine density. Further APLP1 exhibits increased trans-directed binding and elevated cell-surface levels, indicating that APLP1 synaptic function depends on trans-cellular dimerization, as shown for other synaptic adhesion molecules (SAMs). Further, we examined the central and peripheral nervous systems of FE65-KO, FE65L1-KO and FE65/FE65L1-DKO mice. We observed spatial learning and memory deficits, severe motor impairments, hippocampal LTP deficits and neuromuscular junction (NMJ) abnormalities. As the NMJ deficits resemble those of mutant APP/APLP2-DKO mice lacking the FE65/FE65L1 binding site, the NMJs of APLP2/FE65-DKO and APLP2/FE65L1-DKO mice were analyzed. NMJ deficits are aggravated in these mice when compared to single FE65 and FE65L1 KO mice. Together, our data demonstrate distinct functions of APP family members at the synapse and suggest that FE65 proteins function in trans-synaptic APP/APLPs signaling.

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Nanosymposium

200. Alzheimer's Synaptic Dysfunction

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CSC 201307650003

Title: APP maintains dendritic spine plasticity in the adult brain via extracellular D-serine levels

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Abstract: APP proved to be critical in the formation and stabilization of synaptic connections in the developing nervous system. In order to understand if the dynamics of dendritic spine plasticity in the adult brain are regulated by APP as well, we monitored and compared dendritic spine plasticity in cortical pyramidal neurons of 4-5 months old GFP-M (WT) and APP-KO mice crossed with GFP-M *in vivo*. Apical tufts of GFP labeled layer V pyramidal neurons were imaged in the somatosensory cortex over several weeks by the use of *in vivo* two-photon imaging. While the overall spine density of WT and APP-KO mice were similar, both the elimination and formation of new spines were significantly lower in neurons of APP-KO mice compared to controls, resulting in reduced spine turnover rate. To investigate if the reduced spine dynamic has a functional consequence in neural circuit remodeling in adult mouse brain, both WT and APP-KO mice were exposed to environmental enrichment (EE) over 5 weeks. Whereas EE induced a steady increase of spine density in WT mice it failed to increase spine density in APP-KO mice. Since dendritic spine plasticity depends on the release of D-serine from astrocytes, the function of which has been previously shown to be altered in APP-KO mice, we measured cortical extracellular and total D-serine concentrations in APP-KO mice. Using microelectrode biosensors we observed that the extracellular concentration of endogenous D-serine was dramatically decreased in the absence of APP. In order to prove that altered D-serine levels are causatively linked to impaired structural spine plasticity in APP-KO mice we supplemented D-Serine to the drinking water of APP-KO mice housed under standard or EE conditions and analyzed dendritic spine plasticity over several weeks. Indeed D-serine treatment in APP-KO mice increased constitutive spine dynamics under standard housing conditions and also rescued the adaptive gain of spines upon environmental enrichment. These data suggest that constitutive and adaptive structural plasticity of dendritic spines requires extracellular D-serine, which is reduced in the absence of APP.

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Nanosymposium

200. Alzheimer's Synaptic Dysfunction

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ERA-Net Neuron 01EW1305A

LOEWE Center for Cell and Gene Therapy

Title: APPsalpha rescues impaired spine density and synaptic dysfunction in aged Alzheimer model mice and conditional APP/APLP2 double knockout mice

Authors: *U. MULLER[1], R. FOL[2], M. RICHTER[1], S. LUDEWIG[3], T. ABEL[4], J. BRAUDAU[2], S. WEYER[1], M. HICK[1], D. WOLFER[5], C. BUCHHOLZ[4], M. KORTE[3], N. CARTIER[2]; [1]Heidelberg Univ., Heidelberg, Germany; [2]Univ. Paris-Sud, Orsay, France; [3]TU Braunschweig, Braunschweig, Germany; [4]Paul-Ehrlich-Institut, Langen, Germany; [5]Zurich Univ., Zurich, Switzerland

Abstract: Alzheimer's disease (AD) is characterized by synaptic failure, dendritic and axonal atrophy, neuronal death and progressive loss of cognitive functions. It is commonly assumed that these deficits arise due to β-amyloid accumulation and plaque deposition. However, increasing evidence indicates that loss of physiological APP functions mediated predominantly by neurotrophic APPsα produced in the non-amyloidogenic α-secretase pathway may contribute to AD pathogenesis. Upregulation of APPsα production via induction of α-secretase might, however, be problematic as this may also affect substrates implicated in tumorigenesis. Here, we used a gene therapy approach to directly overexpress APPsα in the brain using AAV-mediated gene transfer and explored its potential to rescue structural, electrophysiological and behavioral deficits in APP/PS1deltaE9 AD model mice. Sustained APPsα overexpression in aged mice with already preexisting pathology and amyloidosis restored synaptic plasticity (LTP and PPF) and partially rescued spine density deficits. Importantly, AAV-APPsα treatment also resulted in a functional rescue of spatial reference memory in the Morris water maze. Moreover, we demonstrate a significant reduction of soluble Aβ species and plaque load. In addition, APPsα induced the recruitment of microglia into the vicinity of plaques and upregulated TREM2 expression suggesting enhanced plaque clearance. Collectively, these data indicate that APPsα may mitigate synaptic and cognitive deficits, despite established pathology. To complement our analysis in APP overexpressing mice, we also assessed the synaptic function of APPsα in previously generated conditional APP/APLP2 double knockout (cDKO) mice exhibiting deficits in dendritic branching, spine density, LTP and hippocampus dependent behavior. Interestingly, both acute in vitro application of nanomolar amounts of APPsα (but not APPsβ) and AAV-APPsα gene transfer in vivo restored synaptic plasticity in cDKO mice. Ongoing experiments are aimed at delineating the minimal functional domain of APPsα. Together our data highlight not only the physiological role but also the therapeutic potential of APPsα that may be of relevance for AD treatment.

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Presentation Number: 200.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR

Title: Amyloid-beta42 interacting peptide protects synaptic structure and function

Authors: S. HOSSAIN[1], P. CHANG[1], R. MCKINNEY[1], *G. MULTHAUP[2];
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Abstract: Alzheimer's disease is a neurodegenerative disorder for which neither a cure nor an effective therapy exist. It is characterized by neuroinflammation, intraneuronal tangles and amyloid plaques. At the center of both onset and pathogenesis of this disease, amyloid-β (Aβ) peptides are believed to play significant roles, as supported by the discovery of protective variants in the amyloid gene. Aβ peptides of varying length are produced by the sequential, proteolytic processing of amyloid precursor protein (APP) by β- and γ-secretases. The most common Aβ peptides are those comprised of 40 and 42-amino acids, where Aβ40 is believed to be largely non-toxic and Aβ42, toxic. Moreover, monomeric Aβ peptides form soluble oligomers, which then further aggregate to proto-fibrils, fibrils, and finally, amyloid plaques. Specifically, the 42-amino acid peptide (Aβ42) is prone to form aggregated amyloid oligomers, which allegedly contribute to plaque formation and cognitive decline. In organotypic hippocampal slice cultures, soluble Aβ42 oligomers were found to be highly toxic to neuronal cells, (i.e. impaired synaptic function) and contributed to progressive neuronal dysfunction, loss of synaptic spine density, change in spine shape, and long-term potentiation (LTP). Therefore, rather than simply inhibiting the aggregation of Aβ monomers into oligomers, we specifically targeted a relatively well-defined population of low-n Aβ42 oligomers using an Aβ-oligomer Interacting Peptide (AIP). "Trapping" these toxic Aβ42 species with this short peptide, prevented the loss of Aβ42-induced synaptic spine density and in fact, rescued LTP in organotypic hippocampal slice cultures. Moreover, AIP ameliorated the 'rough-eye' phenotype in a transgenic Aβ42 fly model and significantly improved the function of photoreceptors of these flies. Overall, our results indicate a toxic role for soluble Aβ42-oligomers on synaptic dysfunction, and specifically "trapping" low-n oligomers could be a novel strategy Aβ42-oligomer recognition and removal, while preserving and protecting synaptic structure and function.

Disclosures: **S. Hossain:** None. **P. Chang:** None. **R. McKinney:** None. **G. Multhaup:** None.

Nanosymposium

200. Alzheimer's Synaptic Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PRIN2012SNMJRL to G.D.C.

Title: Recurrent Herpes Simplex type-1 (HSV-1) infections alter synaptic functions in adult mice via amyloid-β protein (Aβ) production and accumulation

Authors: *R. PIACENTINI[1], D. D. LI PUMA[1], A. MASTRODONATO[1], S. COCCO[1], G. DE CHIARA[2], A. PALAMARA[3,4], C. GRASSI[1];
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Abstract: A growing body of evidence suggests that HSV-1 infection is a risk factor for Alzheimer disease's (AD). We recently reported that HSV-1 disrupts synaptic function in cultured hippocampal neurons via glycogen synthase kinase 3 activation and intraneuronal accumulation of amyloid β protein (Aβ) (Piacentini&Li Puma et al., Scientific Reports, 5:15444, 2015). By using a novel viral infection-reactivation protocol, we extended our studies to *in vivo* mouse models to check whether recurrent HSV-1 infections cause Aβ accumulation leading to synaptic plasticity and memory alterations that are reminiscent of AD. Male C57/bl6 mice and transgenic mouse models (3×Tg-AD and APP KO) at 1 month of age were infected with HSV-1 (1×10^6 PFU) by snout abrasion. After 6 weeks mice were subjected to two thermal stresses (delivered at 1-month interval) to induce HSV-1 reactivation in the brain. Immunohistochemistry revealed Aβ accumulation in hippocampi of HSV-1-infected C57/bl6 mice along with reduced expression of synapsin-1 and synaptophysin when compared with mock-infected mice. Long-term potentiation (LTP) at the hippocampal CA3-CA1 synapse was significantly lower in brain slices from HSV-1-infected C57/bl6 mice than in controls (155±9 vs. 193±16% [n=10 brain slices from 3 and 4 mice, respectively], $p<0.05$). LTP inhibition was even greater in slices from infected 3×Tg-AD mice whose age-matched controls did not show detectable LTP impairment yet (144±6 vs. 179±9% [n=10 brain slices from 3 and 4 mice, respectively], $p<0.05$). Instead, HSV-1 infection did not significantly affect LTP in APP KO mice. C57/bl6-infected mice also exhibited a significant reduction of the preference index in the novel object recognition test (56.6±1.9% [n=11] vs. 66.6±2.4% of mock-infected mice [n=12]; $p<0.05$). Greater memory impairment was found in 3×Tg-AD mice (51.7±1.2% [n=5] vs. 66.4 ±1.7% of mock [n=9]; $p<0.05$), whereas APP KO mice showed no significant alterations. Behavioral, functional and

molecular alterations correlated with the presence of HSV-1 in brains that was assessed by PCR amplification of the viral TK gene and real time PCR of cDNA for ICP4. Collectively, our results suggest that repeated HSV-1 infections produce an AD-like phenotype thus supporting their possible involvement in AD pathogenesis.

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Nanosymposium

200. Alzheimer's Synaptic Dysfunction

Location: SDCC 30B

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Presentation Number: 200.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant NS049442

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Title: Amyloid-beta peptide is required for the cGMP-induced long-term potentiation and memory

Authors: *D. PUZZO[1], R. RICCIARELLI[2], W. GULISANO[1], M. TROPEA[1], C. REBOSIO[2], O. ARANCIO[3], E. FEDELE[2], A. PALMERI[1];
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Abstract: Background - Accumulation of amyloid-beta (Aβ) has been related to Alzheimer's disease pathogenesis. However, in the healthy brain, low concentrations of Aβ are necessary for physiological long-term potentiation (LTP) and memory. Because cGMP plays a key role in these processes, here we have investigated whether cGMP might influence Aβ production and function during LTP and memory in physiological conditions.
Methods - We first evaluated whether an increase of cGMP levels by phosphodiesterase-5 inhibitors (PDE5-Is), such as sildenafil and vardenafil, might affect Aβ levels in Neuro-2a (N2a) cells and hippocampal slices. We also evaluated whether PDE5-Is might modify Amyloid Precursor Protein (APP) expression and the interaction between APP and the β-site APP cleaving enzyme-1 (BACE-1), evaluated by the OptiCAB assay. Finally, we performed electrophysiological experiments on hippocampal slices and behavioral studies (novel object recognition) to analyze whether the vardenafil-induced enhancement of LTP and memory was still present when blocking Aβ function.

Results - We showed that the increase of intracellular cGMP after a treatment with sildenafil or vardenafil induced a parallel increase of Aβ levels in N2a cells and hippocampal slices. This effect was reduced by the guanylyl cyclase inhibitor ODQ. Vardenafil did not modify APP full-length expression but increases the approximation of APP and BACE1. Finally, we demonstrated that the cGMP-induced LTP and memory depended upon Aβ production. In fact, the physiological potentiation of LTP and recognition memory induced by vardenafil was not present if blocking Aβ function - by anti-murine Aβ antibodies or APP knock-out mice. Conclusions - The increase of cGMP positively modulates Aβ production, which, in turn boosts synaptic plasticity and memory. The lack of effect of PDE5-Is in APP KO mice suggests that Aβ is needed for the cGMP-induced enhancement of LTP and memory. Thus, PDE5-Is might work as cognitive enhancers via a positive modulation of Aβ at physiological concentrations in the brain.

Disclosures: **D. Puzzo:** None. **R. Ricciarelli:** None. **W. Gulisano:** None. **M. Tropea:** None. **C. Rebosio:** None. **O. Arancio:** None. **E. Fedele:** None. **A. Palmeri:** None.

Nanosymposium

200. Alzheimer's Synaptic Dysfunction

Location: SDCC 30B

Time: Sunday, November 13, 2016, 1:00 PM - 4:30 PM

Presentation Number: 200.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Assessing the effects of $A\beta_{1-42}$ on neuronal excitability and synaptic function using a novel high-throughput assay

Authors: ***J. K. VIRDEE**, Y. SINHA, A. FOUILLET, S. EVERSDEN, M. O'NEILL, J. WOLAK, D. URSU;
Eli Lilly and Co., Windlesham, United Kingdom

Abstract: Loss or alteration of synaptic activity is associated with a number of psychiatric and neurological disorders such as Alzheimer’s disease (AD). In the case of AD excessive accumulation of amyloid-beta (Aβ) oligomers is believed to cause synaptic disruption. Aβ oligomers bind to functional synapses, in particular the postsynaptic compartment, and can negatively affect synaptic plasticity and cause synapse loss. Understanding the effects of Aβ1-42 oligomers on neuronal and synaptic function and how novel mechanisms may either restore or modulate synaptic activity in AD is therefore an attractive pursuit.
Our laboratory has previously described and characterised a high-throughput assay for measuring neuronal excitability and synaptic activity in primary rat cultured neurones (RCNs) in a multi-well plate format (Electrical Field Stimulation - EFS assay). We have shown previously a

thorough pharmacological validation of this assay by evaluating compounds with known pharmacological profiles, acting either directly on neuronal excitability or modulating pre- and post-synaptic activity.
We have now further used this assay to investigate the effects of synthetic Aβ1-42 oligomers on synaptic function and neuronal excitability. We are showing that chronic treatment (48hrs) of RCN's with oligomeric Aβ1-42 produces a consistent disruption of neuronal and synaptic activity, an effect not seen with a control- scrambled peptide. Additionally, we have developed custom EFS protocols that have enabled us to demonstrate Aβ1-42 induced specific synaptic disruption in a dose dependent manner, effects not seen with the scrambled peptide.
Having demonstrated robust Aβ1-42 induced deficits in our model we have further used the assay to profile well characterised pathways and targets involved in Aβ1-42 induced synaptotoxicity. We have evaluated a range of standard tool compounds and observed protective activity against the effects of Aβ1-42. These results suggest that this assay can be used to examine the effects of Aβ1-42 on synaptic physiology, and therefore represents an attractive novel phenotypic screening model that can help us to uncover novel mechanisms involved in Aβ1-42 induced synaptotoxicity.

Disclosures: **J.K. Virdee:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **Y. Sinha:** A. Employment/Salary (full or part-time): Eli lilly and Company. **A. Fouillet:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **S. Eversden:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **M. O'Neill:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **J. Wolak:** A. Employment/Salary (full or part-time): Eli lilly and Company. **D. Ursu:** A. Employment/Salary (full or part-time): Eli lilly and Company.

Nanosymposium

200. Alzheimer's Synaptic Dysfunction

Location: SDCC 30B

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Presentation Number: 200.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Differential effects of $A\beta_{1-42}$ and control oligomers on synaptic toxicity, tau mislocalisation and microglial activation.

Authors: *E. L. MEAD[1], Y. SINHA[2], S. EVERSDEN[2], J. VIRDEE[2], D. URSU[2], M. O'NEILL[2], M. HUTTON[2], J. WOLAK[2];
[1]Mol. Pathology, Eli Lilly & Co, Windlesham, United Kingdom; [2]Mol. Pathology, Eli LIlly and Co., Windlesham, United Kingdom

Abstract: Alzheimer's disease is characterized by the accumulation of tau in neurofibrillary tangles, as well as deposits of Aβ plaques. Whilst these are the typical hallmarks of the disease, emerging evidence suggests that Aβ oligomers may be the toxic species that contribute to Alzheimer's disease pathology. These Aβ species have been proposed to lead to a number of AD-related pathogenic processes including tau mislocalisation, impairment of synaptic function and microglial activation. It is still not clear though which types of Aβ oligomeric species contribute to those processes. It has therefore become increasingly important to develop reproducible methods to generate well-described $A\beta_{1-42}$ oligomers for *in vitro* and *in vivo* use. We have developed reliable methods for generation of $A\beta_{1-42}$, control scrambled and reversed peptide assembles which differ in size and biochemical properties under conditions compatible with experimental approaches. We also applied a range of methods, including dynamic light scattering, MSD, circular dichroism, ThT binding assay and electron microscopy to characterize the $A\beta_{1-42}$ assembles.

We have set up a number of experimental models and demonstrated differential effects of oligomeric species *in vitro* on tau mislocalisation, synaptic function and neuronal excitability, and microglial activation. Treatment of RCN's with oligomeric $A\beta_{1-42}$ causes a disruption of neuronal and synaptic activity, an effect not seen with a control- scrambled peptide. Similarly $A\beta_{1-42}$ but not the control oligomers of a comparable size drive tau mislocalisation into the somatodendritic compartments, as investigated in mature primary hippocampal neuronal cultures. Furthermore, oligomeric $A\beta_{1-42}$ promotes microglial activation which is characterized by elevated CD68 expression, and induces a robust increase in the release of pro-inflammatory cytokines, which is not seen with the control peptide.

The generation of synthetic $A\beta_{1-42}$ oligomers and the control peptides has allowed us to begin to understand the importance of Aβ species on disease-related pathology at a cellular level. It is anticipated that these tools will enable us to explore Aβ oligomer driven pathologies, and will facilitate target validation efforts, in addition to enhancing our disease understanding.

Disclosures: **E.L. Mead:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **Y. Sinha:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **S. Eversden:** A. Employment/Salary (full or part-time): Eli Lilly. **J. Virdee:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **D. Ursu:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **M. O'Neill:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **M. Hutton:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **J. Wolak:** A. Employment/Salary (full or part-time): Eli Lilly and Company.

Nanosymposium

200. Alzheimer's Synaptic Dysfunction

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Presentation Number: 200.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CNPq

FAPERJ

CAPES

INNT

Title: Neurexin and its trans-synaptic partner, neuroligin 1, bind soluble aβ oligomers andmediate oligomer-induced neuronal damage, synapse loss and cognitive impairment in mice

Authors: *M. M. OLIVEIRA, J. BRITO-MOREIRA, M. V. LOURENCO, J. FONTES, M. MAGDESIAN, F. C. RIBEIRO, J. H. LEDO, H. M. MELO, L. DINIZ, F. A. C. GOMES, J. CLARKE, C. P. FIGUEIREDO, F. G. DE FELICE, S. T. FERREIRA;
Inst. of Med. Biochem. Leopoldo de Meis, Federal Univ. of Rio De Janeiro, Rio de Janeiro, Brazil

Abstract: Brain accumulation of amyloid-β and synapse loss are neuropathological hallmarks of Alzheimer disease (AD). Amyloid-β oligomers (AβOs) build up in AD brains and are thought to act as synaptotoxins that drive early memory impairment. Nonetheless, the mechanisms underlying AβO-induced synapse failure and brain damage remain to be fully elucidated. Identifying synaptic targets of AβOs comprises an essential step to allow rational design of novel therapeutic approaches in AD. Trans-synaptic interactions between neurexins (Nrxs) and neuroligins (NLs) are essential for synapse structure, stability and function, and reduced NL levels have been recently associated with AD. Here, we investigated whether Nrx and NL mediate synapse damage and memory loss induced by AβOs in rodents. Results showed that AβOs interact with both α-neurexin (Nrxα) and neuroligin 1 (NL1), and that application of anti-Nrx2α or anti-NL1 antibodies reduced dendritic binding of AβOs in cultured hippocampal neurons. Blocking the interaction between AβOs and Nrx2α or NL1 with anti-Nrx2α or anti-NL1 antibodies, or using a specific peptide corresponding to the Nrx/AβO interaction site, prevented AβO-induced neuronal oxidative stress, loss of dendritic spines and synapses. Significantly, blocking AβO interaction with Nrx2α or NL1 abolished memory impairment triggered by AβOs in mice. Current findings establish that trans-synaptic partners Nrxα and NL1 are targets of AβOs and mediate molecular pathways leading to synapse dysfunction and memory impairment, offering novel perspectives for halting synapse failure and cognitive loss in AD.

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Nanosymposium

200. Alzheimer's Synaptic Dysfunction

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Cure Alzheimer’s Fund

Baila Foundation

Title: Microtubule disruption, BACE1 elevation, and increased Aβ generation in presynaptic dystrophic neurites that surround amyloid plaques in Alzheimer's disease.

Authors: ***K. R. SADLEIR**[1], P. C. KANDALEPAS[1], V. BUGGIA-PRÉVOT[2], D. A. NICHOLSON[3], G. THINAKARAN[2], R. VASSAR[1];
[1]Cell and Mol. Biol., Northwestern Univ., Chicago, IL; [2]Univ. of Chicago, Chicago, IL; [3]Rush Univ., Chicago, IL

Abstract: Alzheimer's disease (AD) is characterized by amyloid plaques composed of the β-amyloid (Aβ) peptide surrounded by swollen presynaptic dystrophic neurites consisting of dysfunctional axons and terminals that accumulate the β-site amyloid precursor protein (APP) cleaving enzyme (BACE1) required for Aβ generation. The cellular and molecular mechanisms that govern presynaptic dystrophic neurite formation are unclear, and elucidating these processes may lead to novel AD therapeutic strategies. Previous studies suggest Aβ may disrupt microtubules, which we hypothesize have a critical role in the development of presynaptic dystrophies. To investigate this further, here we have assessed the effects of Aβ, particularly

neurotoxic Aβ42, on microtubules during the formation of presynaptic dystrophic neurites in vitro and in vivo. Live-cell imaging of primary neurons revealed that exposure to Aβ42 oligomers caused varicose and beaded neurites with extensive microtubule disruption, and inhibited anterograde and retrograde trafficking. In brain sections from AD patients and the 5XFAD transgenic mouse model of amyloid pathology, dystrophic neurite halos with BACE1 elevation around amyloid plaques exhibited aberrant tubulin accumulations or voids. At the ultrastructural level, peri-plaque dystrophies were strikingly devoid of microtubules and replete with multi-lamellar vesicles resembling autophagic intermediates. Proteins of the microtubule motors, kinesin and dynein, and other neuronal proteins were aberrantly localized in peri-plaque dystrophies. Inactive pro-cathepsin D also accumulated in peri-plaque dystrophies, indicating reduced lysosomal function. Most importantly, BACE1 accumulation in peri-plaque dystrophies caused increased BACE1 cleavage of APP and Aβ generation. Our study supports the hypothesis that Aβ induces microtubule disruption in presynaptic dystrophic neurites that surround plaques, thus impairing axonal transport and leading to accumulation of BACE1 and exacerbation of amyloid pathology in AD.

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Nanosymposium

200. Alzheimer's Synaptic Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

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NIH/NIA R01AG044793

Title: Increased neuronal PreP expression improves mitochondrial and synaptic function in old Alzheimer disease’s mouse model

Authors: ***S. YAN**;
Univ. of Kansas, Lawrence, KS

Abstract: Mitochondrial and synaptic dysfunction is the early pathological feature of Alzheimer’s Disease (AD). The underlying mechanisms and strategies to rescue mitochondrial and synaptic injury are not well understood. Presequence protease (PreP), a novel mitochondrial peptidasome, is localized in the mitochondrial matrix in mammals and is responsible for

degradation and clearance of mitochondrial Aβ, which is important for maintaining mitochondrial integrity and function. PreP proteolytic activity is significantly reduced in Aβ-enriched brain mitochondria from AD-affected brains compared to non-AD age-matched controls. The decreased PreP activity is possibly due to enhanced oxidative stress in Aβ- and AD- mitochondria. In the present study, we demonstrate that increased neuronal PreP expression attenuates Aβ accumulation in neuronal mitochondria, amyloid pathology, improves mitochondrial function, suppresses oxidative stress, and alleviates synaptic injury and cognitive decline. Notably, restoring PreP activity significantly attenuates the induction of proinflammatory mediators including cytokine and chemokines in APP mice carrying human mutant APP gene and expressing human Aβ. The protective effects of PreP on Aβ-induced mitochondrial and synaptic injury are even observed in old AD mice at 19-20 month of age. Thus, our results provide *in vivo* evidence that PreP may play an important role in maintaining mitochondrial function and synaptic development along with the improvement in synaptic plasticity by clearance of mitochondrial Aβ. Therefore, enhancing PreP activity or expression may be a new therapeutic target for treatment of AD.

Disclosures: S. Yan: None.

Nanosymposium

200. Alzheimer's Synaptic Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: German center for neurodegenerative diseases

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Tau consortium

Title: The release and trans-synaptic transmission of tau via exosomes derived from cell cultures and CSF

Authors: *Y.-P. WANG[1,2], V. BALAJI[1,3], S. KANIYAPPAN[1,3], L. KRUEGER[1,2], S. IRSEN[2], K. TEPPER[1,2], A. SCHNEIDER[4], E. MANDELKOW[1,2,3], E. MANDELKOW[1,2,3];
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Abstract: Tau pathology in AD spreads in a hierarchical pattern, but the mechanism remains elusive. Exosomes, a type of small extracellular vesicles with a diameter of 50-150nm, have been reported to contribute to the transmission of different pathological proteins in several neurodegenerative diseases including prion diseases and Parkinson disease. However, whether exosomes are involved in Tau pathology spreading is unclear. Here we show that Tau protein can be released via exosomes by cultured primary neurons or by N2a cells overexpressing different Tau constructs. The neuron-derived exosomal Tau protein is hypo-phosphorylated, compared to cytosolic Tau. Activation of neurons by chemical depolarization promotes release of exosomes containing Tau. We find Tau is also present in exosomes isolated from cerebrospinal fluid (CSF) in both AD and control subjects. SDS-stable Tau oligomers with a molecular weight of ~180kD are encapsulated into these human exosomes. By using microfluidic devices which allow the culture of two populations of hippocampal neurons in different compartments, we show that exosomes can mediate the neuron-to-neuron transmission of Tau depending on synaptic connectivity. In organotypic hippocampal slices, Tau-containing exosomes are taken up by neurons and microglia, but not by astrocytes. Finally, we find that in an N2a cell model of Tau aggregation, Tau aggregates can be released via exosomes. Taken together, our study demonstrates that exosomes could play a role in the trans-synaptic transmission of Tau between neurons.

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Nanosymposium

200. Alzheimer's Synaptic Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Tau dependant impairments in hippocampal-cortical network synchrony precede deficits in spatial navigation in the rTg4510 Alzheimer’s mouse model

Authors: *A. BLOCKEEL, M. ALBASSER, G. FERRATI, A. FISHER, S. MEFTAH, T. MURRAY, K. PHILLIPS;
Eli Lilly, Windlesham, United Kingdom

Abstract: The development of Alzheimer’s disease is initially characterised by the accumulation of beta amyloid which is later followed by the aggregation of tau to form neurofibrillary tangles (NFTs). The development of NFTs is most closely correlated with the onset of clinical

symptoms; therefore we focused here on identifying progressive changes in the rTG4510 (Tg) mouse model that may give insights into the tau dependent pathophysiology of the disease. Firstly, spatial working memory was assessed using an automated T-maze apparatus. From the first time point (3 months) of a longitudinal study, female Tg mice (n=20; WT: n=20) were significantly impaired on a delayed non-match to place (DNMTP) task. During the subsequent 9 months there was a further progressive decline in performance that was greatest in the Tg mice. This was prevented by doxycycline treatment, suggesting that it was directly related to tau overexpression. Further evidence for the role of tau in this age related decline was obtained in a cross-sectional study of 8.5 month old Tg mice (n=9; WT: n=9). Again the Tg mice were significantly impaired on the maze, with successful performance negatively correlated with immunohistolocically derived Tau levels in the hippocampus (HPC) & positively correlated with HPC size. Despite the accumulation of NFTs & shrinkage of the brain, Tg mice could successfully perform a simple T-maze task (always left/right) at 12months of age (Tg: n=9; WT: n=9). When the cognitive demands of the task were increased by rule reversal, again the Tg mice were significantly impaired relative to WT controls.
To further investigate how these behavioural effects may be underpinned by altered activity at the neuronal network level, 4 month old mice (Tg: n=8; WT: n=8) were implanted with 16 site (8 per brain region) linear silicon probes targeting the prefrontal cortex (PFC) & multiple subdivisions of the dorsal HPC. The local field potential (LFP) was recorded wirelessly while the mice explored an open field arena at 6, 7 & 8 months of age. At all time points tested, HPC power was lower in the Tg group across the frequency spectrum, likely a result of reduced HPC volume. LFP changes in the PFC were more subtle, with the clearest changes observed in the gamma frequency band (40-80Hz). Similarly to the DNMTP data, there was a progressive decline in gamma power at 7 & 8 months relative to the 6 month time point where both groups exhibited equivalent power in this band. Finally, theta frequency (5-12Hz) HPC-PFC coherence was reduced in Tg mice at all 3 time points tested suggesting that disrupted coordination between brain regions may be a contributory factor in the memory impairments associated with Alzheimer's disease.

Disclosures: **A. Blockeel:** A. Employment/Salary (full or part-time): Eli Lilly. **M. Albasser:** A. Employment/Salary (full or part-time): Eli Lilly. **G. Ferrati:** A. Employment/Salary (full or part-time): Eli Lilly. **A. Fisher:** A. Employment/Salary (full or part-time): Eli Lilly. **S. Meftah:** A. Employment/Salary (full or part-time): Eli Lilly. **T. Murray:** A. Employment/Salary (full or part-time): Eli Lilly. **K. Phillips:** A. Employment/Salary (full or part-time): Eli Lilly.

Nanosymposium

200. Alzheimer's Synaptic Dysfunction

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BrightFocus Foundation (M.I.)

Title: Voltage-gated L-type Ca^{2+} currents play a role in the Aβ-mediated dyshomeostasis of intracellular Ca^{2+} in hypothalamic arcuate (Arc) NPY neurons

Authors: *G. WANG, M. ISHII, M. J. MCGUIRE, C. IADECOLA;
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Abstract: Alterations in leptin signaling in hypothalamic neurons may play a role in the body weight dysfunction seen in pre-symptomatic Alzheimer's disease (AD) (*Cell Metab.,* 2015, 22: 761). We previously reported that the leptin-mediated hyperpolarization of Arc NPY neurons are absent in slices from Tg2576 mice overexpressing the Swedish mutation of amyloid precursor protein (APP) or wild-type (WT) slices treated with amyloid-beta$_{1-42}$ (Aβ_{1-42}) (*J. Neurosci.*, 2014, 34: 9096). Disruption of calcium (Ca^{2+}) homeostasis is one of the major mechanisms by which Aβ alters neuronal function (*Cell* 2012, 148: 1204). Therefore, we tested the hypothesis that APP overexpression or Aβ_{1-42} causes neuronal dysfunction and insensitivity to leptin in Arc NPY neurons by altering intracellular Ca^{2+} homeostasis via L-type Ca^{2+} channel-dependent mechanisms. Intracellular Ca^{2+} levels were measured in WT and APP Arc NPY neurons using Fura2-AM (5 μM) and exciting fluorescence at 340/380 nm. GFP-labeled NPY neurons from APP mice had 2.4-fold higher basal cytosolic-free Ca^{2+} levels than WT mice (APP/WT 340/380nm: 2.4±0.3, $p<0.01$, n=23-55 cells). Similarly, exogenous oligomeric Aβ_{1-42} dose-dependently increased cytosolic-free Ca^{2+} levels in WT neurons (+47% at 300 nM, $p<0.05$, n=5), which was reversed by the L-type Ca^{2+} channel blocker nimodipine (2 μM, +6%, $p>0.05$, n=4). Next, we examined if the Ca^{2+} dysregulation was due to altered voltage-gated L-type Ca^{2+} currents. Using whole-cell voltage-clamp in GFP-labeled Arc NPY neurons from WT or APP slices, L-type Ca^{2+} currents in both WT and APP NPY neurons were blocked by nimodipine and had similar amplitudes ($p>0.05$). However, the L-type Ca^{2+} currents in APP NPY neurons showed a left shift in its current-voltage relationship (I/V) curve, significantly potentiating the currents at relatively hyperpolarized membrane potentials (the potential reaching peak currents: WT at 0 mV, APP at -20 mV, $p<0.05$, n=7-9). Importantly, the CaMKII inhibitor KN93 (10 μM) fully reversed the left shifted I/V curve of the L-type Ca^{2+} currents in APP NPY neurons.

Moreover, leptin (100 nM) partially blocked the L-type Ca^{2+} currents in WT (-41±11%, $p<0.01$ from vehicle, n=7) but not in APP NPY neurons ($p>0.05$, n=7). We conclude that APP overexpression and $A\beta_{1-42}$ can disrupt the intracellular Ca^{2+} homeostasis and responses to leptin in Arc NPY neurons by activating voltage-gated L-type Ca^{2+} influx at hyperpolarized membrane potentials via CaMKII-dependent mechanisms. The data are consistent with the hypothesis that Ca^{2+} dyshomeostasis underlies the mechanisms leading to hypothalamic dysfunction in AD.

Disclosures: G. Wang: None. **M. Ishii:** None. **M.J. McGuire:** None. **C. Iadecola:** None.

Nanosymposium

201. ALS Mechanisms

Location: SDCC 32B

Time: Sunday, November 13, 2016, 1:00 PM - 4:15 PM

Presentation Number: 201.01

Topic: C.05. Neuromuscular Diseases

Title: Development of a novel cell-based model for oligomerization and intercellular transmission of FUS

Authors: *T. HASHIMOTO, T. MATSUMOTO, K. MATSUKAWA, N. WATANABE, T. WAKABAYASHI, T. IWATSUBO;
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Abstract: Intercellular transmission of disease-related proteins is a key process in the progression of a range of neurodegenerative disorders, although the molecular mechanisms of intercellular transmission of pathogenic proteins are still unclear. Cytoplasmic accumulation of fused in sarcoma/translated in liposarcoma (FUS) is a pathologic signature of a subgroup of frontotemporal lobar degeneration (FTLD-FUS) or amyotrophic lateral sclerosis (ALS). Moreover, mutations in *FUS* gene have been identified in the pedigree of familial ALS type 6. To examine whether FUS protein oligomerizes, and subsequently spreads intercellularly, we developed a novel cell-based model using a bi-molecular fluorescent complementation technique. We co-transfected wild-type FUS tagged with amino- or carboxy-terminal fragments of venus into HEK293 cells and found that venus protein was reconstituted and exhibited fluorescence within the nuclei. We also co-transfected fALS-linked P525L mutant FUS tagged with amino- or carboxy-terminal fragments of venus and observed fluorescence in the cytoplasmic granules. These data suggest that fALS mutation of FUS enhanced the cytoplasmic localization of FUS oligomers. Next, to detect intercellular transmission of FUS, we generated stably transfected HEK293 cells with P525L FUS tagged with amino- or carboxy-terminal fragments of venus, and co-cultured the two types of stable transformants. After incubation for 4 days, we observed fluorescence in the cytoplasmic granules in co-cultured cells, which suggested

that split-venus tagged FUS protein were intercellularly transmitted. We thus established a novel cell-based model for monitoring the oligomerization and intercellular transmission of FUS. Application of our model will provide clues to the molecular mechanisms of intercellular transmission of FUS.

Disclosures: **T. Hashimoto:** None. **T. Matsumoto:** None. **K. Matsukawa:** None. **N. Watanabe:** None. **T. Wakabayashi:** None. **T. Iwatsubo:** None.

Nanosymposium

201. ALS Mechanisms

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Time: Sunday, November 13, 2016, 1:00 PM - 4:15 PM

Presentation Number: 201.02

Topic: C.05. Neuromuscular Diseases

Support: CIHR

Title: The connection between microRNAs, RNA-binding proteins and intermediate filament dysregulation in amyotrophic lateral sclerosis (ALS)

Authors: Z. HAWLEY[1], D. CAMPOS-MELO[1], K. VOLKENING[1], *M. J. STRONG[2]; [1]Robarts Res. Inst., Western Univ., London, ON, Canada; [2]London Hlth. Sci. Ctr. - UH, London, ON, Canada

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease that results in loss of motor function and is fatal within 3-5 years of diagnosis. 90-95% of ALS cases are sporadic (sALS). Common pathological features of ALS include cytoplasmic aggregation of RNA-binding proteins (RBPs) and intermediate filaments (IFs) in spinal motor neurons, yet little is known about their etiology.
The 5 main IFs within a mature neuron are neurofilament light, medium and heavy (NFL, NFM and NFH), α-internexin (INA) and peripherin (PRPH); these IFs maintain a specific stoichiometry that ensures cell structure and stability. In sALS, *NEFL*, *PRPH* and *INA* mRNA steady-state levels are selectively suppressed, with no changes to *NEFM* or *NEFH* mRNA. This loss in IF stoichiometry is believed to cause IF aggregation in ALS, yet we lack an explanation as to why we see this selective suppression. Further, Fused in Sarcoma (FUS), Rho guanine nucleotide exchange factor (RGNEF) and TAR DNA-binding protein 43 (TDP-43) are dysregulated RBPs that co-aggregate with each other in sALS. Interestingly, *TDP-43* and *RGNEF* mRNA levels are up- and down-regulated, respectively, while *FUS* mRNA levels have yet to be examined. However, the relationship between FUS, TDP-43 and RGNEF dysregulation in sALS suggests there may be alterations to *FUS* mRNA levels as well.

Recently, microRNAs (miRNA) - small RNA molecules that alter mRNA metabolism - were observed to be massively dysregulated in the spinal cord tissue of sALS patients, providing a possible explanation for the changes we see in RBP and IF mRNA expression. I hypothesize that the dysregulation of specific groups of miRNAs in ALS leads to alterations in levels of IFs (*NEFL, INA* & *PRPH*) and RBPs (*TDP-43, FUS* & *RGNEF*) linked to ALS pathology. Bioinformatic analysis using miRanda software provided 8 miRNA candidates (miR-105, 140-5p, 185, 1179, 1297, 3120, 4306 and b4335) and four miRNA candidates (miR-194, 548x, sb659 and b2122) that potentially regulate the expression of IFs and RBPs, respectively. Real time PCR showed a significant downregulation in only miR-194, b2122, 105 and 140-5p in sALS patients compared to control groups. Currently, *in vitro* studies within HEK293T cells are being executed, which include transfection of our miRNAs and genes of interest to perform gene reporter, RT-qPCR and site-directed mutagenesis assays to determine the effect and specificity of these ALS-linked miRNA candidates on RBP and IF expression.
Overall, this experimental design will determine the miRNAs that participate in the aberrant regulation and expression of RBPs and IFs, and provide a potential pathogenic mechanism for several ALS pathologies.

Disclosures: **Z. Hawley:** None. **D. Campos-Melo:** None. **K. Volkening:** None. **M.J. Strong:** None.

Nanosymposium

201. ALS Mechanisms

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Time: Sunday, November 13, 2016, 1:00 PM - 4:15 PM

Presentation Number: 201.03

Topic: C.05. Neuromuscular Diseases

Title: Synapse loss: An underlying correlate of cognitive decline in amyotrophic lateral sclerosis?

Authors: ***C. HENSTRIDGE**[1], E. CARROLL[1], S. ROTARIU[1], D. SIDERIS[1], J. NEWTON[2], C. SMITH[3], T. GILLINGWATER[4], S. ABRAHAMS[2], T. SPIRES-JONES[1];
[1]Ctr. for Cognitive and Neural Systems, [2]Sch. of Philosophy, Psychology and Language Studies, [3]Ctr. for Clin. Brain Sci., [4]Ctr. for Integrative Physiol., Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease (MND), a fatal neurodegenerative disorder which results from the breakdown of motor neurons in the brain and spinal cord. This manifests as progressive muscle wastage and paralysis, however it is becoming increasingly accepted that ALS is not solely a disorder of the motor

system. Approximately 50% of patients also exhibit cognitive and behavioural deficits that cannot be accounted for by disruption of the motor system. Most common are deficits in verbal fluency and executive function, thought to be controlled by the temporal and frontal lobes respectively. Interestingly, genetic studies have revealed that ALS may lie on a disease spectrum with frontotemporal dementia (FTD). Mutations in c9orf72 or UBQLN2 can result in either ALS or FTD and it's becoming apparent that some FTD patients also exhibit some motor deficits, reinforcing this hypothesis of a disease spectrum.
Synapse loss is a common pathogenic feature of many neurodegenerative disorders, including dementia and we aimed to discover if this was also true for ALS. To do this, we used the high-resolution imaging technique, array tomography to analyse around half a million synapses in total, from 10 control (non-demented) cases and 17 ALS cases. Of the 17 ALS cases, 11 were cognitively tested, 6 were unimpaired and 5 were cognitively impaired. Cognitive testing was performed using the Edinburgh Cognitive and Behavioural ALS Screen (ECAS), to give us an accurate representation of ALS-specific cognitive changes.
There was no significant difference in mean synapse density between all ALS and control cases; however the variability in the ALS group was larger. When the ALS group was split into impaired and unimpaired groups, we discovered that the impaired group had a statistically lower synapse density in the frontal cortex than control non-demented brains. In a separate group of control and ALS cases (not cognitively defined) electron microscopy revealed a significant decrease in synapses from the frontal cortex of ALS brains compared to controls. Furthermore, an increase in degenerating (electron dense) synapses was discovered in the ALS brains.
Taken together, our data suggests that synapse loss may play an important role in the pathogenesis of cognitive decline in ALS patients.

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Nanosymposium

201. ALS Mechanisms

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Presentation Number: 201.04

Topic: C.05. Neuromuscular Diseases

Support: MDA

CIRM

Title: Stem cell modeling of ALS identifies mTOR and mitochondria dysregulation associated to VAPB mutation

Authors: *H. C. MIRANDA[1], J. MORESCO[2], J. M. WARD[1], M. MITNE, Neto[3], J. OKUBO[1], S. MOORE[1], M. ZATZ[4], J. YATES, III[2], A. R. LA SPADA[1], A. R. MUOTRI[1];
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Abstract: Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's disease, is the most common adult-onset motor neuron disease. ALS is characterized by rapidly progressing motor neuron degeneration in the cortex, brainstem and spinal cord accompanied by muscle atrophy, muscular weakness, cramps and fasciculation. Patient's death can occurs between two to five years from the onset of symptoms. To date, the only drug available, Riluzol extends the survival for only 2-4 months and shows mild neuroprotective action. A substantial number of ALS drugs found to alleviate symptoms in animal models have failed in clinical trials, likely due to heterogeneity in the patient cohort. Therefore, disease-specific induced pluripotent stem cells (iPSCs) offer unprecedented potential for the study of rare diseases in vitro, and can provide a platform for drug screening and disease pathway discovery. We pioneer in this field by describing the first ALS in vitro model with a clear molecular phenotype, based on VAMP associated protein B (VAPB) mutant patient-derived cells, derived from ALS8 patients. Recently, we have performed an interactome study in neural progenitor cells (NPCs), motor neurons (MNs) and astrocytes derived from patient and familiar controls iPSCs that identified VAPB interactors. Analyzes of biological processes of the VAPB interactors have shown canonical pathways associated to the mechanistic target of rapamycin (mTOR) and protein translation in the disease context. The mTOR pathway is known to regulate a variety of cell functions, such as protein translation, cell metabolism, mitochondria function and autophagy. Interestingly, we further identified an mTOR dysregulation in ALS derived neural progenitor cells and astrocytes and motor neurons. Our results point to a hyperactivation of the mTOR pathway. Moreover, in our ALS8 patient cells, mTOR dysregulation leads to a protein translation dysregulation, which subsequently culminates with mitochondria depolarization and decreased respiration identified by JC1 and seahorse assays in NPCs and MNs. Finally, electrophysiology investigation using a multi-electrode array (MEA) revealed a decreased neuronal activity in the ALS8 derived MNs compared to controls. Our results thus implicate protein translation and mitochondria dysregulation in ALS8 patients compared to their familial controls, and pinpoint altered mTOR signaling pathway function as a pathological turning point in ALS8 and potentially in other related neurodegenerative proteinopathies.

Disclosures: H.C. Miranda: None. **J. Moresco:** None. **J.M. Ward:** None. **M. Mitne:** None. **J. Okubo:** None. **S. Moore:** None. **M. Zatz:** None. **J. Yates:** None. **A.R. La Spada:** None. **A.R. Muotri:** None.

Nanosymposium

201. ALS Mechanisms

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Time: Sunday, November 13, 2016, 1:00 PM - 4:15 PM

Presentation Number: 201.05

Topic: C.05. Neuromuscular Diseases

Title: Loss of dual leucine zipper kinase signaling is protective in the SOD1 mouse model of ALS

Authors: *C. LE PICHON[1], W. J. MEILANDT[2], S. DOMINGUEZ[2], H. SOLANOY[2], H. LIN[2], H. NGU[2], A. SENGUPTA GHOSH[2], B. WANG[2], Z. JIANG[2], S. LEE[3], M. SIU[2], X. LIU[2], Y. RUDHARD[4], M. BACA[2], A. GUSTAFSON[2], E. J. HUANG[3], O. FOREMAN[2], K. SCEARCE-LEVIE[2], J. W. LEWCOCK[2];
[1]NIH/NINDS, Bethesda, MD; [2]Genentech, South San Francisco, CA; [3]UCSF, San Francisco, CA; [4]Evotec, Hamburg, Germany

Abstract: Hallmarks of chronic neurodegenerative disease include progressive synaptic loss and neuronal cell death, yet the cellular pathways that underlie these processes remain largely undefined. Here we provide evidence that Dual Leucine Zipper Kinase (DLK) is an essential regulator of neurodegeneration in Amyotrophic Lateral Sclerosis (ALS). We demonstrate that DLK/JNK pathway activity is increased in the SOD1 mouse model as well as sporadic ALS patients, and that genetic deletion of DLK protects against axon degeneration, neuronal loss, and functional decline *in vivo*. We have developed DLK inhibitors that preserve neuromuscular junction synapses upon chronic dosing in the SOD1 mouse. Furthermore, in an acute model of neurodegeneration, pharmacological inhibition of DLK pathway signaling is capable of reversing pro-degenerative gene expression changes, suggesting that DLK inhibition could be a viable treatment option even after disease symptoms are already established. Finally, we have identified that pathological activation of DLK is a conserved mechanism that generalizes beyond ALS, regulating neurodegeneration in several disease models, thus making it an attractive target for therapeutic intervention in multiple neurodegenerative indications.

Disclosures: **C. Le Pichon:** None. **W.J. Meilandt:** A. Employment/Salary (full or part-time): Genentech. **S. Dominguez:** A. Employment/Salary (full or part-time): Genentech. **H. Solanoy:** None. **H. Lin:** A. Employment/Salary (full or part-time): Genentech. **H. Ngu:** A. Employment/Salary (full or part-time): Genentech. **A. Sengupta Ghosh:** A. Employment/Salary (full or part-time): Genentech. **B. Wang:** None. **Z. Jiang:** A. Employment/Salary (full or part-time): G. **S. Lee:** None. **M. Siu:** A. Employment/Salary (full or part-time): Genentech. **X. Liu:** A. Employment/Salary (full or part-time): Genentech. **Y. Rudhard:** A. Employment/Salary (full or part-time): Evotec. **M. Baca:** A. Employment/Salary (full or part-time): Genentech. **A. Gustafson:** A. Employment/Salary (full or part-time): Genentech. **E.J. Huang:** None. **O.**

Foreman: A. Employment/Salary (full or part-time): Genentech. **K. Scearce-Levie:** None. **J.W. Lewcock:** None.

Nanosymposium

201. ALS Mechanisms

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Presentation Number: 201.06

Topic: C.05. Neuromuscular Diseases

Support: Emmy Noether

Synergy

Title: The role of TDP-43 and FUS in dendritic RNA transport and regulation.

Authors: *H. BOWDEN, D. DORMANN;
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Abstract: FUS and TDP-43 are RNA-binding proteins that play a key role in the pathogenesis of ALS and FTD. Although primarily nuclear, both proteins are found in neuronal processes and neuronal RNA transport granules and therefore are thought to be involved in mRNA transport in dendrites and dendritic spines. However, their dendritic mRNA targets are not well-described. The aim of this study is to identify the protein and RNA components of cytosolic FUS and TDP-43 granules, as well as elucidate the potential role of TDP-43 and FUS in dendritic mRNA localization and translational control. Here we report first insights into the protein and RNA composition of FUS and TDP-43 RNP granules immunoprecipitated from the cytosolic fraction of mouse brain. For FUS, we will compare our results to knock-in mice carrying a NLS truncation mutation in order to study how disease-associated mutations may alter the granule composition and therefore RNA target regulation, e.g. dendritic localization and local translation at synapses.

Disclosures: H. Bowden: None. **D. Dormann:** None.

Nanosymposium

201. ALS Mechanisms

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Presentation Number: 201.07

Topic: C.05. Neuromuscular Diseases

Support: CIHR

MDA

NSERC

Title: Implication of TDP 43 in the stress response in neurodegenerative disease

Authors: *Y. KHALFALLAH[1], C. PELLETIER[2], C. VANDE VELDE[2];
[1]Biochem. and molecular medicine, [2]Neurosciences, CRCHUM- Univ. of Montreal, Montreal, QC, Canada

Abstract: Environmental factors are suspected of playing a major role in neurodegenerative diseases. The affected cell type (neurons) is more sensitive to stressful stimuli such as oxidative stress compared to other cell types. One of the genes implicated is TARDBP, which encodes TAR DNA binding protein 43 (TDP-43). TDP-43 is a principal component of neuronal and glial inclusions in ALS and FTD (frontotemporal dementia) patients and disease-causing mutations in TDP-43 are well documented. Whether mutations yield a gain or loss of TDP-43 function and the effect of TDP-43 nuclear depletion associated with cytoplasmic inclusion formation is not yet resolved. We have shown that depletion of TDP-43 impairs stress granule (SG) assembly through the depletion of another core component of SGs, G3BP1. This protective pathway comprises the rapid and transient formation of granules that store translationally repressed mRNA during stress conditions. SGs are dynamic and form via an initial nucleation event followed by assembly where small SGs fuse to form larger SGs, and then disassemble. In order to directly test our hypothesis that TDP-43 depletion from the nucleus and mislocalisation to the cytoplasm, impair SG signalling through a loss of function, we have evaluated this mechanism *in vitro* in cell types relevant to ALS/FTD including cortical neurons and astrocytes. Also, to assess the consequences of the possible mislocalisation to the cytoplasm of TDP-43 on SG components, we have an *in vivo* axonal injury model. Our results demonstrate a conserved phenotype in the default of SG assembly due to TDP-43 depletion in neurons and astrocytes. Moreover, in conditions of lower levels of TDP-43, we note an acceleration of SG disassembly in astrocytes. Using osmotic stress, astrocytes demonstrate a robust dependence on TDP-43 for efficient SG dynamics. These findings are one step further towards understanding the selective cellular vulnerability that is characteristic of ALS.

Disclosures: **Y. Khalfallah:** None. **C. Pelletier:** None. **C. Vande Velde:** None.

Nanosymposium

201. ALS Mechanisms

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Title: Calcineurin protects against pathological phosphorylated TDP-43 by direct dephosphorylation

Authors: ***N. LIACHKO**[1,3], A. D. SAXTON[1], P. J. MCMILLAN[1,2,4], T. J. STROVAS[1], J. M. WHEELER[1], A. L. OBLAK[7], B. GHETTI[7], T. J. MONTINE[5], C. D. KEENE[5], M. A. RASKIND[2], T. D. BIRD[1,6,3], B. C. KRAEMER[1,3,4];
[1]GRECC, [2]Mirecc, VA Puget Sound Hlth. Care Syst., Seattle, WA; [3]Med., [4]Psychiatry and Behavioral Sci., [5]Pathology, [6]Neurol., Univ. of Washington, Seattle, WA; [7]Pathology and Lab. Med., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Proteinaceous detergent insoluble inclusions of TDP-43 protein are hallmarks of the neuropathology in over 90% of amyotrophic lateral sclerosis (ALS) cases and approximately half of frontotemporal dementia (FTLD-TDP) cases. In TDP-43 proteinopathy disorders, lesions containing aggregated TDP-43 protein are extensively post-translationally modified, with phosphorylated TDP-43 (pTDP) being the most consistent and robust marker of pathological TDP-43 deposition. Abnormally phosphorylated TDP-43 has been shown to mediate TDP-43 protein stability and turnover, cellular localization, protein aggregation, and neurotoxicity in neurodegenerative disease models. To date several different kinases have been implicated in the genesis of pTDP, but no phosphatases have been shown to reverse pathological TDP-43 phosphorylation. We have identified the phosphatase calcineurin as an enzyme binding to and catalyzing the removal of pathological C-terminal phosphorylation of TDP-43 *in vitro*. In *C. elegans* models of TDP-43 proteinopathy, genetic elimination of calcineurin results in accumulation of excess pTDP, exacerbated motor dysfunction, and accelerated neurodegenerative changes. In cultured human cells, treatment with FK506 (tacrolimus), a

calcineurin inhibitor, results in accumulation of pTDP species. Lastly, calcineurin co-localizes with pTDP in disease relevant brain regions in subjects with FTLD-TDP and in spinal cord motor neurons from subjects with ALS. Taken together these findings suggest calcineurin acts on pTDP-43 as a phosphatase in neurons. Furthermore, patient treatment with calcineurin inhibitors may have unappreciated adverse neuropathological consequences.

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Nanosymposium

201. ALS Mechanisms

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Title: Endogenous MIF reduces the accumulation and toxicity of misfolded SOD1 in a mouse model of ALS

Authors: ***A. ISRAELSON**, M. LEYTON, C. BENAIM, S. ABU-HAMAD;
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Abstract: Mutations in superoxide dismutase (SOD1) cause amyotrophic lateral sclerosis (ALS) - a fatal neurodegenerative disease characterized by the loss of upper and lower motor neurons in the brain and spinal cord. It has been suggested that the toxicity of mutant SOD1 results from its misfolding and accumulation on the cytoplasmic faces of intracellular organelles, including the mitochondria and endoplasmic reticulum (ER) of ALS-affected tissues. Recently, macrophage migration inhibitory factor (MIF) was shown to directly inhibit the accumulation of misfolded SOD1 and its binding to intracellular membranes, but the role of endogenous MIF in modulating SOD1 misfolding *in vivo* remains unknown. To elucidate this role, we bred MIF-deficient mice with $SOD1^{G85R}$ mice, which express a dismutase-inactive mutant of SOD1 and are considered a model of familial ALS. We found that the accumulation of misfolded SOD1, its association with

mitochondrial and ER membranes, and the levels of sedimentable insoluble SOD1 aggregates were significantly higher in the spinal cords of $SOD1^{G85R}$-$MIF^{-/-}$ mice than in their $SOD1^{G85R}$-$MIF^{+/+}$ littermates. Moreover, increasing MIF expression in neuronal cultures inhibited the accumulation of misfolded SOD1 and rescued from mutant SOD1-induced cell death. In contrast, the complete elimination of endogenous MIF accelerated disease onset and late disease progression and shortened the lifespan of the $SOD1^{G85R}$ mutant mice. These findings indicate that MIF plays a significant role in the folding and misfolding of SOD1 *in vivo,* and they have implications for the potential therapeutic role of upregulating MIF within the nervous system to modulate the selective accumulation of misfolded SOD1.

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Nanosymposium

201. ALS Mechanisms

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Minna-James-Heineman-Stiftung

French Muscular Dystorphy Association (AFM)

Alexander von Humboldt Foundation

Title: *Drosophila* FUS mutant phenotypes are mediated by increased Xrp1 expression leading to gene expression dysregulation

Authors: *E. STORKEBAUM[1], M. MALLIK[1], M. CATINOZZI[1], C. B. HUG[2], M. WAGNER[1], L. ZHANG[1], S. MERSMANN[1], M. FRICKENHAUS[1], O. SENDSCHEID[1], J. M. VAQUERIZAS[2];
[1]Mol. Neurogenetics Lab., [2]Regulatory Genomics, Max Planck Inst. For Mol. Biomedicine, Muenster, Germany

Abstract: Defects in RNA biogenesis may causally contribute to neurodegenerative diseases, as genetic mutations in several RNA-binding proteins (RBPs) give rise to the motor neurodegenerative disorder amyotrophic lateral sclerosis (ALS), and RBP-containing inclusions

are a pathological hallmark of both ALS and frontotemporal dementia (FTD). Among these RBPs, the FET family proteins FUS, TAF15 and EWSR1 are DNA- and RNA-binding proteins involved in regulation of transcription, mRNA splicing and mRNA subcellular localization. Loss of function of the single *Drosophila* FET orthologue *cabeza (caz)* results in inability of fully differentiated adult flies to eclose from the pupal case due to motor deficits. Here, we performed a genetic modifier screen for rescue of *caz* mutant pupal lethality, which identified *Xrp1* as a key modifier gene. Heterozygosity for *Xrp1* not only rescued *caz* mutant pupal lethality, but also adult motor performance and life span. Interestingly, selective knock-down of Xrp1 in neurons was sufficient to rescue *caz* mutant phenotypes. Xrp1 expression is strongly upregulated in *caz* mutants, and selective Xrp1 overexpression in neurons of otherwise wild type flies results in developmental lethality, with adult escaper flies displaying motor performance defects and shortened life span. The genetic interaction between *caz* and *Xrp1* was dependent on the functionality of the AT-hook DNA-binding domain in Xrp1. Finally, high-throughput RNA sequencing revealed profound gene expression dysregulation in *caz* mutant animals, which was substantially mitigated by *Xrp1* heterozygosity. Together, our findings indicate that *caz* mutant phenotypes are mediated by increased neuronal Xrp1 levels, leading to gene expression dysregulation and neuronal dysfunction.

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Nanosymposium

201. ALS Mechanisms

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Topic: C.05. Neuromuscular Diseases

Support: Target ALS

Title: iPSC disease modeling of the excitability phenotype of ALS patient derived motor neurons

Authors: *K. C. ROET[1], O. WISKOW[2], S. LEE[1], X. HUANG[1], J. SANDOE[2], A. GRANTHAM[1], D. BAKER[2], L. BARRETT[1], K. EGGAN[2], C. J. WOOLF[1];
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Abstract: Induced pluripotent stem cell (iPSC) technologies provide the opportunity for both human disease modeling and the execution of phenotypic drug screens. This is especially true for

poorly understood neurodegenerative neurological disorders that are without effective treatments such as Amyotrophic Lateral Sclerosis (ALS). Our labs have recently discovered that motor neurons (MNs) derived from ALS patient induced pluripotent stem cells (IPSC) carrying SOD1, C9orf72 and FUS mutations are hyperactive relative to those from control subjects as measured by Multi-ElectrodeArray (MEA) recordings (Wainger et al, 2014). We have now set out to validate the disease modeling aspects of these observations and their relevance for phenotypic screens for which three criteria have been suggested: (i) Disease relevance of the assay system. (ii) Disease relevance of the stimulus. And (iii) assay readout proximity to the clinical end point. Clinically, ALS is associated with development of neuronal hyperexcitability at many levels, including cortical motor neurons, spinal motor neurons, and peripheral axons. Importantly, the degree of hyperexcitability in ALS patients correlates with their survival. The highly penetrant SOD1_A4V mutation was selected for an in depth characterization of the hyperexcitability phenotype in spinal motor neurons. Two motor neuron differentiation protocols from iPSC lines were employed, a 3D method based on embryoid bodies and a 2D method based on monolayer differentiation. Motor neuron specificity was ensured by generation of Hb9-GFP reporter lines which allowed FACS based purification of motor neurons. Both protocols from two independent patient lines yielded motor neurons that were hyperactive relative to their isogenic corrected controls, albeit with slight differences in time of onset. Hyperexcitability of the motor neurons was measured by a patch clamp current ramp protocol, calcium level measurements using GCaMP6 and by MEA, and for all three measurements the diseased motor neurons were more active than the isogenic controls. We conclude that iPSC derived motor neurons of ALS patients carrying the SOD1_A4V mutation show a robust hyperexcitability and hyperactivity phenotype. The three criteria for conducting a phenotypic screen are met and therefore a valuable tool is provided for the identification of compounds that can normalize the hyperactivity. *Part of this work was supported by GlaxoSmithKline*

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Nanosymposium

201. ALS Mechanisms

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Time: Sunday, November 13, 2016, 1:00 PM - 4:15 PM

Presentation Number: 201.12

Topic: C.05. Neuromuscular Diseases

Support: National Science Foundation Graduate Research Fellowship

Axol Science Scholarship

Thomas Shortman Graduate Scholarship

The Solomon H. Snyder Department of Neuroscience Graduate Program

Target ALS

NIH

ALSA

Title: The nuclear pore complex is compromised in ALS

Authors: *J. C. GRIMA[1], K. ZHANG[1], J. DAIGLE[1], J. T. PHAM[1], J. C. GLATZER[2], A. D. MATLOCK[3], V. J. DARDOV[3], Y. ZHANG[4], J. CHEW[4], M. J. ELRICK[1], Y. HUO[1], J.-P. RICHARD[1], L. OSTROW[1], N. J. MARAGAKIS[1], C. J. DONNELLY[5], J. VAN EYK[3], L. PETRUCELLI[4], T. E. LLOYD[1], J. D. ROTHSTEIN[1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease and Frontotemporal dementia (FTD) is the second most common form of early-onset dementia. Interestingly, both of these devastating neurodegenerative diseases share a common genetic mutation in chromosome 9 open reading frame 72 (C9orf72). An expanded hexanucleotide repeat (GGGGCC) in intron 1 of the C9orf72 gene is the most common genetic cause of familial and sporadic ALS and FTD, along with Huntington's disease phenocopies. Until recently, very little was known about the underlying mechanisms by which this expanded repeat causes neurodegeneration until 5 independent labs published 3 papers including one from our own group simultaneously showing that dysfunction in Nucleocytoplasmic Transport (NCT) may be a fundamental pathway for C9orf72 ALS-FTD pathogenesis. NCT, the trafficking of protein and RNA between the nucleus and cytoplasm, is critical for signal transduction and is especially arduous for neurons due to their highly polarized biology. Efficient regulation of this process is mediated by the Nuclear Pore Complex (NPC), an extraordinary molecular machine that serves as the main gateway to the nucleus. In order for any cell to function properly, it is imperative that RNA and protein be efficiently and selectively exchanged between the nucleus and the cytoplasm. This critical task is achieved by the ~2000 NPCs that span the entire nuclear envelope. Each NPC consists of multiple copies of 30 different proteins called Nucleoporins (NUPs) that differ in anatomical location, function, domain, post-translational modification and residence time. Mutations in various NUPs result in tissue-specific diseases. Additionally, some of the longest-lived proteins in the mammalian brain are specific NUPs and may represent the "weakest link" in the aging proteome. We now present data using human brain and iPS neurons that the NPC may also be compromised in sporadic ALS (sALS). We have surveyed the majority of NUPs in transgenic and BAC C9orf72 mice, iPS neurons/astrocytes, HEK293 cells and human postmortem brain tissue using IF, IHC, super resolution imaging, western blot, FRAP, shRNA, overexpression constructs and proteomic analysis. We have identified a unique set of

NUPs with critical and disease relevant functions that are consistently affected across not only models of C9orf72 but also sALS indicating that NPC dysfunction may be a common insult and pathogenic mechanism in the majority of ALS. This suggests that NPC dysfunction may be a critical global mechanism of neurodegeneration and what distinguishes one disease from another is the unique set of NUPs that are differentially affected.

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Nanosymposium

201. ALS Mechanisms

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Time: Sunday, November 13, 2016, 1:00 PM - 4:15 PM

Presentation Number: 201.13

Topic: C.05. Neuromuscular Diseases

Title: Ataxin-2 is a common mediator of nucleocytoplasmic transport defects in ALS and dementia

Authors: ***K. ZHANG**[1], J. GRIMA[2], G. DAIGLE[1,3], K. CUNNINGHAM[4], T. LLOYD[1,4], J. ROTHSTEIN[1,2,3];
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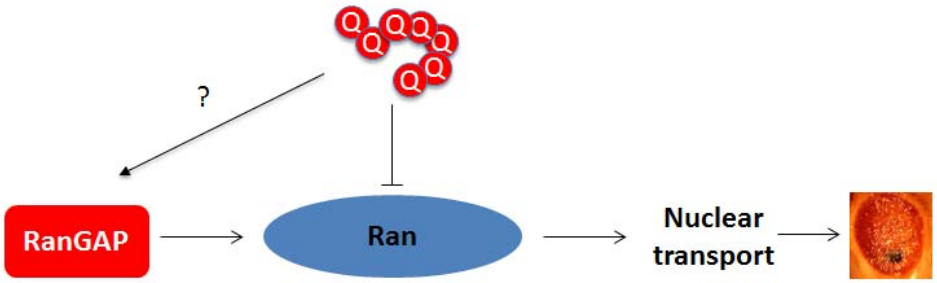
Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating motor neuron degenerative disease that is often associated with frontotemporal dementia (FTD). A GGGGCC hexanucleotide repeat expansion (HRE) in C9orf72 is the most common genetic cause of ALS/FTD. Recently, we reported defects in nucleocytoplasmic transport as a fundamental pathogenic mechanism of C9orf72-mediated ALS/FTD (C9-ALS) in model systems including *Drosophila* and human induced pluripotent stem neurons as well as patients. We showed that the HRE directly interacts with and sequesters RanGAP, a master regulator of nucleocytoplasmic transport. In C9-ALS, both RanGAP and its downstream target, Ran GTPase (Ran), are mislocalized, causing impaired nuclear import and neurodegeneration that can be suppressed by nuclear export inhibitors. Here we show that nucleocytoplasmic transport is also disrupted in a large fraction of sporadic ALS cases, raising the possibility that some risk factors of ALS may also affects nucleocytoplasmic transport. Indeed, we discovered that mutant Ataxin-2 with an expansion of polyglutamine (polyQ) repeats, an ALS risk factor, causes cytoplasmic

mislocalization and accumulation of Ran. Importantly, accumulated Ran co-localizes with cytoplasmic aggregates of Ataxin-2-polyQ, suggesting that Ataxin-2-polyQ may directly bind and sequester Ran. Consistently, we show that Ran co-immunoprecipitates with Ataxin-2-polyQ *in vitro*. Moreover, Ataxin-2-polyQ enhances the nucleocytoplasmic transport defects as well as neurodegenerative phenotypes in *Drosophila* eyes caused by the HRE, suggesting that they interact genetically. Hence, we hypothesize that Ataxin-2-polyQ contributes to ALS by disrupting nucleocytoplasmic transport via sequestration of Ran. As polyQ has been implicated in many neurodegenerative diseases, including spinocerebellar ataxia, Huntington's disease, and spinal-bulbar muscular atrophy, our findings suggest a general role for dysfunctional nucleocytoplasmic transport in repeat-associated neurodegenerative disorders.



Disclosures: **K. Zhang:** None. **J. Grima:** None. **G. Daigle:** None. **K. Cunningham:** None. **T. Lloyd:** None. **J. Rothstein:** None.

Nanosymposium

202. Stroke and Injury: Optogenetic and Chemogenetic Approaches

Location: SDCC 24A

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Presentation Number: 202.01

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

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The Dana Foundation

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Title: Microinfarcts produce widespread functional deficits in perilesional tissues

Authors: *A. Y. SHIH[1], P. M. SUMMERS[1], D. A. HARTMANN[1], R. L. DEARDORFF[2], X. NIE[2], J. A. HELPERN[2], E. S. HUI[3], J. H. JENSEN[2];
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Abstract: A greater microinfarct burden at autopsy is linked to a higher risk for vascular cognitive impairment and dementia during life. The mechanisms by which microinfarcts contribute to cognitive impairment remain unclear. While individually miniscule in size, microinfarcts could induce pathological changes that contribute to brain dysfunction distant from the lesion core. We tested this possibility by examining the core and perilesional regions of microinfarcts in a mouse model using *in vivo* MRI, two-photon imaging and *post-mortem* histology. To model microinfarcts, single pial penetrating vessels supplying the cortex were non-invasively occluded by focal photothrombosis. The resulting mouse lesions bore remarkable similarity to a subset of microinfarcts found in human brain and could be detected with structural and diffusion MRI for 5 to 7 days post-onset. However, MRI reported only the nonviable microinfarct core. When microinfarcts were strategically generated in the vibrissa sensory cortex, they induced deficits in neural and hemodynamic responses in viable perilesional tissues surrounding the core. This region of deficit occupied a volume estimated to be 12 to 25-fold greater than that of the core and was most prominent during peak MRI signal at 1 to 3 days post-occlusion. Thus, microinfarcts in the human brain may induce broad functional deficits that are secondary to the spatially restricted cell death that occurs within their ischemic cores.

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Nanosymposium

202. Stroke and Injury: Optogenetic and Chemogenetic Approaches

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Presentation Number: 202.02

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Heart & Stroke Foundation of Canada (EH)

Canadian Institutes of health research (EH, MOP-142417)

Canadian Institutes of health research (AS, MOP-102599).

Title: Optogenetic activation of DBH-neurons in the Locus Coeruleus impact cortical neurovascular coupling responses

Authors: *C. LECRUX[1], Z. YAO[3], E. MITCHELL[1], A. LIM[1], L. GALLET[1], A. ADAMANTIDIS[2,4,5], A. SHMUEL[3], E. HAMEL[1];
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Abstract: Neurovascular coupling (NVC), which allows a precise match between activated brain region and blood supply, is a fundamental brain function that is the basis of functional brain imaging relying on hemodynamic signals. However, the impact varying brain states on NVC responses remains poorly characterized. In particular, the activity of noradrenergic neurons originating from the locus coeruleus (LC) is linked to changes in cortical activity underlying brain states, as seen during sleep-wake cycles, and may influence NVC. We therefore investigated the effects of increased NA neurotransmission on the well-characterized whisker-evoked NVC response. Dopamine-β hydroxylase (DBH)-Cre mice injected with AAVdj-EF1α-DIO-CHETA-eYFP and optogenetic stimulation (blue laser, 473 nm) were used to specifically target LC NA neurons. Cortical maps of hemodynamic responses were measured by optical imaging of intrinsic signals over the barrel cortex. Whisker stimulation (4Hz, 5sec) increased cerebral blood volume (CBV, assessed by 570nm reflectance) in the contralateral barrel cortex. In contrast, optogenetic LC NA neurons activation (5-20mW, 5Hz, 10sec) induced a consistent intensity-dependent decrease in the CBV map (570nm reflectance), an effect not seen in control animals. Sub-threshold stimulation of LC (5mW, 5Hz, 10sec), defined as having no significant effect on baseline CBV, led to a significantly decreased CBV response to whisker stimulation (-35%, $p<0.05$, $n=8$). Overall, our results demonstrate a role for LC NA neurons as potential modulators of the NVC response to sensory stimulation. Current work is in progress to identify the effects of optogenetic activation of LC NA on the activity of the neuronal network underlying the sensory-evoked hemodynamic responses.

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Nanosymposium

202. Stroke and Injury: Optogenetic and Chemogenetic Approaches

Location: SDCC 24A

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Presentation Number: 202.03

Topic: C.08.Stroke

Support: CIHR MOP-12675 and Foundation Grant 143209

Canadian Partnership for Stroke Recovery

Leduc Collaborative Grant

Title: Assessing the cortical contribution to recovery after focal ischemia in awake, behaving mice using optogenetic indicators and actuators

Authors: *G. SILASI[1], M. VANNI[2], R. KATREDDI[2], Y. SEKINO[2], F. BOLANOS[2], J. BOYD[2], S. SCOTT[3], T. MURPHY[2];
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Abstract: Functional recovery after injury to the motor cortex can be facilitated by changes in multiple CNS regions, including cortical, sub-cortical and spinal circuits. This is especially true for behaviours requiring whole limb movements, such as pressing a lever or positioning a robotic arm in space, as these movements may be partially generated outside the motor cortex. Such tasks are readily performed by rodents even while headfixed, and offer the ability to assess motor learning or recovery from injury such as stroke.
In the current experiment we trained head-fixed mice to displace a robotic lever to a rewarded position in order to receive a water reward. We then confirmed the necessity of the motor cortex in executing the task by inactivating M1 in VGAT-ChR2 expressing mice. The probability of performing successful auditory cue-initiated trials was significantly decreased during contralateral, but not ipsilateral M1 inactivation (~65% reduction, p=0.004). This effect was mostly driven by the inability of the mice to initiate task execution, as the mice attempted ~50% fewer trials during contralateral M1 inhibition (p=0.001; vs. ipsilateral stimulation). These data demonstrate the necessity of contralateral M1 in moving a robotic lever with the forelimb by headfixed mice.
We then applied this task in mice recovering from M1 focal ischemic injury in order to track mesoscale changes in functional cortical activation through widefield calcium imaging. Imaging was performed transcranially through a chronic window that did not require removal or thinning of the skull. Transgenic mice (Ai94 or Ai93; Jackson Labs) expressing the GCaMP6 calcium indicator completed an average of 128±27 successful trials per 20-minute session. Ca^{2+} imaging revealed a time-locked increase in activity of the limb region (8.5% $\Delta F/F$) while barrel cortex

and other sensory areas showed less activity (1.8% ΔF/F) during pulling. Compared to a sham group (n=6), mice with a stroke in the limb region of the cortex (n=14) produced smaller responses during successful trials in the first week of recovery (ΔF/F =2.6±0.5% and 7.4±1.1%, $p<0.001$). After 2 months, a partial recovery of the responses was observed (4.1±0.4% and 6.1±1.0% p=0.045), presumably driven by increased activity in peri-infarct cortex.
Our results demonstrate the feasibility to monitor post-stroke recovery with a simple, robotic arm displacement task that requires contralateral M1 activity. Concurrent quantification of cortical activity through calcium imaging and behavioural performance allows us to identify peri-infarct as well as distal regions that may contribute to spontaneous recovery in this task over days and weeks.

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Nanosymposium

202. Stroke and Injury: Optogenetic and Chemogenetic Approaches

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Presentation Number: 202.04

Topic: C.08.Stroke

Support: "GIST-Caltech Research Collaboration" Project through a grant provided by GIST in 2015

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Title: Optogenetic stimulation for cell-specific activation of sensory-parietal cortex in chronic subcortical capsular infarct model

Authors: ***R. KIM**[1], J.-Y. PARK[1], H. SONG[1], J. REE[2], J. CHO[1], M.-C. LEE[3], P. ROSA-NETO[4], H.-I. KIM[1];
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Abstract: Subcortical capsular stroke causes severe disability. Our previous study revealed that motor deficit can be overcome by administering the electrical stimulation on sensory-parietal cortex, regardless of extent of infarction in capsular infarct model. However, electrical stimulation influences all types of neurons and glial cells randomly, so that stimulation-responsive neural circuits cannot be clarified. In this study, we employed the optogenentic stimulation and microPET imaging to determine the cell-specific activation of neural circuit as well as to find the imaging biomarker which may indicate the progress of behavioral recovery in capsular infarct. We used twenty-two male Sprague Dawley rats which capsular infarct was created by photothrombosis. The addeno-asscoiated viral vector encoding channelrhodopsin was combined with promoters, CamKIIa (CamKIIa group), GFAP (GFAP group), or hSyn (hSyn group) to differentially stimulate pyramidal, glial or pan-neuronal cells. The virus construct was injected and expressed in sensory-parietal cortex, followed by blue laser stimulation. Optrode recording was conducted to validate that >1mW of 473nm light pulses at 10Hz induced local neuronal firing. Two times of one hour stimulation per day were delivered for 14 days, along with rehabilitative training using single pellet reaching task (SPRT) in capsular infarct model rats. In addition, microPET scans were performed longitudinally prior to stimulation, and at 7 and 14 days of stimulation. The images were analyzed using a group-level linear mixed-effect model. Our results showed that CamKIIa, GFAP, and hSyn groups had motor improvement up to 15%, 50%, and 65% of pre-lesional SPRT scores, respectively. MicroPET images demonstrated that diaschisis was observed at the 7^{th} day of stimulation in all of the groups, however, the reduction of diaschisis was correlated with behavioral recovery. The reduction of diaschisis was greater in GFAP and hSyn groups (>50%) whereas CamKII group did not show the change in its extent at the 14^{th} day. In addition, subcortical activations circuits were differentially constructed for each group. It is concluded that motor recovery after capsular stroke can be augmented by cell-specific stimulation. Stimulation of pan-neuronal and glial cells were more effective than pyramidal neuronal stimulation, and the motor recovery was correlated with the reduction of diaschisis.

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Nanosymposium

202. Stroke and Injury: Optogenetic and Chemogenetic Approaches

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Presentation Number: 202.05

Topic: C.08.Stroke

Support: 1R01NS093057

R21NS082894

Title: Cerebellar dentate nucleus is a promising brain stimulation target for enhancing post-stroke recovery

Authors: *M. Y. CHENG, A. M. SHAH, S. ISHIZAKA, E. H. WANG, A. R. BAUTISTA, S. LEVY, D. SMERIN, G. SUN, G. STEINBERG;
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Abstract: Objective: Functional recovery after stroke has been observed in both human and animals. Post-stroke brain stimulations are promising neurorestorative techniques as they allow direct manipulation of the target area's excitability. Previously we have demonstrated that optogenetic neuronal stimulation of the ipsilesional primary motor cortex (iM1) promotes functional recovery. In an effort to identify optimal brain stimulation targets, we investigate the effects of stimulation in the cerebellar dentate nucleus (cDN) on poststroke recovery. We hypothesize that stimulation of cDN may be more effective than iM1, as it sends major excitatory outputs to multiple motor and non-motor areas.
Methods: Thy-1-ChR2-YFP line-18 transgenic male mice were used. Mice underwent stereotaxic surgery to implant an optical fiber in cDN or iM1. One week after implant, stroke was induced using an intraluminal middle cerebral artery suture occlusion (30 minutes). Three groups of mice were used: control non-stimulated stroke mice, short-stimulated stroke mice (short-stim, poststroke day5-14) and long-stimulated stroke mice (long-stim, poststroke day5-28). Sensorimotor behavior tests (rotating beam tests) were used to assess their recovery at day 0, 4, 7, 10, 14, 21 and 28 post-stroke.
Results: Our results demonstrate that repeated cDN stimulations result in robust recovery in motor sensory functions as early as poststroke day7 in distance traveled ($p<0.001$) and speed ($p<0.05$). Comparison of iM1 and cDN-stimulated mice indicate that cDN-stimulated mice recovered significantly faster than iM1-stimulated mice (speed, $p<0.01$). The effect of cDN stimulation was also persistent, as stimulated mice maintained their recovery state after day14 without further stimulations ($p<0.001$). The long-stim group did not further enhance recovery, suggesting that prolonged stimulations may not be necessary to achieve permanent recovery.
Conclusion: Our data demonstrate that cDN stimulations post-stroke can promote robust and persistent functional recovery. Furthermore, cDN-stimulated mice recovered faster than iM1-stimulated mice, indicate that cDN is a more effective brain stimulation target. Current studies examine the brain activation patterns of cDN-stimulated mice, as well as examining their transcriptome using RNAseq, which will provide insight on the molecular pathways involved in cDN-stimulation induced recovery.

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Nanosymposium

202. Stroke and Injury: Optogenetic and Chemogenetic Approaches

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Presentation Number: 202.06

Topic: C.08.Stroke

Support: 1R01NS093057

Title: Optogenetic neuronal stimulation reduces expression of neuronal nitric oxide synthase in the contralesional motor cortex after stroke

Authors: *D. L. SMERIN, M. CHENG, L. GONZALES, S. LEVY, E. WANG, S. WANG, S. SHUNSUKE ISHIZAKA, G. SUN, G. STEINBERG;
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Abstract: Objective: Nitric oxide (NO) is a gaseous messenger that mediates many physiological functions, including neuronal excitability, vascular tone and angiogenesis. Nitric oxide synthases (NOS) are a family of enzymes that catalyzes the production of NO. While activation of endothelial NOS (eNOS) has been shown to be neuroprotective, activation of neuronal NOS (nNOS) or inducible NOS (iNOS) worsens ischemic damage. Although the roles of NOS in acute stroke are well-established, their contribution to stroke recovery is less clear. Previously we demonstrated that optogenetic neuronal stimulation increases cerebral blood flow (CBF) and enhances post-stroke recovery. Given the role of NO in cerebral blood flow regulation, we investigated whether NOS is involved in stimulation-enhanced recovery. We hypothesized that neuronal stimulations promote recovery in part by altering NOS expression.
Methods: Thy-1-ChR2-YFP line-18 transgenic male mice were stereotaxically implanted with an optical fiber in the contralesional cerebellar dentate nucleus (cDN). One week later, mice underwent experimental stroke induced by an intraluminal middle cerebral artery suture occlusion (30min). Repeated optogenetic stimulations in the cDN were administered from post-stroke day5-14. Functional recovery was evaluated by the rotating beam test at post-stroke day 0, 4, 7, 14. Mice were sacrificed at post-stroke day15 and ipsilesional and contralesional motor and sensory cortices were processed for quantitative PCR to examine expression of nNOS, iNOS and eNOS. GAPDH was used as a housekeeping control.
Results: Our data showed that cDN-stimulated mice traveled a significantly longer distance ($p<0.001$) at a significantly faster speed ($p<0.05$) as early as day7 post-stroke compared to non-stimulated mice. Quantitative PCR revealed that stroke significantly increased nNOS expression in the contralesional motor cortex (cM1) at day15 post-stroke ($p<0.01$), and cDN-stimulations significantly reduced this nNOS expression ($p<0.001$). Interestingly, no change was detected for eNOS and iNOS. Western blot analysis further confirmed the reduction of total nNOS protein in cM1 of cDN-stimulated mice.

Conclusion: Optogenetic stimulations promoted post-stroke recovery and selectively reduced nNOS expression in the contralesional motor cortex. Our results further highlight the involvement of nNOS and the contralesional cortex in stroke recovery. We are currently examining the nNOS signaling pathway after stimulation, and the effects of manipulating nNOS expression on stroke recovery.

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Nanosymposium

202. Stroke and Injury: Optogenetic and Chemogenetic Approaches

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Presentation Number: 202.07

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant NS087198-03

Title: Astrocytes regulate the spatiotemporal pattern of glutamate released after brain injury

Authors: *S. A. LEVINSON[1], J. CICHON[1], M. SANTELLO[2], W. GAN[1];
[1]New York Univ., New York, NY; [2]Physiol., Univ. of Zurich, Zurich, Switzerland

Abstract: Traumatic brain injury (TBI) and stroke have different underlying causes but a common mechanism contributing to neuronal damage is glutamate excitotoxicity. Ischemia leads to large increases in neuronal intracellular calcium, excessive glutamate release, and necrosis or apoptosis. Current treatments for ischemia focus on reinstating the blood supply, however a better understanding of the source and mechanisms underlying glutamate released after brain injury could lead to therapeutic strategies to more specifically target excitotoxicity. Visualizing glutamate directly *in vivo* has been made possible by the development of a genetically encoded fluorescent probe, iGluSnFr (Marvin et al., 2013), now allowing us to obtain a high resolution view of the previously undescribed spatial and temporal properties of injury-induced glutamate release. We expressed the iGluSnFr probe on the extracellular membrane of astrocytes in the anesthetized mouse cortex and found that brain injury caused by a two-photon laser burn immediately increased extracellular glutamate in a ring surrounding the site of damage. This was followed by small and transient bursts of glutamate around the ring, henceforth termed "glutamate clouds", which we have never observed in the undamaged brain. Pharmacologically inhibiting astrocytic glutamate transporters with TBOA increased both the size of the initial glutamate ring as well as the number of glutamate clouds. Application of a high concentration of

TBOA induced glutamate clouds prior to a lesion in otherwise healthy brain tissue. Together with the fact that clouds tend to occur near the lesion site, these results suggest that structural damage to astrocytes may compromise glutamate transport, leading to a localized and temporary failure of uptake and resulting in a "glutamate cloud". By imaging neuronal structure over the course of multiple days following the lesion in the absence or presence of TBOA, we have also seen that the extent of damage is positively correlated with the amount of glutamate present after injury. In summary, our data provide direct observation of excitotoxic glutamate induced by brain injury, demonstrate a key role for astrocytes in controlling levels of extracellular glutamate and consequently, neuronal damage, and highlight astrocytic glutamate transporters as a potential therapeutic target in the treatment of TBI and stroke.

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Nanosymposium

202. Stroke and Injury: Optogenetic and Chemogenetic Approaches

Location: SDCC 24A

Time: Sunday, November 13, 2016, 1:00 PM - 3:00 PM

Presentation Number: 202.08

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Canadian Institutes of Health Research

National Science and Engineering Research Council (Canada)

Research Manitoba

Canada Foundation for Innovation

Winnipeg Health Sciences Center

Title: Endothelial NMDA receptors mediate astrocyte-induced cortical vasodilation

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Abstract: Functional hyperemia (FH) is a regulatory mechanism that ensures active brain regions receive proportional delivery of blood flow. Glutamatergic neurotransmission is a key functional bellwether of neuronal activity that initiates FH by activating post-synaptic N-methyl-

D-aspartate (NMDA) receptors linked to neuronal nitric oxide synthase (nNOS), or by inducing release of vasodilatory gliotransmitters from perisynaptic astrocytes with process endfeet resting on local arterioles or capillaries. However, neither of these mechanisms is endothelium-dependent, leaving neuro-endothelial coupling as a key conceptual deficit in understanding FH. Our cumulative results indicate that brain endothelial cells also express NMDA receptors that are activated subsequent to astrocytic neurotransmitter input, leading to eNOS-dependent vasodilation. Experiments in primary mouse brain microvascular endothelial cultures revealed expression of the pan-NMDA receptor subunit, GluN1, and NO production in response to NMDA receptor co-agonists, glutamate and D-serine, in a manner sensitive to NMDA receptor antagonists, eNOS inhibition and chelation of intracellular Ca^{2+}. Using immuno-electron microscopy, GluN1 immunoreactivity was also detected in brain endothelium *in situ*, with preferential localization to brain-facing (abluminal) endothelial membranes. Direct exposure of cortical slice arterioles to glutamate and D-serine by local pressure ejection and two-photon (TP) flash photolysis of NP-EGTA (o-Nitrophenyl EGTA; Ca^{2+} uncaging) in perivascular astrocytes both produced increases in lumen diameter. This effect was significantly mitigated by competitive antagonists of NMDA receptor glutamate (AP5) or co-agonist (DCKA) binding sites, and by eNOS loss of function. To distinguish between neuronal and endothelial NMDA receptors, we created conditional endothelial NMDA receptor loss of function mice by crossing "floxed" GluN1 animals with a Tie-2 Cre recombinase driver line. This strategy resulted in greater than 50% loss of endothelial GluN1 (eGluN1) expression in cultures and cortical slice arterioles. In cortical slices, conditional eGluN1 loss of function significantly mitigated vasodilatory responses and endothelial NO generation *in situ*, produced by stimuli including TP astrocytic Ca^{2+} uncaging, direct vascular application of NMDA receptor agonists and bath-applied metabotropic glutamate receptor agonist (t-ACPD). Our results identify a novel mechanism of neuro-endothelial coupling by showing that endothelial NMDA receptors mediate activity-dependent, glutamatergic neurovascular signaling.

Disclosures: **A.D. Hogan-Cann:** None. **L. Lu:** None. **A.K. Globa:** None. **S.X. Bamji:** None. **C.M. Anderson:** None.

Nanosymposium

203. Molecules and Circuits of Somatosensation

Location: SDCC 23A

Time: Sunday, November 13, 2016, 1:00 PM - 3:45 PM

Presentation Number: 203.01

Topic: D.03. Somatosensation: Touch

Support: Klingenstein Foundation

Title: Inactivation tunes response of Piezo1 ion channels to cellular membrane tension

Authors: *A. H. LEWIS, J. GRANDL;
Neurobio., Duke Univ., Durham, NC

Abstract: Piezo ion channels mediate the conversion of mechanical forces into electrical signals and are critical for responsiveness to touch in metazoans. The apparent mechanical sensitivity of Piezos varies substantially across cellular environments and with different stimulation methods, raising the fundamental questions of what precise physical stimulus activates the channel and how sensitivity to this stimulus is regulated. To answer these questions, we combined high-resolution, high-contrast imaging with electrophysiology to distinguish between membrane curvature and lateral membrane tension as potential physical stimuli for activation of Piezo1. Piezo1-mediated currents were measured in HEK293t cells in three patch configurations using a high-speed pressure clamp to induce membrane curvature and tension, while simultaneously imaging membrane geometry within the patch pipette. We found that both positive and negative pressure, which elicit opposite membrane curvature, activate Piezo1 channels in all three patch configurations (cell-attached, inside-out, and outside-out), albeit with different sensitivities. Further, we quantified the lateral membrane tension produced at different pressures and found that Piezo1 activity is well-described by a Boltzmann function with a tension of half-maximal activation (T_{50}) of 2.7±0.1 mN/m in cell-attached patches and 4.7±0.3 mN/m in inside-out patches. Building on this approach, we next developed a protocol to minimize resting membrane tension in cell-attached patches prior to probing Piezo activity. Specifically, we used a "pre-pulse" of positive pressure (~+5 mmHg) that precisely flattens the membrane and allows channels to recover from inactivation. We find that in the absence of prior, resting membrane tension, Piezo1 responds to increases in lateral membrane tension with exquisite sensitivity (T_{50} = 1.4±0.1 mN/m in cell-attached patches) as compared to other mechanically activated channels. Our results explain how Piezo1 sensitivity can be tuned by resting membrane tension and channel inactivation, thus priming the channel to respond to physiologically relevant changes in tension in diverse cellular contexts. We are now using our approach to investigate the response of wild-type and inactivation-deficient Piezo1 to dynamically modulated stimuli.

Disclosures: **A.H. Lewis:** None. **J. Grandl:** None.

Nanosymposium

203. Molecules and Circuits of Somatosensation

Location: SDCC 23A

Time: Sunday, November 13, 2016, 1:00 PM - 3:45 PM

Presentation Number: 203.02

Topic: D.03. Somatosensation: Touch

Support: the Howard Hughes Medical Institute

NIH grant R01DE022358

a Giovanni Armenise-Harvard Foundation Grant

Title: Mechanosensation mediated by Piezo ion channels

Authors: *K. NONOMURA[1], S.-H. WOO[1], R. B. CHANG[2], A. GILLICH[3], Z. QIU[1], A. G. FRANCISCO[1], S. S. RANADE[1], S. D. LIBERLES[2], A. PATAPOUTIAN[1];
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Abstract: Mechanotransduction, the conversion of physical forces into biochemical signals, is essential for various physiological processes such as touch, hearing and sensing blood flow. However, the molecular mechanisms of mechanotransduction or physiological/biological responses triggered by mechanical stimuli have not fully understood. Mechanically activated ion channels have been proposed as sensors of physical force, while the identity of these channels had been elusive for decades. The Patapoutian group has identified Piezo1/2, mechanically activated cation channels, in mammalian cells. Piezos are activated by mechanical indentation or suction stretch on cell membrane and shear stress. In mammals, Piezos are broadly expressed in a wide range of mechanosensitive cells and are enriched in the lungs, kidneys, and DRG neurons. We have demonstrated that Piezo2 is the principle mechanotransducer for cutaneous light touch sensing and proprioception in mice. Piezo-deficient animal models provide us an opportunity to study involvement of mechanostransduction in various physiological processes. In this presentation, the key role of Piezo in respiration will be shown.

Disclosures: K. Nonomura: None. **S. Woo:** None. **R.B. Chang:** None. **A. Gillich:** None. **Z. Qiu:** None. **A.G. Francisco:** None. **S.S. Ranade:** None. **S.D. Liberles:** None. **A. Patapoutian:** None.

Nanosymposium

203. Molecules and Circuits of Somatosensation

Location: SDCC 23A

Time: Sunday, November 13, 2016, 1:00 PM - 3:45 PM

Presentation Number: 203.03

Topic: D.03. Somatosensation: Touch

Support: Emmy Noether-Program of the Deutsche Forschungsgemeinschaft (DFG)

DFG Collaborative Research Center 889 (project A9)

MPI PhD Fellowship

Title: Identification and characterization of native Piezo2 interactors

Authors: *P. NARAYANAN[1], J. SONDERMANN[1], T. ROUWETTE[1], H. URLAUB[2], D. GOMEZ-VARELA[1], M. SCHMIDT[1];
[1]Max Planck Inst. For Exptl. Med., Goettingen, Germany; [2]Max Planck Inst. of Biophysical Chem., Goettingen, Germany

Abstract: Somatosensation (sense of touch, pain, temperature etc.) is pivotal to our interactions with the internal and external environments. Peripheral somatosensory neurons are equipped to respond to stimuli of different nature ranging from chemical, thermal to mechanical. The ability of somatosensory neurons to perceive mechanical stimuli relies on specialized mechanotransducing proteins and their molecular environment. Recently, a major transducer of mechanical forces in vertebrate somatosensation was revealed by the discovery of Piezo2. Piezo2 is a non selective cation channel which is expressed in Merkel cells and a wide range of low threshold mechanoreceptors (LTMRs) in mice. Further work has established its pivotal role for innocuous touch in mice as well as a role in proprioception. It is widely recognized that ion channels operate as part of dynamic multiprotein complexes. Elegant work in other mechanosensory systems (e.g. *D. melanogaster and C. elegans*) demonstrated the involvement of such a complex molecular machinery in the detection of mechanical stimuli. In vertebrates some evidence of similar protein complexes has come to light but so far only few of the components have been identified. Therefore, the characterization of Piezo2-associated protein complexes in peripheral sensory neurons holds the promise to elucidate novel proteins implicated in somatosensory mechanotransduction. Towards this goal, we performed a quantitative mass spectrometry-based interactomics screen on native Piezo2, immunoaffinity-purified from somatosensory neurons of mouse dorsal root ganglia (DRG). By means of stringent statistical analysis, we identified 36 binding partners of Piezo2. The biological significance of this dataset is reflected by functional experiments demonstrating a role for selected candidate proteins in modulating Piezo2 activity and membrane expression in somatosensory neurons. Collectively, our findings provide a framework for understanding Piezo2 physiology and serve as a rich resource for the molecular dissection of vertebrate somatosensory mechanotransduction.

Disclosures: **P. Narayanan:** A. Employment/Salary (full or part-time): Max Planck Institute of Experimental Medicine. **J. Sondermann:** None. **T. Rouwette:** None. **H. Urlaub:** None. **D. Gomez-Varela:** None. **M. Schmidt:** None.

Nanosymposium

203. Molecules and Circuits of Somatosensation

Location: SDCC 23A

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Presentation Number: 203.04

Topic: D.03. Somatosensation: Touch

Support: Spanish Mineco SAF2013-45608-R project

Title: Piezo2 channels are essential for mechanotransduction in mouse proprioceptive sensory neurons

Authors: *D. M. FLOREZ PAZ[1], K. KUMAR BALI[2], R. KUNER[2], A. GOMIS[1];
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Abstract: Proprioceptors innervate muscle spindles, Golgi tendons and joints and mediate conscious sensation of limb position, muscle tension and balance. Characterization of mechanotransducer channels indicates that Piezo2 is the principal mechanoreceptor in touch sensation. To determine the role of Piezo2 in proprioception, we have characterized the electrical and mechanical properties of neurons in the mesencephalic trigeminal nucleus (MTN), the unique primary sensory neurons located in the central nervous system. These neurons receive signals originating from the masseter muscle and dental pressoreceptors and are involved exclusively in processing proprioceptive information from the face and oral cavity. Neurons were identified by injecting the retrograde dye DiI into the masseter muscle. The MTN neurons in mice respond to a current injection with a phasic discharge pattern, have a big soma size and show narrow action potentials, which are abolished by tetrodotoxin. All these characteristics indicate that MTN neurons belong to the low threshold mechanoreceptors group of primary sensory neurons. Mechanical indentation in MTN neurons exclusively displayed rapidly adapting currents (RA). Piezo2 was detected in almost every MTN neurons by using single cell RT-PCR and immunohistochemistry. To study whether RA currents in MTN neurons were mediated by Piezo2, we silenced its expression by infecting MTN neurons of C57Bl/6j mice with an adeno-associated viral vector (AAV) carrying a shRNA against Piezo2. We also used a conditional Piezo2 KO mouse in which Piezo2 was eliminated exclusively in proprioceptive neurons. The results showed that RA mechanically-activated currents in proprioceptive neurons were fully depending on Piezo2. Furthermore, functional characterization of Piezo2^{cKO} mice showed that the lack of Piezo2 in proprioceptive neurons produced severe defects in limb position and impaired performance in different balance and coordination behavioral tests. All those findings provided directly evidence that the ion channel Piezo2 is essential for mammalian proprioceptive mechanotransduction.

Disclosures: **D.M. Florez Paz:** None. **K. Kumar Bali:** None. **R. Kuner:** None. **A. Gomis:** None.

Nanosymposium

203. Molecules and Circuits of Somatosensation

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Presentation Number: 203.05

Topic: D.03. Somatosensation: Touch

Support: 1T32HL120826

P30AR044535

R25NS076445

Title: Somatosensory contributions to food oral processing

Authors: ***Y. MOAYEDI**[1,2], L. DUENAS-BIANCHI[2,3], C.-K. TONG[1], S. MICHLIG[4], J. LE COUTRE[4], B. LE RÉVÉREND[4], E. A. LUMPKIN[1,2];
[1]Dermatol., Columbia Univ., New York, NY; [2]Dept. of Physiol. and Cell. Biophysics, [3]SPURS Biomed. Res. Program, Columbia Univ. Med. Ctr., New York, NY; [4]Perception Physiol., Nestlé Res. Ctr., Lausanne, Switzerland

Abstract: The reception of tactile stimuli in mammalian skin is mediated by an intricate mechanosensitive machinery involving dedicated receptors, cells and neurons. This signal transduction setup is coupled with the topology and mechanical properties of skin, thereby providing for a highly sensitive and accurate perception system to detect stress fluctuations (texture) from the external environment. Although textural attributes are important in the sensory experience of food, the underlying physiology relevant to oral texture perception is not well understood. The proper functioning of oral texture perception is of high significance in various food intake scenarios ranging from breastfeeding, to intake control and possibly even in the prevention of presbyphagia. We hypothesize that subsets of tactile receptors are tuned to encode specific textural qualities of foodstuffs. Input from such a system will encode the properties of a food bolus during oral manipulation until swallowing as well as textural palatability. To test this hypothesis, we mapped the distribution of anatomically distinct neuronal endings in the mouse oral cavity (tongue, palate and gums) using transgenic reporters, molecular markers and quantitative histomorphometry. We found that the hard palate and gums are densely populated with four classes of putative tactile afferents that are organized in discrete patterns. These included Merkel cell-neurite complexes and Meissner's corpuscles, which have well-defined

roles in discriminative touch reception in skin. Third, glomerular corpuscles were localized to the *lamina propria* of hard palate. Finally, an unusual subset of neurofilament heavy (NFH)-positive afferents innervating superficial epithelial layers were identified. We find that the murine tongue is devoid of Merkel cells. Rather, the tongue is equipped with a unique set of putative mechanosensory afferents. These studies lay the groundwork for physiological studies to define functional differences between anatomically distinct somatosensory neurons in the oral cavity. Ongoing studies aim to identify the behavioral contributions of mechanosensitivity to food texture discrimination and to identify the response properties of subsets of putative touch receptors.

Disclosures: Y. Moayedi: 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; Nestec. **L. Duenas-Bianchi:** None. **C. Tong:** 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; Nestec. **S. Michlig:** A. Employment/Salary (full or part-time): Nestec. **J. le Coutre:** A. Employment/Salary (full or part-time): Nestec. **B. Le Révérend:** A. Employment/Salary (full or part-time): Nestec. **E.A. Lumpkin:** 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; Nestec.

Nanosymposium

203. Molecules and Circuits of Somatosensation

Location: SDCC 23A

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Presentation Number: 203.06

Topic: D.03. Somatosensation: Touch

Support: NIH Grant R01AR059385

Title: Neutrophils play a key role in CXCL1-induced acute itch and atopic dermatitis

Authors: *J. K. SCHWENDINGER-SCHRECK, J. DEGUINE, C. M. WALSH, E. C. BROCK, G. M. BARTON, D. M. BAUTISTA;
MCB, Univ. of California Berkeley, Berkeley, CA

Abstract: Chronic itch is a highly prevalent and often debilitating disorder with few effective treatments. During chronic itch, multicellular interactions between keratinocytes, immune cells,

and neurons act to maintain and exacerbate inflammation and itch. However, the initiating factors of this disorder, as well as the mechanisms of intercellular communication, remain poorly understood. Here, we find that CXCL1, a keratinocyte-derived chemokine, is highly upregulated in the vitamin D mouse model of atopic dermatitis. Further, acute injection of CXCL1 causes significant scratching behaviors (58 seconds versus 24s vehicle, $p<0.005$) and neutrophil infiltration (41% of cells versus 5% control by 30 minutes) in the mouse cheek model of itch. To determine the molecular mechanisms by which CXCL1 triggers itch and inflammation, we probed calcium responses in primary cultured sensory neurons and neutrophils, and explored itch behaviors in wild type, neutrophil-depleted, and TRP channel mutant mice. Robust calcium signaling was observed in neutrophils, but not sensory neurons in response to application of 1μg/ml CXCL1 (n=2 and 9 biological replicates, respectively), supporting a model whereby CXCL1 acts on neutrophils to trigger itch. Consistent with this, we found that neutrophil-depleted mice display no CXCL1-induced itch (scratch time of 20s versus 21s for vehicle). Neutrophil-dependent signals likely mediate itch through the ion channel TRPA1, as TRPA1-deficient animals displayed attenuated itch behaviors that are statistically indistinguishable from vehicle (36s versus 22s vehicle, $p>0.1$). Finally, in the vitamin D model of atopic dermatitis, we identified a striking phenotype whereby early loss of neutrophils greatly ameliorates both itch and later recruitment of myeloid lineage immune cells during chronic itch. These data support the importance of neutrophil-neuron interactions in both acute and chronic itch.

Disclosures: **J.K. Schwendinger-Schreck:** None. **J. Deguine:** None. **C.M. Walsh:** None. **E.C. Brock:** None. **G.M. Barton:** None. **D.M. Bautista:** None.

Nanosymposium

203. Molecules and Circuits of Somatosensation

Location: SDCC 23A

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Presentation Number: 203.07

Topic: D.03. Somatosensation: Touch

Support: AR063772

NS023735

Title: Neural circuit for inhibition of itch by scratching

Authors: ***J. HACHISUKA**, L. M. SNYDER, Y. OMORI, X. CAI, H. R. KOERBER, S. E. ROSS;
Dept. of Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Counter stimuli such as scratching and cooling are known to reduce itch. However, the neural circuit mechanisms underlying this phenomenon remain unclear. To address this question, we developed a novel semi-intact preparation that allows us, for the first time, to record from lamina I spinal output neurons while controlling somatosensory input though natural stimulation of the skin and manipulating the activity of spinal interneurons though optogenetic approaches. We identify spinal projection neurons that are tuned for itch, and show that scratching the receptive field of these cells reduces itch-related responses. Moreover, we reveal that nNOS inhibitory interneurons provide strong feed forward inhibition onto these spinal projection neurons. Thus, nNOS inhibitory interneurons provide a feed forward mechanism through which counter stimuli inhibit itch.

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Nanosymposium

203. Molecules and Circuits of Somatosensation

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Presentation Number: 203.08

Topic: D.03. Somatosensation: Touch

Support: HHMI

Title: Traditional and atypical presynaptic inhibition in the spinal cord dorsal horn

Authors: *A. L. ZIMMERMAN[1,2], V. E. ABRAIRA[1], D. GINTY[1,2];
[1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Howard Hughes Med. Inst., Chevy Chase, MD

Abstract: For over 60 years, presynaptic inhibition of sensory terminals within the spinal cord has been identified as a key mechanism by which sensory inflow is modulated. The primary method of presynaptic inhibition is through depolarization of the primary afferent terminals, hypothesized to be dependent on a trisynaptic circuit involving last order GABAergic axo-axonic synapses. Using mouse molecular genetics and identified low-threshold cutaneous mechanoreceptors and dorsal horn interneurons, we show that this inhibition is not monolithic. While low-threshold cutaneous stimulation evokes a dorsal root potential that depends on $GABA_A$ receptors, optogenetic activation of Aδ- low threshold mechanoreceptors and C-afferents produces a primary afferent depolarization which is $GABA_A$ receptor independent. By selectively activating or silencing interneurons in the spinal cord dorsal horn, we identify two distinct populations of excitatory interneurons, one which activates the 'traditional' $GABA_A$ receptor dependent pathway and one which activates a $GABA_A$ receptor independent pathway.

The pathways involved and the functional implications of having two different forms of presynaptic inhibition are discussed.

Disclosures: **A.L. Zimmerman:** None. **V.E. Abraira:** None. **D. Ginty:** None.

Nanosymposium

203. Molecules and Circuits of Somatosensation

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Title: Modulation of multiple modalities of somatosensory information by peripheral kappa opioid receptors

Authors: ***L. SNYDER**[1], H. HUANG[1], X. CAI[1], J. HACHISUKA[1], P. ADELMAN[1], Z. HU[1], Y. OMORI[1], S. FULTON[1], M. GOLD[2], H. R. KOERBER[1], S. ROSS[1];
[1]Neurobio., [2]Anesthesiol., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Peripherally selective kappa opioids are emerging as a novel treatment for pain and itch that have shown efficacy in several recent clinical trials. Yet, the subtypes of somatosensory neurons that express KOR remain unclear. Using a newly developed KOR-cre knockin allele, viral tracing, and single-cell PCR we reveal that that KOR is expressed in a specific subset of peptidergic afferents that are tuned for inflammatory pain and itch, but not heat or mechanical force. Consistent with this, peripherally restricted KOR agonists inhibit behavioral responses to chemical pain and itch, but not acute heat responses nor punctuate mechanical sensitivity. Unexpectedly, we also find that KOR is expressed in subsets of primary afferents that form lanceolate or circumferential endings around hair follicles, suggesting an unappreciated role for KOR signaling in the modulation of low-threshold mechanosensation. At a functional level, optogenetic experiments reveal that dynorphin inhibits glutamate release from the central terminals of KOR-expressing afferents, and genetically-labeled afferents show inhibited calcium

influx in response to kappa agonists. These experiments provide key insight for the rationale use of peripherally selective KOR agonists for the modulation of inflammatory pain, itch, and potentially mechanical allodynia.

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Nanosymposium

203. Molecules and Circuits of Somatosensation

Location: SDCC 23A

Time: Sunday, November 13, 2016, 1:00 PM - 3:45 PM

Presentation Number: 203.10

Topic: D.03. Somatosensation: Touch

Support: CIHR

Brain Canada

Quebec Pain Research Network

Title: A genetic and functional analysis of nociceptive somatotopy

Authors: ***A. KANIA**[1], H. U. ZEILHOFER[2], R. V. DA SILVA[1];
[1](IRCM) Inst. de recherches cliniques de Montreal, Montreal, QC, Canada; [2]Univ. of Zurich, Zurich, Switzerland

Abstract: Somatotopic organisation is a common feature of sensory neural circuits yet its functional relevance is unclear. One of its advantages is that it allows the localisation of the sensory stimulus relative to the body of the animal. This is particularly significant in the context of nociception where the survival of an organism depends on the accurate localization of a painful sensation. In vertebrates, noxious stimuli are transferred from sensory neurons to dorsal spinal cord or trigeminal nuclei neurons, and then on to the sensory cortex via the thalamus. The spinothalamic (STT) projection neurons in the spinal cord and their terminals in the thalamus form somatotopic maps, but their importance for nociceptive stimulus localisation is not clear. We are using genetic approaches to disrupt the left-right segregation of STT connections. Since the majority of STT axons cross the midline of the nervous system at the level of their spinal cell bodies, we hypothesised that disrupting axon guidance signals that promote commissure formation might result in erroneous STT axon projection. Using a Cre driver transgenic mouse line, we have generated a spinal-cord specific knockout of a commissural axon guidance receptor. Axonal tracing experiments reveal that in such mice, some STT neurons

inappropriately innervate the ipsilateral thalamus. Primary afferent innervation and spinal c-Fos induction in response to unilateral noxious stimuli applied to the limb of such mutants are unchanged, suggesting that nociceptive signal relay at the level of the spinal cord is normal. However, such stimulation elicits bilateral behavioural responses, suggestive of abdnormal left-right localisation of pain. We are currently studying the laterality of thalamic and cortical neuron activities in such mutants, and the relationship between nociceptive and innocuous touch somatotopic maps.

Disclosures: **A. Kania:** None. **H.U. Zeilhofer:** None. **R.V. Da Silva:** None.

Nanosymposium

203. Molecules and Circuits of Somatosensation

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Presentation Number: 203.11

Topic: D.03. Somatosensation: Touch

Title: Identification of early RET+ deep dorsal spinal cord interneurons in gating pain

Authors: ***L. CUI**[1], X. MIAO[2], L. LIANG[2], I. ABDUS-SABOOR[1], W. OLSON[1], M. S. FLEMING[1], M. MA[1], Y. TAO[2], W. LUO[1,2];
[1]neuroscience, Univ. of Pennsylvania, Philadelphia, PA; [2]Rutgers, the State Univ. of New Jersey, Newark, NJ

Abstract: The gate control theory (GCT) of pain proposes that pain- and touch-sensing neurons antagonize each other through spinal cord dorsal horn (DH) gating neurons. However, the exact neural circuits underlying the GCT remain largely elusive. Here, we identified a new population of deep layer DH (dDH) inhibitory interneurons that express the receptor tyrosine kinase Ret neonatally. These early RET+ dDH neurons receive excitatory as well as polysynaptic inhibitory inputs from touch- and/or pain-sensing afferents. In addition, they negatively regulate DH pain and touch pathways through both pre- and postsynaptic inhibition. Finally, specific ablation of early RET+ dDH neurons increases basal and chronic pain, whereas their acute activation reduces basal pain perception and relieves inflammatory and neuropathic pain. Taken together, our findings uncover a novel spinal circuit that mediates crosstalk between touch and pain pathways and suggest that early RET+ dDH neurons function as pain "gating" neurons.

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Nanosymposium

204. Higher-Order Processing of Taste and Olfactory Stimuli

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Time: Sunday, November 13, 2016, 1:00 PM - 3:15 PM

Presentation Number: 204.01

Topic: D.04. Olfaction and Taste

Support: Colton Foundation

Title: Short time-scale basolateral amygdala activity and its role in modulating taste learning

Authors: E. ARIELI[1], D. UDI[1], I. HARPAZ[2], *A. MORAN[3,2,1];
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Abstract: The basolateral amygdala (BLA) is known to play a major role in taste-related learning. Most of this knowledge comes from lesion or long-term pharmacological inhibition of the BLA during different epochs of conditioning taste aversion (CTA) paradigm, wherein a novel palatable taste becomes aversive following pairing with malaise. Less is known, however, about the importance of short time-scale BLA activity during taste processing and its impact on learning acquisition. Recent evidence suggested the importance of such short time-scales of the BLA activity in shaping GC neuronal responses. Taste palatability (hedonic value) information for instance is detected in the BLA about 500 msecs earlier than in the GC. Same short time-scale ensemble dynamics were also reported in the GC, where they faithfully follow behavior through leaning and extinction, and were hypothesized to be driven by the BLA. To further examine the fine temporal contribution of the BLA to CTA acquisition we have utilized optogenetics tools to inhibit the BLA for short time-scales (seconds) during training and tested its impact on CTA learning. We have hypothesized that inhibiting the BLA, thus blocking palatability information from reaching the GC, only during the short time of taste experience, but leaving it intact otherwise, will attenuate taste-malaise association. To test this hypothesis we first infected the BLA of rats (n=4) bilaterally with an optogenetic neural silencer (AAV-CamKII-ArchT-GFP), and in another surgery 3 weeks later implanted fiber optics above the BLAs, as well as intraoral cannula (IOC) for taste deliveries. Following recovery rats were water deprived and habituated to poke for water (for 20 min) delivered through the IOC for 3 days. On the 4th, training, day sucrose replaced water and the BLA was briefly (3 secs) optogenetically inhibited with each taste delivery. LiCl IP injection was performed at this session termination to induce malaise. Reduced number of pokes for sucrose the next day served as a measure of learning and were compared to a control group (n=3) which followed the same procedure but without the BLA inhibition. We have found that the experimental group poked significantly more for sucrose than the control group (t-test, $p<0.05$, normalized to training day), with no significant difference between their training day sucrose consumption (t-test, $p>0.1$). These results show that short time-scale BLA activity is essential for intact acquisition of learning.

Follow-up experiments will investigate the short time-scale single and ensemble neuronal activity in the GC that respond to the BLA drive and its role in memory formation.

Disclosures: **E. Arieli:** None. **D. Udi:** None. **I. Harpaz:** None. **A. Moran:** None.

Nanosymposium

204. Higher-Order Processing of Taste and Olfactory Stimuli

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Presentation Number: 204.02

Topic: D.04. Olfaction and Taste

Support: NIH Grant DA035025

Title: Sweet and bitter taste in the brain of awake behaving animals

Authors: ***Y. PENG**[1], S. GILLIS-SMITH[1], N. J. P. RYBA[2], C. S. ZUKER[1];
[1]Columbia Univ., New York, NY; [2]3National Inst. of Dent. and Craniofacial Res., NIH, Bethesda, MD

Abstract: Taste is responsible for evaluating the nutritious content of food, guiding essential appetitive behaviors, preventing the ingestion of toxic substances, and helping ensure the maintenance of a healthy diet. Sweet and bitter are two of the most salient sensory percepts for humans and other animals; sweet taste permits the identification of energy-rich nutrients while bitter warns against the intake of potentially noxious chemicals. In mammals, information from taste receptor cells in the tongue is transmitted through multiple neural stations to the primary gustatory cortex in the brain. Recent imaging studies have shown that sweet and bitter are represented in the primary gustatory cortex by neurons organized in a spatial map, with each taste quality encoded by distinct cortical fields. Here we demonstrate that by manipulating the brain fields representing sweet and bitter taste we directly control an animal's internal representation, sensory perception, and behavioral actions. These results substantiate the segregation of taste qualities in the cortex, expose the innate nature of appetitive and aversive taste responses, and illustrate the ability of gustatory cortex to recapitulate complex behaviors in the absence of sensory input.

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Nanosymposium

204. Higher-Order Processing of Taste and Olfactory Stimuli

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Presentation Number: 204.03

Topic: D.04. Olfaction and Taste

Support: NSF PHY-1444273

KIBM IRG 2015

Title: Coding high dimensional stimuli with distributed maps

Authors: *S. SRINIVASAN[1,2], T. SCHIKORSKI[3], C. F. STEVENS[1,2];
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Abstract: Neural circuits such as the hippocampus or olfactory cortex (OC) encode high dimensional stimuli (e.g. faces or odors) by activating sparse and distributed neuronal ensembles. How is such ensemble activity produced, and how does it encode stimuli? The olfactory circuit is ideal for addressing these questions for 2 reasons. First, elegant studies have shown that input from the bulb forms a distributed map, i.e. it innervates the OC without any spatial preference. Second, such distributed input and activity is conserved in mouse and numerically simpler fly olfactory circuits. We use a quantitative model of the fly circuit - whose connection characteristics are known - to reveal 2 properties. First, the distributed input naturally gives rise to sparse and distributed responses. Second, such responses form a maximum entropy code that aids in discrimination, even in the presence of noise. We extend these lessons to our quantitative characterization of the mouse olfactory cortex. We measured the inhibitory and excitatory input into the cortex, using stereology with light and electron microscopes. A quantitative model based on our measurements shows that sparse distributed activity in the cortex too can naturally arise from distributed input. If function follows form, our findings might shed light on coding in the hippocampus and the cerebellum, which have similar distributed maps.

Disclosures: **S. Srinivasan:** None. **T. Schikorski:** None. **C.F. Stevens:** None.

Nanosymposium

204. Higher-Order Processing of Taste and Olfactory Stimuli

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Topic: D.04. Olfaction and Taste

Support: NIH R01 DC005813

NIH T32 DC000044

Florida State University

Title: Functional circuitry of the medial amygdala: A mechanism for discrimination of social and heterospecific chemosignals?

Authors: L. M. BIGGS[1], *M. MEREDITH[2];
[1]Program in Neurosci. and Dept. Biol. Sci., [2]Dept. Biol. Sci., Florida State Univ., Tallahassee, FL

Abstract: The amygdala is thought to assign affective value to incoming sensory signals of all modalities, strongly influencing behavioral responses. The most direct sensory input to the amygdala complex is chemosensory input to medial amygdala (Me) from the accessory olfactory bulb in animals with a functional vomeronasal system and from the main olfactory bulb in all animals with a functional olfactory system. Normal Me function is essential for many social behaviors in rodents and different social chemosignals generate different characteristic patterns of immediate early gene activity across the anterior (MeA) and posterior (MeP) subdivisions, and the adjacent main intercalated nucleus (mICN) group of GABAergic cells. However, the underlying circuitry has not been well studied. MeA is activated by all chemosignals tested but GABA-receptor-ir cells in MeP are selectively suppressed by some stimuli, while GABAergic cells in adjacent mICN are activated. We are using intracellular patch-clamp recording in both coronal and horizontal brain slices from male hamsters to examine the circuit connections in Me. We have shown both functional excitatory and inhibitory connections from MeA to MeP, predominantly excitatory connections from MeA to mICN and functional inhibitory connections from mICN to MeP, in both horizontal and coronal slices. These results are consistent with a role for mICN in chemosignal processing and suggest MeA may also modulate MeP activity via excitatory input to mICN. This MeA/main-ICN/MeP triangular-circuit shares intriguing similarities with the triangular-circuit of Basolateral (BLA)/paracapsular-ICN/Central (Ce) amygdala involved in regulation of fear conditioning and extinction. In both, the main nuclei (BLA/Ce or MeA/MeP) communicate directly and also indirectly via distinct locally-adjacent groups of intercalated nucleus cells. In the fear circuit, the ICN cells receive DA and mPFC input which modulates the indirect path. We find DA also modulates mICN of the medial amygdala

circuit and there are traceable connections from mPFC. These similarities suggest a possible modular organization within the amygdala for evaluation of different sensory stimuli. Additional regulation of this circuit via dopamine (DA) and infralimbic (IL) cortex input may also be involved in producing behavioral responses. My data provides a mechanism that may be responsible for the production of different Me output patterns to basal forebrain that can engage different behavioral responses appropriate to the pattern of input (to MeA and MeP) and the animal's internal state, via DA and IL cortex modulation.

Disclosures: L.M. Biggs: None. **M. Meredith:** None.

Nanosymposium

204. Higher-Order Processing of Taste and Olfactory Stimuli

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Topic: D.04. Olfaction and Taste

Support: Howard Hughes Medical Institute

Jane Coffin Childs Memorial Fund

Title: Novelty and familiarity in a mushroom body compartment

Authors: *D. HATTORI[1], Y. ASO[2], G. RUBIN[2], L. ABBOTT[1], R. AXEL[1];
[1]Dept. of Neurosci., Columbia Univ., New York, NY; [2]Janelia Farm Res. Campus, Ashburn, VA

Abstract: Exposure to a novel sensory stimulus often leads to attention and exploration that may be an adaptive response to uncertainty. Continued exposure to a stimulus affords an organism a more predictable knowledge of its environment, thereby relieving uncertainty. We have identified a plastic neural circuit that may encode novelty and familiarity in the olfactory system of the fruit fly, *Drosophila melanogaster*. In flies, olfactory sensory information is transmitted to the mushroom body (MB), a structure essential for associative learning. The principal neurons of the MB, Kenyon cells (KCs), encode different odors as distinct ensemble activity, and converge upon a small number of output neurons (MBONs). The dendrites of different MBONs form 15 non-overlapping compartments. Each compartment is innervated by distinct dopaminergic neurons (DANs) that are activated by unconditioned stimuli. Dopamine release can induce plasticity at the KC-MBON synapse, thereby modulating the MBON output. We have performed functional imaging to identify a class of MBONs innervating the α'3 compartment, MBON-α'3, that exhibit strong responses to novel odors and diminish significantly upon repeated exposure. This state of decreased response elicited by repeated odor exposure persists for over 10 minutes.

The upstream KCs as well as another class of MBONs do not diminish their response upon repeated odor exposure; they respond equally to the presentation of novel or familiar odors, suggesting that this property is specific to MBON-α'3. The decrease in MBON-α'3 activity requires odor-evoked excitation of the DANs innervating the α'3 compartment, DAN-α'3, and a dopamine receptor in the MBONs. These data suggest that the α'3 compartment may afford flies with the ability to detect novelty and familiarity; elevated MBON-α'3 activity may signal novelty and the suppression of MBON-α'3 activity by DAN-α'3 may result in familiarity. In accord with this model, we have developed a behavioral assay that may reflect novelty and familiarity (interruption of grooming by novel odors), and this behavior is dependent upon odor-evoked activity in MBON-α'3. Thus, we have identified a neural circuit in the MB that may encode novelty and familiarity.

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Nanosymposium

204. Higher-Order Processing of Taste and Olfactory Stimuli

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Topic: D.04. Olfaction and Taste

Support: SFB 874

Title: Show me what I will smell: Early sensory activations in primary olfactory cortex triggered by emotional facial expressions before olfactory stimulation - an fMRI study

Authors: ***P. SCHULZE**[1], A.-K. BESTGEN[2], R. K. LECH[3], L. KUCHINKE[4], B. SUCHAN[1];
[1]Neuropsychology, Inst. of Cognitive Neurosience, Ruhr Universi, Bochum, Germany;
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[4]Methodology and Evaluation, Intl. Psychoanalytic Univ., Berlin, Germany

Abstract: The perception and processing of emotions, in the form of facial expressions appears to be substantial, in evolutionary terms, for reactions and interactions with the social environment but gives also vital informations for avoidance or approach strategies for an appropriate coping with the physical environment. Especially for odorants, as being potentially harmful, it is of great importance to be able to decide between avoidance or approach as early as possible. However yet it is less examined, whether it is possible that an emotional facial expression as a visual stimulus is preprocessed within regions of the primary olfactory cortex and

thus can change the perception of an emotional odor. Therefore, a cross-modal priming task with emotional faces (disgust, neutral or happy) as visual primes and odors (positive and negative) as target stimuli was used to investigate the influence of an emotional facial expression on the perception and cognition of the emotional quality of an odor. This study directly addresses the question whether visual information is processed in regions of the primary olfactory cortex before the perception of an odor (ROI analysis, prior to odor perception), whether this information is later on integrated into regions of the extended olfactory network (whole-brain analysis, while odor perception) and whether this integration influences the perceived valence of an odor (behavioral analysis of odor valence rating). ROI analysis of the primary olfactory cortex revealed piriform cortex activation for emotional visual information before odor perception. Transfer and integration of this information was revealed by responses of the amygdala, the hippocampus and the orbitofrontal cortex for the whole-brain analysis for odor perception and behavioral results show that odors were rated as more unpleasant after seeing a disgusted face than after seeing a happy face. Thus, seeing emotional faces can shift the valence perception of an odor towards the emotional quality of that facial expression. Referring to the fMRI data, visual information (facial expressions) is preprocessed in the primary olfactory cortex and this information is than transferred and integrated into the extended olfactory network, leading to a changed perception of emotional olfactory stimuli.

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Nanosymposium

204. Higher-Order Processing of Taste and Olfactory Stimuli

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Presentation Number: 204.07

Topic: D.04. Olfaction and Taste

Title: Top-down modulation of olfactory-guided behaviours by the anterior olfactory nucleus pars medialis and ventral hippocampus

Authors: ***A. AQRABAWI**[1], C. BROWNE[2], J. KIM[2];
[1]Cell and Systems Biol., [2]Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: Sensory perception is not simply a feed-forward mechanism. Higher cortical regions actively modulate information processing in lower regions via diverse ‘feedback’ connections. This allows the cortex to suppress or enhance responses in peripheral structures depending on the relevance of the stimuli. In the context of the olfactory system, the anterior olfactory nucleus provides first-order feedback in the form of direct excitatory inputs to inhibitory interneurons of

the olfactory bulb. However, the role of this feedback has not been directly demonstrated in awake behaving animals, leaving its relevance to olfactory processing elusive. To examine the behavioural function of olfactory cortical feedback, we virally expressed the chemogenetic activity modulators hM4D or hM3D bilaterally in CaMKIIa-positive neurons of the anterior olfactory nucleus pars medialis (mAON). We found that feedback from the mAON is capable of bidirectionally modulating olfactory sensitivity and performance on olfaction-dependent tasks. To reveal higher-order structures which may tune this bidirectional control of olfactory sensitivity, we infused the retrograde tracer cholera toxin subunit B in the mAON. As a result, we observed dense labelling in the field CA1 of the ipsilateral ventral hippocampus (vHPC). We further demonstrated that optogenetic stimulation of vHPC axon terminals at the mAON is sufficient to alter olfaction-dependent behaviours. Together, these results highlight an important behavioural gain-control function of the vHPC-mAON pathway among other roles they may serve in olfaction.

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Nanosymposium

204. Higher-Order Processing of Taste and Olfactory Stimuli

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Title: Fast and stable discrimination in accentuated divergent-convergent synaptic connectivities

Authors: *T. MOSQUEIRO[1], M. F. STRUBE-BLOSS[3], B. SMITH[4], R. HUERTA[2]; [1]BioCircuits Inst., Univ. of California San Diego, LA Jolla, CA; [2]Univ. of California San Diego, La Jolla, CA; [3]Biocenter Univ. of Würzburg, Würzburg, Germany; [4]Sch. of Life Sci., Arizona State Univ., Tempe, AZ

Abstract: The coding basis for decision making and survival is often provided by a minimal number of higher-order neurons [1]. Before reaching premotor decision layers, sensory information travels through several neural layers. This multi-layered organization is often

composed of (i) divergent connectivities, which are essential for pattern recognition and stimulus codification, and (ii) convergent connectivities, which filter down information. However, multi-layered processing induces a time lag between peripheral input and adaptive behavior, which is inconsistent with the need for speed. Furthermore, an accentuated divergent-convergent architecture may also amplify noise and generate unstable dynamics, which impairs the sensory representation of external stimuli. We propose a simple feedback mechanism that presents robust gain-control, sustains sparse coding, and accelerates the information transfer through layers. An example of such synaptic organization is the early olfactory processing stage of all insects, the Mushroom Bodies (MBs), where a strong divergence from 2k to 300k neurons is followed by a convergence to only 400 neurons [2]. In this case, it is evident that controlling the level of noise is crucial [3,4]. We used in-vivo recordings [5] of Projection Neurons (Antennal Lobe) and MB Output neurons to fit and validate our model predictions. Analytical and numerical solutions prove the existence of a robust gain-control condition. Under this gain-control condition, we calculated the response times [6] of the Output Neurons with respect to their input and observed a 50ms anticipation in odor discrimination in agreement with previous experiments [5]. Thus, because such connectivities are ubiquitous to many brains, we believe divergent-convergent networks play a central role in stable and fast decision-making processes.

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Nanosymposium

204. Higher-Order Processing of Taste and Olfactory Stimuli

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Presentation Number: 204.09

Topic: D.04. Olfaction and Taste

Support: ANR-15-CE37-0004

Title: GABAB receptors tune cortical feedback to the olfactory bulb

Authors: ***C. MAZO**, G. LEPOUSEZ, A. NISSANT, M. T. VALLEY, P.-M. LLEDO; Insitut Pasteur, Paris Cedex 15, France

Abstract: Sensory perception emerges from the confluence of sensory inputs that encode the composition of external environment, and top-down feedback that conveys information from higher brain centers. In olfaction, sensory inputs activity is initially processed in the olfactory bulb (OB), serving as the first central relay, before being transferred to the olfactory cortex. In addition, the OB receives dense connectivity from feedback projections, thus the OB has the capacity to implement a wide array of sensory neuronal computation. However, little is known about the impact and the regulation of this cortical feedback. Here we describe a novel mechanism to selectively gate glutamatergic feedback from the anterior olfactory cortex (AOC) to the OB. Combining *in vitro* and *in vivo* electrophysiological recordings, optogenetics and fiber photometry-based calcium imaging, applied to wild-type and conditional transgenic mice, we explore the functional consequences of circuit-specific GABA type-B receptors ($GABA_BRs$) manipulation. We found that activation of presynaptic $GABA_BRs$ specifically depresses synaptic transmission from the AOC to OB inhibitory interneurons but spares direct excitation to principal neuron. As a consequence, feedforward inhibition of spontaneous and odor-evoked principal neuron activity is diminished. We also show that tunable cortico-bulbar feedback is critical for generating beta but not gamma OB oscillations. Together, these results indicate that $GABA_BRs$ on cortico-bulbar afferents gate excitatory transmission in a target specific manner and as such, shape how the OB integrate sensory inputs and top-down information.

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Nanosymposium

205. Circadian Rhythms: Timely Topics

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Topic: F.08. Biological Rhythms and Sleep

Support: Velux Foundation

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Swiss National Science Foundation

Title: Network dynamics mediate circadian clock plasticity

Authors: *S. BROWN[1], A. AZZI[2], J. A. EVANS[3], T. LEISE[4], J. MYUNG[5], T. TAKUMI[5], A. J. DAVIDSON[6];
[1]Inst. of Pharmacol. and Toxicology, Univ. of Zürich, Zürich, Switzerland; [2]Inst. of Pharmacol. and Toxicology, Univ. of Zürich, University of Zürich, Switzerland; [3]Dept. of Biomed. Sci., Marquette Univ., Milwaukee, WI; [4]Dept. of Mathematics and Statistics, Amherst Col., Amherst, MA; [5]RIKEN Brain Sci. Inst., Wako, Japan; [6]Dept. of Neurobio., Morehouse Sch. of Med., Atlanta, GA

Abstract: A circadian clock governs most aspects of mammalian behavior and physiology. Its basic mechanism is cell-autonomous. Although its properties are in part genetically determined, an altered light-dark environment can change circadian period length stably for prolonged periods. We show that transient exposure of mice to such lighting stably alters global transcription in the suprachiasmatic nucleus of the hypothalamus (the SCN, the "master clock" tissue determining circadian behavior in mammals), and that these behavioral changes require reversible SCN-specific DNA methylation. More broadly than for circadian function, however, changes in lighting periodicity globally change DNA methylation to alter expression of genes required for many aspects of neurophysiology. Investigating these changes, we made the surprising discovery that these epigenetically mediated changes in period are effected not via cell-autonomous clock properties, but rather through altered networking within the SCN, which is DNA-methylated in a region-specific manner. As a result, circadian phasing within individual cells of the SCN is temporally reorganized to change the period length of the network as a whole. Interruption of neural communication by chemical inhibitors of neuronal firing or by physical cutting suppresses SCN reorganization and restores period. Mathematical modeling suggests, and experiments confirm, that SCN reorganization depends upon GABAergic signaling. Our results show that basic circadian clock properties like period length in mammals are governed by dynamic interactions among SCN neurons, with neuroadaptations in network function driven by the environment.

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Nanosymposium

205. Circadian Rhythms: Timely Topics

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Topic: F.08. Biological Rhythms and Sleep

Support: Medical Research Council (MC_U105170643)

Title: Electrophysiological characterisation of the vasoactive intestinal polypeptide microcircuit (VIP-VPAC2) in the suprachiasmatic nucleus

Authors: *A. P. PATTON, J. E. CHESHAM, M. H. HASTINGS;
Neurobio., MRC Lab. of Mol. Biol., Cambridge, United Kingdom

Abstract: The suprachiasmatic nucleus (SCN) is the master circadian clock of the brain. It entrains to solar time via retinal input, but when isolated in slice culture it will free-run indefinitely with a ca. 24-hour period. Two properties confer this robustness: a cell-autonomous transcriptional-translational feedback loop, and interneuronal circuit-level coupling. They are mediated by oscillations in gene expression, which direct oscillations of SCN electrical activity, which in turn reinforce gene expression rhythms. The SCN is a heterogeneous network comprising anatomically segregated sub-regions defined by their neuropeptidergic identity, but the circadian properties of specific circuits are not known. A critically important local circuit consists of neurons expressing vasoactive intestinal polypeptide (VIP) and those expressing its receptor (VPAC2). Loss of either factor compromises SCN timekeeping. This study aimed to characterise the circadian electrical properties of this microcircuit by making genetically targeted whole-cell recordings of VIP+ and VPAC2+ neurons. Organotypic SCN slices from mice expressing VIP-Cre or VPAC-Cre and carrying the bioluminescent circadian reporter PER2::LUC were transduced with AAVs expressing a flexed fluorescent reporter (tdTomato) to identify VIP+ or VPAC2+ neurons. Bioluminescence rhythms were monitored in photon multiplier tubes (PMTs) and electrophysiological recordings made at either subjective day (CT4-8) or subjective night (CT16-20) (CT12 = peak PER2::LUC) to assess intrinsic membrane properties. Surprisingly, recordings of VIP+ neurons revealed no diurnal variation in electrical properties of this subpopulation. Recordings from VPAC2+ neurons, by comparison, reported a robust variation in electrical parameters at CT4-8 vs CT16-20, with a resting membrane potential depolarisation of ~6mV, an increase in spontaneous firing rate (~5Hz vs ~2Hz) and decrease in input resistance (~1GΩ vs ~1.5GΩ). These differences in the VPAC2+ population represent a shift in the day-time state of these neurons away from the VIP+ population that maintain a state closer to the VPAC2+ night-time state. These results reveal that the VIPergic signalling axis in the SCN consists of a microcircuit with contrasting degrees of circadian organisation, and that VIP neurons are weak, if at all, circadian oscillators whereas their targets are strong oscillators.

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Nanosymposium

205. Circadian Rhythms: Timely Topics

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Topic: F.08. Biological Rhythms and Sleep

Support: Medical Research Council (MC_U105170643)

Title: The vasoactive intestinal polypeptide microcircuit (VIP-VPAC2) dictates period and rhythm stability in the mammalian suprachiasmatic nucleus

Authors: *R. HAMNETT, J. E. CHESHAM, M. H. HASTINGS;
Neurobio., MRC Lab. of Mol. Biol., Cambridge, United Kingdom

Abstract: The mammalian suprachiasmatic nucleus (SCN) of the hypothalamus is a light-entrainable circadian (ca. 24h) pacemaker, co-ordinating physiology and behaviour. In single cells, a transcriptional-translational feedback loop controls autonomous cellular rhythms, involving a delayed negative feedback loop of Bmal1/Clock transcriptional activators and Per/Cry inhibitors. *Ex vivo*, the SCN organotypic slice can maintain a strong circadian rhythm because its multiple cellular clocks are synchronous and express a daily wave of *Per* and *Cry* gene expression across the circuit. This directs a circadian rhythm of electrical firing that in turn provides a coherent output to the rest of the body *in vivo*. Neuronal synchrony depends on intercellular communication across the SCN. Vasoactive intestinal peptide (VIP) signalling via the VPAC2 receptor is known to be central to this circuit-level communication. However, the relative contributions of the VIP and VPAC2 cell populations to SCN rhythmicity and period-setting are not known.

Here we used an intersectional genetics approach in mice utilising the Cre-LoxP system combined with behavioural recording and bioluminescence rhythms to dissect the contributions of VIP and VPAC2 cells, as components of this essential micro-circuit, to SCN rhythmicity. In Experiment 1, a floxed $Ck1\varepsilon^{Tau/Tau}$ allele, a mutation that shortens period from 24 to 20 hours, was deleted selectively in either VIP or VPAC2 cells to generate a temporally chimaeric SCN with 20h (non-Cre) and 24h (Cre) cells. Deletion of Tau from the VPAC2 cells caused the period of mouse wheel-running behaviour to revert to an average 23.5±0.1h period, whereas VIP Cre-Tau mice remained at 20±0.2h. Thus VPAC2 but not VIP cells can act as pace-setters to the circuit. In Experiment 2, a floxed allele of the essential clock gene *Bmal1* was deleted from VIP or VPAC2 cells to create chimaeric SCN containing rhythmic (non-Cre) and arrhythmic (Cre) cells. Here 50% (4/8) of VPAC2Cre-$Bmal1^{flx/n}$ mice had a highly disrupted rhythm in constant conditions. Equally, deletion of *Bmal1* from VIP cells caused 3/8 mice to become arrhythmic in constant conditions, while 5 were unaffected. Thus circadian competence in both elements of the VIP-VPAC2 micro-circuit is necessary for completely reliable circadian behaviour.

In conclusion, as a network, the SCN can exhibit appreciable plasticity when challenged by circadian chimerism within its constituent cellular populations. The VIP-VPAC2 micro-circuit directs a series of circadian properties to the SCN network beyond cellular synchrony, including period setting (VPAC2 cells) and the maintenance of a coherent rhythm (VIP and VPAC2 cells).

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Title: Optogenetic investigation of SCN communication and photoperiodicity

Authors: *M. TACKENBERG[1], D. G. MCMAHON[2];
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Abstract: The ways in which electrical activation of retinorecipient SCN neurons communicate with non-retinorecipient regions of the nucleus, and how that communication results in coordinated output, remain unclear despite considerable advancements in recent years. Previous interrogations have been limited technically by the lack of suitable methods for evoking electrical activity in appropriate SCN cell populations in a way that mimics endogenous firing. Using optogenetics in vivo, we have elicited electrical activity in the SCN with precise temporal and spatial resolution by genetically targeting ChR2 expression to VIP-expressing SCN neurons. Using this system, we have explored the role of different durations of high firing rate (6-10 Hz) within subpopulations of the SCN on photoperiodic behavior.

After 7 days of recording locomotor activity in short LD, we began daily 8-hour, 8-Hz blue light stimulations of the SCN using an intracranial fiber optic implant directed to the SCN following lights-off for 7 additional days. This extension of the high-firing portion of the photoperiod induces long-photoperiod-like behavior phenotypes, with optogenetically stimulated animals exhibiting a 2.83 hour decrease in α during stimulated days as compared with pre-stimulation days while unstimulated control animals showed an increase of 0.82 hours (negative controls: 0.49 hour decrease, positive controls: 4.99 hour decrease).

This result expands our interest in further examining the dependence of photoperiodic behavior on the electrical activity, including the potential compression of behavior duration during optogenetic inhibition, and has implications for future research into the role of electrical activity in intra-SCN signaling and photoperiodic/seasonal influences on physiology.

Disclosures: M. Tackenberg: None. **D.G. McMahon:** None.

Nanosymposium

205. Circadian Rhythms: Timely Topics

Location: SDCC 2

Time: Sunday, November 13, 2016, 1:00 PM - 3:45 PM

Presentation Number: 205.05

Topic: F.08. Biological Rhythms and Sleep

Support: NINDS 095367

Hope Center Just-in-Time Grant

Title: Circadian regulation in and by SCN astrocytes

Authors: *C. TSO[1], M. MIEDA[2], T. SIMON[1], T. PURI[1], A. GREENLAW[1], E. HERZOG[1]; [1]BIology, Washington Univ. in St. Louis Dept. of Biol., Saint Louis, MO; [2]Kanazawa Univ., Kanazawa, Japan

Abstract: Astrocytes are heterogeneous glial cells that play fundamental roles in brain functions, including ion and neurotransmitter homeostasis, synaptic modulation and cerebrovascular control. Astrocytes within the mammalian master circadian pacemaker, the hypothalamic Suprachiasmatic Nucleus (SCN), are among the cells that are activated by a phase-shifting light pulse and show day/night differences in surface area *in vivo*. It is not known if these rhythms are intrinsic to the astrocytes of the SCN or if they contribute to daily behaviors. To test whether daily rhythms in clock gene expression are intrinsic to astrocytes or depend on neural input, we infected cultured SCN with a novel *iBmal1::Luc* virus. This virus carries a Cre-activated bioluminescent reporter of *Bmal1* gene expression. We imaged cells in the cultured SCN for at least 5 days. We found that astrocytes in SCN slices have functional, synchronized circadian rhythms in Bmal1 expression with a mean period of 23.6 ± 0.2h. Preliminary results indicate that these daily rhythms are disrupted when the SCN is treated with tetrodotoxin, indicated that neural signals influence astrocyte rhythms. Next, we tested whether circadian rhythms in SCN astrocytes play a role in daily rhythms in the SCN and behavior. By stereotactic injection of single guide RNA-carrying AAV (Adeno-Associated Virus) into the SCN of mice expressing astrocyte-specific Cas9 (Aldh1L1::Cre/+;LSL-Cas9-eGFP/+), we specifically disrupted Bmal1 expression only in astrocytes within the SCN. Wheel-running in mice with astrocyte-specific *Bmal1* deletion had a 0.5 h longer circadian period than littermate controls. These preliminary results indicate that Bmal1 in SCN astrocytes is critical for circadian rhythms in behavior. This work was supported by NINDS grant 095367 and a Hope Center Just-in-time Grant.

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Nanosymposium

205. Circadian Rhythms: Timely Topics

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Time: Sunday, November 13, 2016, 1:00 PM - 3:45 PM

Presentation Number: 205.06

Topic: F.08. Biological Rhythms and Sleep

Title: The role of hdac1 in modulating circadian rhythmicity and alzheimer's disease

Authors: *T. X. PHAN[1], F. GAO[2], A. NOTT[2], S. GOEL[2], P.-C. PAO[2], S. J. BARKER[2], R. VASSAR[1], L.-H. TSAI[2];
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Abstract: Histone acetylation is a form of post-translational modification that is dynamically orchestrated by two opposing groups of enzymes: histone acetyl transferases (HATs) and histone deacetylases (HDACs). HATs add an acetyl group while HDACs remove it. HDACs have been demonstrated to play a significant role in developments, cancer biology, DNA repair, memory formation and more. In particular, our lab has shown that HDAC1 gets recruited to DNA double stranded break sites for proper DNA repair. HDAC1 was thus shown to play a protective role against insults that cause DNA damage. In this study, we report that HDAC1 plays an important role in mediating circadian regulation in vivo. Mice with exon 5-7 genetically ablated exhibited period (Tau) deviating from the normal wild type Tau of 23.5 hours, suggesting a novel critical role for HDAC1 in regulating the core molecular clock. In addition, we assessed the sleep architecture in these HDAC1 KO animals by electroencephalogram (EEG). We also assessed their cognitive functions with multiple behavioral assays. Upon further investigation, we observed that HDAC1 binds to the promoter of circadian clock genes Per1 (Period1) and Bmal1 (Brain and Muscle ARNT-Like protein), highlighting the important role of HDAC1 in modulating the expression of core molecular clock proteins. We also have direct evidence supporting the notion that HDAC1 binds to promoters of Alzheimer's disease (AD) risk genes and regulates their expression, implicating a role for HDAC1 in facilitating Abeta clearance. Microdialysis approach was also employed to assess the amount of Abeta throughout circadian cycle to determine whether this pattern of oscillation is altered when we knockout HDAC1. Notably, we did further analysis from previous published work and revealed that the oscillatory expression pattern of AD risk genes in aging healthy (>60 yo) human brain samples were dampened. Collectively, these results reveal the novel and fascinating mechanism by which HDAC1 regulates circadian rhythm and suggest that disruption in circadian rhythm could potentially precede cognitive decline in Alzheimer's disease patients.

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Nanosymposium

205. Circadian Rhythms: Timely Topics

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant 1R01MH103361

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Title: Forebrain Bmal1 deletion abrogates time-of-day dependent learning and memory

Authors: *K. SNIDER[1], S. ATEN[1], J. LOESER[1], F. E. NORONA[1], K. HOYT[2], K. OBRIETAN[1];
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Abstract: Within the mammalian brain, cellular time-keeping capacity has been identified in an array of regions, including forebrain circuits that underlie complex cognitive processes. These forebrain oscillator populations appear to be entrained to the master circadian oscillator located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Here, we sought to examine the contribution of these forebrain oscillator cell populations to the rhythmic gating of learning and memory by selectively deleting the core clock gene *Bmal1* from forebrain circuits. To this end a *Bmal1* floxed conditional knockout mouse line was crossed with an α*CaMKII-Cre* driver line that selectively expresses Cre recombinase in forebrain excitatory neuronal cell populations. Importantly, with this approach, the functionality of the SCN oscillator was preserved, thus allowing us to test the specific contribution of the forebrain oscillator populations to the time-of-day modulation of cognition and affect. Here we show that clock-gating of novel object location memory efficiency is disrupted in *Bmal1* forebrain knock-out (fKO) mice. Animals were given five minutes to explore two novel objects in an arena with visuospatial cues; following a thirty-minute delay, they were returned to the arena with the same two novel objects, one of which had been moved. *Bmal1* WT animals exhibited strong novel object location discrimination at circadian time (CT) 16 but no discrimination at CT4; in contrast, *Bmal1* fKO animals did not discriminate at either timepoint. We also tested this transgenic model on the Barnes maze. Animals were trained over four days (three trials per day) to find the correct escape hole on a circular maze; they were returned to home cage housing for a further seventeen days, then given a probe test for long-term recall on day twenty-one. We found that *Bmal1* fKO mice were impaired on both acquisition and recall of spatial location in the Barnes maze. However, no differences were found between *Bmal1* WT and *Bmal1* fKO animals on tests of affect, including the open field assay, the elevated plus maze, and the tail suspension test. Altogether, these data indicate that clock timing in forebrain circuits functions in a coordinated manner with the SCN clock to modulate time-of-day learning and memory efficiency.

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Nanosymposium

205. Circadian Rhythms: Timely Topics

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Topic: F.08. Biological Rhythms and Sleep

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Natural Sciences and Engineering Research Council of Canada

Title: Circadian rhythm disruption induces long lasting hippocampal dysfunction in rats

Authors: ***S. H. DEIBEL**, N. S. HONG, R. J. MCDONALD;
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Abstract: Long Evans rats that experience circadian rhythm disruption induced by daily phase advances of the light dark can acquire a spatial memory in the standard version of the water task, but it appears that memory consolidation is impacted as they do not retain this memory. Similar findings have been documented in male and female rats, several rat strains, and various rodent species. Nonetheless, the rat learning and memory literature is rife with sex and strain differences. The current study investigated the effects of circadian rhythm disruption on learning and memory in male and female Fisher-Brown-Norway rats. In contrast to our previous reports with Long Evans rat, Fisher-Brown-Norway rats were able to retain a spatial location that was acquired during circadian rhythm disruption. Despite this, these rats running-wheel behaviour suggested that there were slight deviations in re-entrainment that persisted well after exposure to a normal light dark cycle. With this in mind we assessed their ability to rapidly acquire a new spatial memory in the water task acquired during one training session in a novel room. Amazingly the rats that experienced circadian rhythm disruption months ago were not able to retain the rapidly acquired spatial memory 24 hours after acquiring this information. It appears that for Fisher-Brown-Norway rats, the impact of circadian rhythm disruption on hippocampal function is subtler compared to Long Evans rats. To the best of our knowledge hippocampal dysfunction observed months after six days of phase advances of the light dark cycle has never been reported before, nor has the rapid acquisition water task been used to assess hippocampal function in circadian rhythm disrupted rodents. This task might be particularly sensitive to the

effects of circadian rhythm disruption on memory and offers a unique opportunity for future endeavors.

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Nanosymposium

205. Circadian Rhythms: Timely Topics

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Topic: F.08. Biological Rhythms and Sleep

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Title: Mechanisms of fast resetting of clocks following rhythm bifurcation

Authors: ***T. NOGUCHI**[1,3], E. M. HARRISON[3,4], J. SUN[2], D. MAY[2], D. K. WELSH[1,3,5], M. R. GORMAN[1,3];
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Abstract: The suprachiasmatic nucleus (SCN), a master circadian clock in the hypothalamus, controls circadian rhythms in the whole body through multiple output systems including the nervous system, the endocrine system, and body temperature cycles. Under a permissive 24-h light:dark:light:dark (LDLD) cycle, rodents show bifurcation in activity patterns and melatonin release, and altered SCN oscillatory patterns. Following bifurcation, animals rapidly re-entrain to new LD cycles and can entrain to extreme T-cycles. In this study, we examined oscillatory stability of the SCN and peripheral tissues in LDLD-bifurcated mice using the dissection procedure as an external stimulus. PER2::Luciferase (PER2::LUC) knock-in mice were entrained to either LDLD or a normal LD cycle. The SCN, lung, liver, and adrenal gland were extracted at various time points in a day. Although the SCN explants of bifurcated animals showed bimodal *per1* activity in a previous study using *per1*::luc transgenic mice, none of the SCN explants showed bimodal PER2 expression in our study. However, interestingly, the phases of explants were significantly affected by dissection time. The phase of the SCN explants was strongly set by dissection in LDLD mice but not in normal LD mice. The phase of lung explants was also strongly set by dissection in LDLD mice but not in normal LD mice. The phase of liver explants showed variable resetting patterns in LDLD mice but was independent of dissection time in normal LD mice. The phase of adrenal glands was strongly set by dissection in both LDLD and LD mice. Furthermore, we examined expression patterns of canonical clock genes in the lung, liver, and kidney under LDLD and LD by real-time PCR. Rhythmicity of all three peripheral

organs in LDLD was significantly lower than LD. These results suggest that decreased SCN oscillatory amplitude caused by bifurcation results in fast resetting of cultured tissues, consistent with previously reported mathematical models.

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Nanosymposium

205. Circadian Rhythms: Timely Topics

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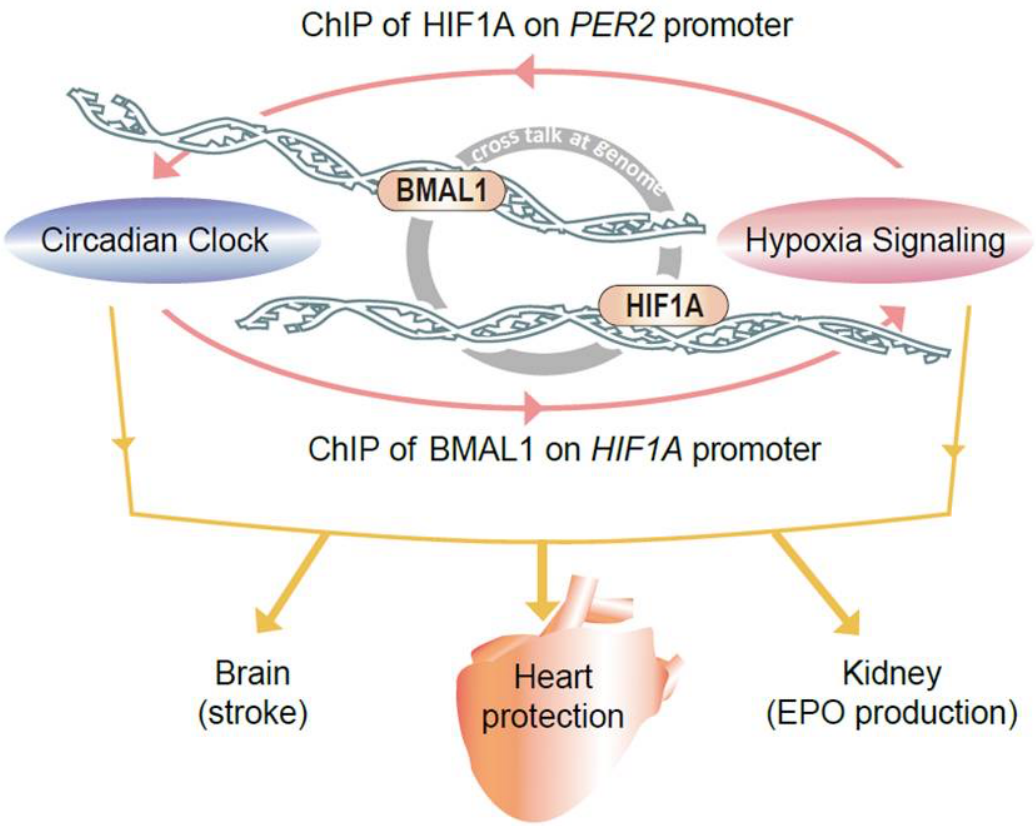
Topic: F.08. Biological Rhythms and Sleep

Support: 973 program (2012CB837700) of M.O.S.T. in China

Title: A ChIP-seq analysis reveals reciprocal regulation between the circadian clock and hypoxia signaling at the genome level in mammals

Authors: ***E. ZHANG**;
Physiol. & Neurosciences, Natl. Inst. of Biol. Sci., Beijing, China

Abstract: Circadian regulation in mammals plays important roles in maintaining metabolic and physiological homeostasis. However, little is known about possible influence of the clock on physiological abnormalities that occur under particular pathological conditions. Here, we report the discovery that hypoxia, a condition that causes catastrophic damage to the body, is gated by the circadian clock *in vivo*. Hypoxia signals conversely regulate the clock by slowing down the circadian cycle and dampening the amplitude of oscillations in a dose-dependent manner. ChIP-seq analyses of hypoxia-inducible factor HIF1A and the core circadian component BMAL1 revealed crosstalk between hypoxia and the clock at the genome level. Further, we found that the severe consequences caused by acute hypoxia, such as those that occur with heart-attacks or strokes, were correlated with defects in circadian rhythms. We propose that the clock plays an important role in fine-tuning hypoxic responses under pathophysiological conditions. We argue that the clock can and likely should be exploited therapeutically to reduce the severity of fatal hypoxia-related diseases.



Disclosures: **E. Zhang:** None.

Nanosymposium

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Topic: F.08. Biological Rhythms and Sleep

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Title: A role for CrebA in regulation of *Drosophila* circadian locomotor behavior

Authors: *Y. ZHANG[1], Z. LIU[2], R. KWOK[3], Y. XIA[4], P. EMERY[4];
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[3]Univ. of California Davis, DAVIS, CA; [4]Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: Circadian clocks have a profound effect on animal behavior and physiology. Circadian rhythms are generated by a conserved negative transcriptional translational feedback loop among species. In *Drosophila*, PERIOD (PER) is the critical regulator of this feedback loop. Our previous studies showed that ATAXIN-2 (ATX2) and TWENTY-FOUR (TYF) are essential for PER accumulation in specific circadian pacemaker neurons-small ventral lateral neurons (sLNvs). How ATX2 and TYF specifically regulate PER in pacemaker neurons is unknown. Here we identified a novel function of CrebA in regulation of *Drosophila* circadian locomotor behavior by interacting with ATX2 and TYF. Depletion of CrebA in the pacemaker neurons lengthened the circadian period, which is due to the lack of PER. Immunostainning indicated that CrebA is only expressed in sLNvs in fly brain. Furthermore, CrebA genetically interacted with TYF, and formed a complex with both ATX2 and TYF. Lastly we showed that CrebA bound to *period* promoter region. Taken together, our data demonstrated that CrebA modulates Drosophila locomotor behavior by regulating PER abundance with ATX2.

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Nanosymposium

206. Stress and Cognition

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Topic: F.04. Stress and the Brain

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DARPA ElectRX

Title: Targeting arousal systems to enhance memory and neural plasticity

Authors: *C. K. MCINTYRE;
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Abstract: The stress response is adaptive in many ways. Release of stress hormones from the adrenal glands into the blood stream readies the body for “fight-or-flight” behaviors that offer obvious protective merits. This sympathetic stress response allows individuals to run farther and to fight harder when in danger. The stress response can also prevent repetition of dangerous behaviors by temporarily enhancing cognitive processes such as attention and memory consolidation, helping the individual to identify threats and store relevant information into long-term memory. One pathway for stress modulation of memory involves the vagus nerve. Both administration of adrenaline and electrical stimulation of the vagus nerve (VNS) modulate memory consolidation and promote release of noradrenaline in the amygdala. Since VNS is FDA-approved for prevention of seizures and treatment of depression, we are testing the hypothesis that pairing VNS could facilitate many kinds of rehabilitation, including exposure therapy for treatment of anxiety disorders and addiction. Our recent findings suggest that VNS promotes plasticity and memory consolidation but bypasses the sympathetic response. In rats, acute VNS increases time spent in the open arms of an elevated plus maze, and this effect depends on the peripheral parasympathetic cholinergic system. This research suggests that VNS may be used to take advantage of mechanisms that are in place to promote memory and neural plasticity without the negative effects of stress.

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Nanosymposium

206. Stress and Cognition

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Topic: F.04. Stress and the Brain

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Title: An examination of beneficial effects of acute post-learning stress on memory, and the moderating factors of those effects

Authors: *A. M. MCCULLOUGH[1], M. RITCHEY[2], G. SHIELDS[2], M. SAZMA[2], C. RANGANATH[2], A. YONELINAS[2];
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Abstract: Recent work has shown that acute stress can positively impact memory performance, particularly when the stress occurs shortly after learning, and yet there is a great deal of variability in the effects reported in the literature. I first present data from a series of experiments in which participants that experienced acute stress after learning exhibited enhanced memory performance compared to control participants who did not experience post-learning stress. Participants completed a learning phase followed by either the cold-pressor task or a control task, and recognition memory was tested 1, 24, or 48 hours later. The results illuminate how different aspects of memory (e.g., recollection, familiarity) can be enhanced by stress, and how the enhancements are related to a physiological marker of stress (i.e., cortisol). The results also illuminate how post-learning stress influences memory-related BOLD activity in brain regions known to be involved in memory encoding and retrieval (i.e., the hippocampus and amygdala).In conjunction, I describe results of a meta-analysis examining the effects of post-learning stress on subsequent memory. These results corroborate the empirical results with respect to the beneficial effects of post-learning stress on memory. However, the results also show that the effects of stress are moderated by a number of factors, such as the time of day that stress is experienced, whether learning and stress occur in the same context, and whether participants are currently taking hormonal contraceptives. Thus, stress can be beneficial for memory when experienced shortly after learning, particularly when learning and stress are experienced in the same context and during the afternoon. These results help to synthesize a vast literature examining the effects of acute post-learning stress on memory, explain some of the variability in reported effects, and provide useful directions and novel predictions for future research.

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Nanosymposium

206. Stress and Cognition

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Presentation Number: 206.03

Topic: F.04. Stress and the Brain

Title: Emotional memories are more accurately remembered over time

Authors: E. ATUCHA[1], V. VUKOJEVIC[4], R. V. FORNARI[5], G. RONZONI[1], P. ATSAK[1], M. COOLEN[2], A. PAPASSOTIROPOULOS[4], D. J. F. DE QUERVAIN[4], *B. ROOZENDAAL[3,1];

[1]Cognitive Neurosci., [2]Human Genet., [3]Radboud Univ. Nijmegen, Nijmegen, Netherlands; [4]Psychology, Univ. of Basel, Basel, Switzerland; [5]Núcleo de Cognição e Sistemas Complexos, Federal do ABC, Santo André, Brazil

Abstract: Emotionally arousing experiences tend to be remembered for long periods of time. Even though such overly strong memories might lead to undesirable and maladaptive long-term consequences, it also helps us remembering significant life events which is a highly adaptive mechanism. Evidence indicates that stress and emotional arousal effects on modulating memory consolidation are mediated by the basolateral amygdala (BLA). This study focuses on the positive effects of an emotionally arousing training experience on the strength and accuracy of the memory. Male Spraque-Dawley rats were trained on a novel inhibitory avoidance discrimination task in which they were subsequently exposed to two similar inhibitory avoidance (IA) apparatuses, but footshock was only delivered in the second IA apparatus. Norepinephrine (NE) (1 μg) or saline was microinfused into the BLA immediately after the training session. Retention of the training was tested either 2 (recent) or 28 (remote) days later by measuring the latencies to enter the dark compartment of both IA apparatuses and of a novel similar apparatus. At 2 days, the NE group had longer latencies in the shock apparatus than the saline group; but both groups remembered in which apparatus they had received footshock (i.e., they showed good discrimination). At 28 days, the saline group no longer showed discrimination between the two training contexts whereas the NE group still showed good discrimination. In order to examine whether this episodic-like discrimination effect requires an intact hippocampus (HPC) during retention testing, the HPC was inactivated with muscimol (0.5 μg) 20 min prior to the testing session. At 2 days, muscimol blocked the discrimination in both the saline and NE groups whereas at 28 days, muscimol had no effect in the saline group, but markedly impaired retention in the NE group. These findings show that emotional experiences via noradrenergic activation of the BLA enhance the strength and accuracy of memories and that even at a remote interval these effect depend on the HPC, suggesting a systems level mechanism. These data provide evidence for stress-induced positive enhancement of memory and opens new directions for clinical applications.

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NSF GRFP DGE1322106

Title: Error monitoring moderates the relation between externalizing but not internalizing behaviors amongst children with a history of institutionalization: Implications for risk and resilience

Authors: *S. TROLLER-RENFREE[1], C. A. NELSON[2,3,4,5], C. ZEANAH[6], N. A. FOX[1]; [1]Human Develop. and Quantitative Methodology, Univ. of Maryland, College Park, MD; [2]Harvard Med. Sch., Boston, MA; [3]Boston Children's Hosp., Boston, MA; [4]Harvard Grad. Sch. of Educ., Cambridge, MA; [5]Harvard Ctr. on the Developing Child, Cambridge, MA; [6]Tulane Med. Sch., New Orleans, LA

Abstract: Exposure to toxic stress through early psychosocial deprivation has been linked to a number of deleterious developmental outcomes. Children raised in institutions are at increased risk of developing internalizing and externalizing problems. However, even after exposure to these early stressors, not all children raised in institutions develop psychopathology. As such, there is much interest in protective factors that may buffer against the effects of early life stress. Proficiency in error monitoring may be one protective pathway for children with a history of institutionalization given that deficits in these skills are related to both internalizing and externalizing psychiatric disorders. Error monitoring and the neural circuitry that supports it have a protracted developmental time course and are highly susceptible to the effects of early life stress. As such, proficiency in error monitoring may moderate the known relation between institutional rearing and subsequent psychopathology. We investigated the impact of psychosocial deprivation on behavioral and neural responses (event-related potentials: ERPs) to a Flanker task assessing error monitoring and the relations between these measures and psychopathology for 12-year-old children in the Bucharest Early Intervention Project (BEIP). The BEIP involves two groups of institutionalized children randomly assigned in infancy to receive either a foster care intervention (FCG) or care as usual (CAUG). Results showed that children who experienced institutional care, particularly those in the CAUG, showed perturbed error monitoring on the Flanker task. Additionally, an ERP measure of error monitoring (the ERN) moderated the relations between time spent in institutional care and externalizing and ADHD behaviors. Results suggested that children who experienced institutional care that had an enhanced ERN were protected against the development of externalizing problems (β=-0.007, p=.110), while children who had a reduced ERN amplitude had increased risk (β=.010, p=.021). Neural correlates of error monitoring did not moderate the relations between time spent in institutionalized care and internalizing behaviors. Perturbations in the neural circuitry associated with error monitoring in combination with psychosocial deprivation is a possible pathway to the development of externalizing and ADHD problems, while proficiency in error monitoring may serve as a protective factor. Implications for future research and evidence-based intervention will be discussed.

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206. Stress and Cognition

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Title: Cortisol response to acute stress predicts more optimal persistence behavior

Authors: ***K. M. LEMPERT**[1], J. T. MCGUIRE[3], D. B. HAZELTINE[2], J. W. KABLE[4], E. A. PHELPS[2];
[1]Dept. of Psychology, [2]New York Univ., New York, NY; [3]Boston Univ., Boston, MA; [4]Univ. of Pennsylvania, Philadelphia, PA

Abstract: People often fail to wait for delayed rewards, even after expressing a preference for them. While this failure to persist is sometimes maladaptive, there are environments in which limiting persistence is advantageous. Persistence should be calibrated to the statistics of delay times in a given environment. Previous work has shown that individuals can calibrate how long to persist for delayed rewards after experience with an environment, and optimal calibration depends on a signal in the ventromedial prefrontal cortex, which tracks the subjective value of waiting over time. Since acute stress can impair prefrontal cortex function, here we tested whether stress would impact the calibration of persistence, in a between-subjects design (four groups; n = 30 each). Half the participants performed a task in which persistence was optimal (high persistence, HP), either after an acute physiological stressor or no stress. The other half performed a task in which it was optimal to quit waiting for a reward soon after each trial began (limited-persistence, LP), either under stress or no stress. Replicating previous work, there was a main effect of environment on persistence, showing that individuals are able to adjust their wait times according to the statistics of the task. There was no main effect of stress on persistence, however, nor was there a stress x environment interaction. Thus, calibration of persistence is preserved under stress. Moreover, among the stressed participants, both the cortisol response to stress ($\beta = 0.38$; $p = 0.01$) and baseline cortisol ($\beta = 0.39$; $p = 0.01$) independently predicted better calibration under stress (less waiting in LP, more waiting in HP). This suggests that increased systemic cortisol output after stress predicts more optimal behavior in this task.

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Nanosymposium

206. Stress and Cognition

Location: SDCC 5B

Time: Sunday, November 13, 2016, 1:00 PM - 3:45 PM

Presentation Number: 206.06

Topic: F.04. Stress and the Brain

Support: NIH Grant R01MH097085

NSF GRFP

Title: Lifetime stress exposure modulates multiple memory systems

Authors: ***E. V. GOLDFARB**[1], G. S. SHIELDS[2], N. D. DAW[3], G. M. SLAVICH[4], E. A. PHELPS[1];
[1]New York Univ., New York, NY; [2]Univ. of California, Davis, Davis, CA; [3]Princeton Univ., Princeton, NJ; [4]UCLA, Los Angeles, CA

Abstract: Although stress can have profound effects on later life, higher levels of stress exposure are not always maladaptive. In fact, moderate stress exposure has been shown to predict better mental health and well-being than no stress exposure. However, the effects of lifetime stress exposure on cognitive function are not well understood. Given that recent acute and chronic stress has been shown to influence how memories are formed and retrieved, we investigated whether experiences of stress over the life course similarly influence memory. We hypothesized that, as with recent stress, higher levels of lifetime stress exposure would cause individuals to be biased toward using striatal rather than hippocampal associations. To test this hypothesis, we screened 894 undergraduates for lifetime stress exposure using the Stress and Adversity Inventory, and invited participants from the high (top 25%, N = 35) and low (bottom 25%, N = 35) ends of this distribution to participate in our visual search task. Our previous neuroimaging data (Goldfarb, Chun & Phelps, *Neuron*, 2016) has demonstrated that we can use visual search performance to measure use of hippocampal (context) and striatal (stimulus-response; SR) memory to guide attention. As each memory cue suggested that the search target would be in a distinct location, at the end of the experiment we combined the context and SR cues to see which association was dominant. We found that more high-stress participants used SR associations, while more low-stress participants used context associations. These biases were driven by differences in the extent to which participants learned to use these associations to guide visual search. Although participants in both stress groups used context to guide visual

search, the high-stress participants were significantly better at using SR associations. Using computational modeling, we separated distinct learning processes that could contribute to these differences. Participants in both groups were able to form predictions about where the target would appear based on the memory cues, but low stress participants were significantly less reliant on predictions from the SR cues. These data provide evidence that individuals with higher lifetime stress exposure have better striatal memory.

Disclosures: E.V. Goldfarb: None. **G.S. Shields:** None. **N.D. Daw:** None. **G.M. Slavich:** None. **E.A. Phelps:** None.

Nanosymposium

206. Stress and Cognition

Location: SDCC 5B

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Presentation Number: 206.07

Topic: F.04. Stress and the Brain

Support: DFG Grant KA-2675/4-1

Title: Neuroendocrine signatures of social discounting

Authors: *T. KALENSCHER[1], Z. MARGITTAI[1], L. SCHWABE[2], M. JOELS[3], M. VAN WINGERDEN[1];
[1]Univ. of Duesseldorf, Duesseldorf, Germany; [2]Hamburg Univ., Hamburg, Germany; [3]Rudolf-Magnus Inst. for Neurosci., Utrecht, Netherlands

Abstract: Despite the still frequently made assumption that human decision makers are exclusively motivated by their material self-interest, decades of research in the behavioural sciences suggest that individuals are often much less selfish than originally assumed. However, people are not equally altruistic to everyone alike. Recent evidence suggests that the propensity to forego personal benefits in exchange for another person's advantage declines hyperbolically as a function of social distance, i.e., how much a subject cares about her interaction partner. The decrease in generosity as a function of social distance is dubbed social discounting. We have recently shown that the social discount function is affected by psychosocial stress. We found that men tested shortly after stressor onset showed increased generosity towards close but not distant others compared to non-stressed men or men tested 90 minutes after stressor onset. Stress goes along with a particular endocrine response that follows a distinct timeline: immediately after stress, rapid actions by non-genomic cortisol and noradrenaline signalling pathways exert their effects on brain functioning in concert, but later on, delayed corticosteroid actions affect neural processing alone. Here, we asked if the psychosocial stress effects on social discounting were

mediated by isolated or combined action of cortisol and/or noradrenaline. To address this question, healthy participants received placebo, cortisol (hydrocortisone) or yohimbine, a drug that increases noradrenergic stimulation, or combinations thereof, before performing a social discounting task. We find that the shape of the social discount function was differentially affected by hydrocortisone action alone or in combination with yohimbine. More specifically, hydrocortisone administration alone increased generosity towards socially close, but not distant, others. However, this generosity-boosting effect was offset when hydrocortisone was administered in combination with yohimbine. Our findings suggest a putative neuroendocrine mechanism underlying the 'tend-and-befriend' response to acute stress.

Disclosures: T. Kalenscher: None. **Z. Margittai:** None. **L. Schwabe:** None. **M. Joels:** None. **M. van Wingerden:** None.

Nanosymposium

206. Stress and Cognition

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Presentation Number: 206.08

Topic: F.04. Stress and the Brain

Support: NSF BCS 1430799

Title: Affect contagion: Physiological covariation among strangers and close others

Authors: *W. MENDES[1], E. SIEGEL[2];
[1]Psychiatry, [2]UC San Francisco, San Francisco, CA

Abstract: Emotions, thoughts, and intentions are not simply concepts that live privately in one's minds, but rather, affective states emanate from us via multiple channels - voice, posture, facial expressions, and behavior - and influence those around us. *Affect contagion*, or the spread of affective states—including stress, emotions, evaluations—from one person to another, is studied in a variety of ways in the social sciences. In this talk I will discuss a series of experiments exploring the antecedents and consequences of affect contagion using dynamic peripheral psychophysiological measurement. The experiments include ones focusing on mothers and children and explore how infants "catch" their mothers' stress reactivity and how *touch* potentiates stress contagion. Another series of experiments explore how recently acquainted individuals can catch each others' affective state and how moderators such as racial/ethnic group, social standing, valence and empathetic tendencies moderate affect contagion.

Disclosures: W. Mendes: None. **E. Siegel:** None.

Nanosymposium

206. Stress and Cognition

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Presentation Number: 206.09

Topic: F.04. Stress and the Brain

Support: European Research Council (ERC_StG 2012_313749

Title: Activity in human V1 reflects changes in spatial frequency perception during freezing.

Authors: *M. LOJOWSKA[1], K. ROELOFS[2], E. J. HERMANS[3];
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Abstract: Prioritization of relevant perceptual information during threatening situations is essential for an optimal behavioural response. Indeed, recent behavioral work suggests that low-spatial frequency (LSF) visual information is preferentially boosted in threat-related perception, potentially acting to facilitate threat detection in perceptually degraded conditions such as long distances or peripheral vision. This improved perception of LSF appears to be associated with a parasympathetically dominated state of freezing, characterized by heart rate deceleration. Animal studies have also shown that freezing depends on efferent connections from the amygdala to the periaqueductal gray (PAG), and also that LSF perception may involve magnocellular connections between the amygdala and early visual regions. However, the neural mechanisms by which freezing alters neural processing of LSF visual information remain unclear, particularly in humans. In the current study, we investigated whether threat anticipation results in functional changes in early visuocortical areas in response to LSF and HSF visual stimuli, and to what extent this response pattern is mediated by physiological responses and activation of the amygdala and PAG. To this end, we used functional MRI and a visual orientation discrimination task during which participants had to indicate the orientation of LSF or HSF Gabor gratings (100 ms), presented in the periphery. Gratings were presented during a 7-s display of a central fixation, the color of which signaled either a 50% chance of receiving an electric shock (threat anticipation condition) or no shock (safe condition). Physiological responses, i.e., heart rate, pupil dilation, and skin conductance, were recorded throughout the whole task for offline assessment of arousal and freezing responses. Consistent with previous findings, threat anticipation resulted in significantly higher skin conductance and pupil dilation responses as well as lower heart rate, which confirmed the efficacy of freezing induction. The preliminary fMRI analysis showed that the magnitude of perceptual improvement to LSF gratings by threat was related to lower event-related BOLD responses in V1. These findings suggest that spatial frequency-specific changes in visual perception during freezing are mediated by state-dependent

changes in responsiveness of early visual areas. In further analyses, we will explore the relationship between the amygdala and PAG activation with the LSF- and HSF- evoked responses in early visual areas under threat.

Disclosures: **M. Lojowska:** None. **K. Roelofs:** None. **E.J. Hermans:** None.

Nanosymposium

206. Stress and Cognition

Location: SDCC 5B

Time: Sunday, November 13, 2016, 1:00 PM - 3:45 PM

Presentation Number: 206.10

Topic: F.04. Stress and the Brain

Title: Prior chronic life stress exposure predicts better decision-making competence

Authors: ***G. S. SHIELDS**[1], G. M. SLAVICH[2];
[1]Psychology, Univ. of California, Davis, Davis, CA; [2]Psychiatry and Biobehavioral Sci., UCLA, Los Angeles, CA

Abstract: Prior research on stress and decision-making has been largely restricted to examining the effects of stress on component processes supporting decision-making because, until recently, no measures of real-world decision-making ability have existed. However, the development of ecologically valid, performance-based measures of decision-making competence have enabled researchers to examine for the first time the effects that acute and chronic life stress exposure has on real-world decision-making abilities. Recently, we reported the unexpected finding that acute stress enhances decision-making competence, and that this enhancement is correlated with stress-induced changes in the hormones cortisol and DHEA (Shields et al., 2016, Psychoneuroendocrinology, 67, 51-60). To date, however, no studies have examined how exposure to chronic stress is associated with decision-making competence. To address this gap in the literature, we recruited 55 healthy young adults and assessed their lifetime exposure to chronic stress using the Stress and Adversity Inventory (STRAIN). We also measured their decision-making competence using the Adult Decision-Making Competence (ADMC). Overall, greater lifetime chronic stress exposure was associated with better decision-making competence, Beta=.31, *p*=.023, *R*-squared=.094. Follow-up analyses designed to examine links between the timing of stress exposure and decision-making competence revealed that this association was driven by chronic stressors that were not presently ongoing in participants' lives. Specifically, exposure to chronic stressors that were not ongoing was strongly associated with better decision-making competence, Beta=.35, *p*=.009, *R*-squared=.121. In contrast, exposure to ongoing chronic stressors was unrelated to participants' decision-making competence, Beta=.04, *p*=.789, *R*-squared=.001. These data are thus the first to suggest that chronic stress exposure is strongly

related to better decision-making competence, but that these effects depend on the exact timing of exposure.

Disclosures: **G.S. Shields:** None. **G.M. Slavich:** None.

Nanosymposium

206. Stress and Cognition

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Presentation Number: 206.11

Topic: F.04. Stress and the Brain

Support: PITN-GA-2013-607652

Title: Neural activity under Psychosocial stress detected by Magnetoencephalography

Authors: *H. WANG.[1,2,3], I.-S. LEE[1,2,3], P. ENCK[2], C. BRAUN[1,4];
[1]Univ. Hosp. Tuebingen MEG Ctr., Tuebingen, Germany; [2]Dept. of Psychosomatic Med. and Psychotherapy, Univ. of Tuebingen, Tuebingen, Germany; [3]Grad. Training Ctr. of Neuroscience, IMPRS for Cognitive and Systems Neurosci., Tuebingen, Germany; [4]Ctr. for Mind/Brain Sciences, Univ. of Trento, Trento, Germany

Abstract: Background: Social stress is a common psychological strain in daily life, which can range from ostracism and social exclusion. Previous studies using fMRI and EEG have revealed a large distributed network when experiencing social exclusion induced by the 'Cyberball' paradigm - a computerized ball-tossing game. The network comprises: the insula, anterior cingulate cortex, temporal gyrus, and prefrontal cortex. **Method**: The present study used 275 channel MEG to detect neural activity of social exclusion in 16 healthy volunteers. Source analysis localized the generation of neural oscillations in theta, alpha and beta bands (6, 11, 16, 21 and 26Hz) comparing 'exclusion' vs. 'inclusion' conditions. Averaged power in significantly activated clusters was correlated with self-report scores of Need Threat Scale and Mood questionnaire. **Result**: Results showed increased activity in left temporal cortex in all frequency bands (bilaterally in 26Hz). Additional activations were found in left fusiform and rolandic operculum in 11Hz band; left supramarginal, rolandic operculum, postcentral cortex, right superior parietal and right posterior cingulum in 16Hz band; left Heschl, insula, rolandic operculum, and hippocampus in 26Hz band. Averaged power of activated clusters in 6 and 11Hz bands showed significantly negative correlations with mood scores, and power in 26Hz band negatively correlated with Need scores. **Discussion**: Activities in the left temporal and hippocampus cortex suggested memory related process of social exclusion, activation in the fusiform reflected increased valuation of faces, activation in the supramarginal area was

associated somatosensory process of 'social pain', and activations of the left insula and rolandic operculum suggest distress and negative emotion caused by social exclusion. Taken together, different frequency oscillations in certain brain areas may reflect various respects of neural process during social exclusion.

Disclosures: H. Wang.: None. **I. Lee:** None. **P. Enck:** None. **C. Braun:** None.

Nanosymposium

207. Transcranial Stimulation and MRI Techniques

Location: SDCC 7B

Time: Sunday, November 13, 2016, 1:00 PM - 2:45 PM

Presentation Number: 207.01

Topic: I.04. Physiological Methods

Title: Activation mechanisms of recently reported magnetogenetic proteins

Authors: *G. DURET, S. POLALI, J. T. ROBINSON;
Electrical and Computer Engin., Rice Univ., Houston, TX

Abstract: The development of magnetogenetic tools permits the wireless stimulation of specific neurons located deep inside the brain. This new capability has the potential to improve the exploration of neural networks, the mapping of regions of the brain that cannot be reached with optogenetic tools, and the stimulation of neurons in freely moving animals. Recently, several proteins have been reported to induce transmembrane depolarization in response to magnetic fields either by magnetothermal or magnetomechanic stimulation of membrane proteins. The first approach takes advantage of the thermosensitivity of certain TRPV proteins that can be gated by a local increase in temperature using magnetic nanoparticles and RF magnetic fields (Chen *et al*., *Science* 2015). The second approach aims to open mechanosensitive channels by applying forces directly on the protein via magnetic nanoparticles. Based on that assumption, the fusion of the mechanosensitive channel TRPV4 to the iron-sequestering protein ferritin has produced a magnetically responsive protein (named Magneto) that is able to provoke membrane depolarization upon stimulation by a static magnetic field (Wheeler *et al*., *Nat. Neurosci.* 2016). Finally, the expression of the magnetically sensitive protein ISCA1 (also called MagR) has been reported to cause an influx of calcium under the application of a static magnetic field (Long *et al*., *Sci. Bull.* 2015).These exciting results have prompted stimulating discussions between physicists and neurobiologists on the actual mechanism by which an applied static field can generate sufficient force at the protein level and trigger the gating of membrane-embedded channels. Calculations and simulations point to a discrepancy between the assumed mechanism for Magneto and MagR and the force that can be produced by magnetic nanoparticles (Meister, *arXiv:1604.01359v2* 2016). In an effort to reconcile the biological observations with the physical

properties of artificial and genetically coded magnetic nanoparticles, we characterized these recent magnetogenetic proteins by applying static magnetic fields of varying intensities and durations, while we recorded transmembrane ion influx by electrophysiology and calcium-sensitive fluorescence imaging. Here we discuss these results and the implications for the mechanisms behind the magnetic sensitivity of TRPV4-ferritin (Magneto) and the protein ISCA1 (MagR).

Disclosures: **G. Duret:** None. **S. Polali:** None. **J.T. Robinson:** None.

Nanosymposium

207. Transcranial Stimulation and MRI Techniques

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Presentation Number: 207.02

Topic: I.04. Physiological Methods

Support: Samsung Research Funding Center of Samsung Electronics SRFC-IT1401-05

Title: Neuronal Resonance-MRI (NR-MRI): A new method to detect weak oscillating magnetic field

Authors: ***K. KIM**, H.-I. HEO, S.-H. PARK;
KAIST, Daejeon, Korea, Republic of

Abstract: Neuronal activity induces transient magnetic fields, and previous works have attempted to directly measure such magnetic field changes using MRI. The feasibility of various MR techniques for detecting neuronal oscillations and related MR signal dephasing was demonstrated in phantom and *in vitro* studies, but their application *in vivo* is still in a debate. In order to detect biphasic spontaneous neuronal oscillations *in vivo*, MR methods should be free from temporal cancellation between positive and negative episodes of the neuronal oscillations and be able to improve sensitivity by temporal averaging effects, in the whole brain wave frequency up to ~200 Hz. In this regards, we propose a new approach, termed neuronal resonance MRI (NR-MRI) for detecting the neuronal oscillations through multi-phase acquisition and Fourier analysis. A time series of images acquired by multi-phase scheme encoded periodic neuronal phase shifts based on the relative phase difference between the neuronal oscillation and the sequence. In both simulation and phantom studies, the signals from the oscillating magnetic field could be detected at the target frequency under the conditions of random phases, on/off intervals, and random frequencies of oscillation, and the range of detectable frequency could be manipulated by adjusting repetition time. We also introduced spin preparation method with multiple 180^{o} refocusing pulses, in order to accumulate the phase shift by neuronal oscillation

without temporal cancellation. In combination with multiple 180° refocusing pulses, NR-MRI showed the improved sensitivity in simulation and phantom studies. NR-MRI can potentially be applied to any MRI sequences that have been used for neuronal currents imaging so far. These results demonstrate that NR-MRI is a strong strategy to enhance detectability of weak neuronal oscillation and thus it may enable us to directly detect *in vivo* neuronal currents in a reproducible manner.

Disclosures: K. Kim: None. **H. Heo:** None. **S. Park:** None.

Nanosymposium

207. Transcranial Stimulation and MRI Techniques

Location: SDCC 7B

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Presentation Number: 207.03

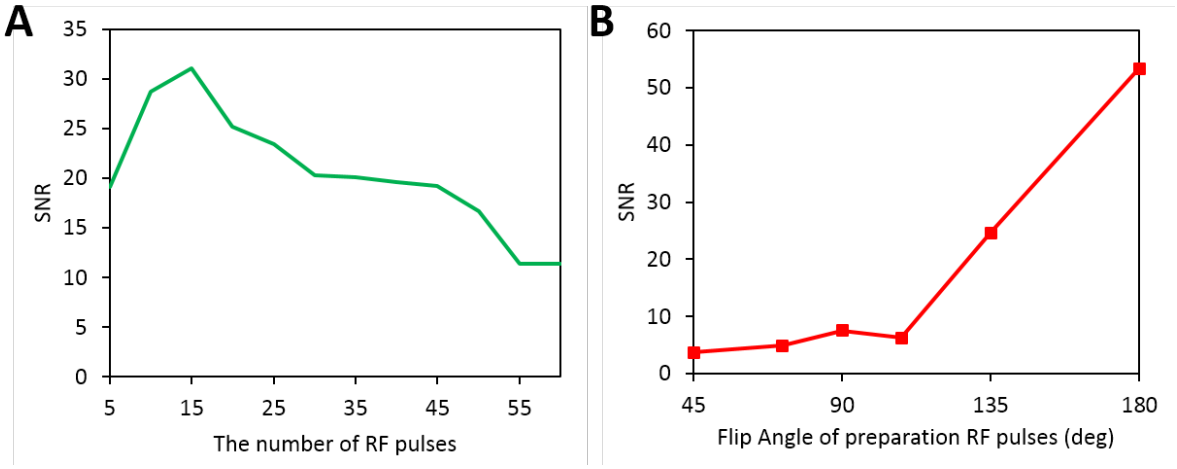
Topic: I.04. Physiological Methods

Title: Investigating effects of multiple refocusing radiofrequency pulses for direct detection of neuronal currents using MRI.

Authors: *H.-I. HEO, K.-H. KIM, S.-H. PARK;
KAIST, Daejeon, Korea, Republic of

Abstract: Neuronal activity has been measured with imaging modalities such as EEG, MEG, and fMRI, which have limited spatial and/or temporal resolution. Many researchers have tried to directly detect the neuronal activity using MRI, which is different from the hemodynamic-based conventional fMRI. However, magnetic fields induced by neuronal oscillation in-vivo is weak to be detected in current MRI methods, and strategies to amplify the neuronal current signals are needed. In this study, we investigated spin preparation with multiple radiofrequency (RF) pulses as a method to increase sensitivity to neuronal current, using phantom experiments. A fast spin echo (FSE) sequence was used to evaluate effects of number of RF pulses on neuronal signal enhancement. Echo planar imaging (EPI) with the spin preparation at various flip angles was also tested with corresponding inter-pulse intervals maintaining the same effective RF power level, which corresponds to the spin rotation frequency of 25 Hz. The FSE and EPI datasets were acquired as a repeated time series of multiple phase offsets between the data acquisition and the neuronal oscillation (25 Hz, 1nT) and then the acquired data were analyzed after temporal Fourier transform. Increasing the number of RF pulses increased sensitivity to neuronal oscillation for the initial 15 RF pulses because of phase accumulation effects, and then decreased the sensitivity for the higher number of RF pulses because of T_2 relaxation effects (Fig. 1A). In Fig. 1B, SNR increased with flip angle of individual RF pulses at the same effective power. The study results provided the optimal conditions for the multiple RF pulse preparation to increase

sensitivity to neuronal current, when MR image acquisition was performed with multiple phase offsets and Fourier analysis. The study results are also potentially applicable to accelerating image acquisition and/or increasing spatial coverage at the same scan time by acquiring data in between the multiple RF pulses.



Disclosures: **H. Heo:** None. **K. Kim:** None. **S. Park:** None.

Nanosymposium

207. Transcranial Stimulation and MRI Techniques

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Topic: I.04. Physiological Methods

Support: Univ. Tübingen fortune Junior Grant 2287

IZST Industry-on-Campus Project 211

Title: Modulation of brain networks with brain-oscilliation synchronized brain-stimulation: Real-time EEG triggered TMS with sub-millisecond precision

Authors: ***C. ZRENNER**, P. BELARDINELLI, D. DESIDERI, U. ZIEMANN;
Ctr. for Neurol., Univ. Hosp. Tübingen, Tuebingen, Germany

Abstract: Why are evoked cortical responses so variable? The explanation is not “noise”, but the dynamics of ongoing activity that lead to a different brain state at the time of each stimulus. However, synchronizing the timing of the individual stimuli with instantaneous state parameters

such as the phase of localized ongoing 10 Hz or 20 Hz oscillations has so far been challenging: Signal analysis (e.g. band-pass filtering) requires a window both backward and forward around the time point of interest; the "now" is elusive since the future hasn't happened yet. We present a technique that addresses this: Source-level brain activity is computed in real-time from high-density online streamed EEG data using a spatial filter based on a pre-computed forward model from the individual's segmented MRI data and precise EEG electrode locations. The resulting sliding window of brain network activity, localized to relevant cortical atlas parcels, is zero-phase filtered at the frequency band of interest, forward predicted using an autoregressive model, and Hilbert transformed, thus yielding oscillatory phase-state at multiple nodes as a measure of "instantaneous brain network state". This parameter is then used to trigger the TMS pulse based on a pre-defined brain-state condition. The entire algorithm is implemented on commodity hardware and software (Mathworks Simulink Real-Time) and executed at a fixed sample time of 0.5 milliseconds. The efficacy of the method with respect to modulating brain networks is validated in a study with 12 healthy volunteers that receive closed-loop single pulse and 100 Hz triple pulse TMS stimulation to left primary motor cortex, phase-locked to the "up phase" and the "down phase" of the spatially filtered local EEG signal in a double blind cross-over design with an open-loop "replay" control condition. The results show that brain-state triggered stimulation is phase-locked to the individual local ipsi- and contralateral sensorimotor 10 Hz μ-rhythm phase with an accuracy of ±30 degrees. Furthermore, the magnitude of TMS evoked motor and EEG potentials demonstrate a reliable differential effect of local 10 Hz μ-rhythm "up phase stimulation" vs. "down phase stimulation" with respect to both cortical excitability as well as the induction of long lasting plasticity in cortico-spinal and cortico-cortical network pathways. Importantly, the effects would have averaged out in a standard "open loop" stimulation protocol. These results suggest that such an approach may be relevant for both an investigation of basic cortical neurophysiology as well as for the development of more effective and specific personalized therapeutic brain stimulation protocols.

Disclosures: **C. Zrenner:** None. **P. Belardinelli:** None. **D. Desideri:** None. **U. Ziemann:** None.

Nanosymposium

207. Transcranial Stimulation and MRI Techniques

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Zhejiang NSF LZ15H180001

Title: Brain network alterations induced by high-frequency rTMS

Authors: X. LIU, P. WEI, D. LI, J. XIE, J. ZHANG, Q. GE, *Z. WANG;
Ctr. for cognition and brain disorders, Hangzhou Normal Univ., Hangzhou, China

Abstract: This study was to assess human functional connectome (FCN) using transcranial magnetic stimulation (TMS), which themselves are two hot brain research topics. Human brain is a highly integrated complex network. Any local brain alteration is likely to induce a network-wise variation regardless the variation strength. The macro-level network properties can be characterized using the graph theoretical analysis (GTA) in the so-called FCN analysis. TMS is a reversible and relatively focal approach for creating "virtual lesions" in the healthy brain and provides an ideal approach to assess the nodal effects on FCN but has yet been done. The purpose of this study was to examine FCN alterations in response to focal applications of repetitive TMS (rTMS). Our hypothesis is that focal rTMS will change FCN topology properties. fMRI data were acquired before and after applying 20 Hz rTMS or the corresponding SHAM stimulation from 40 young healthy subjects (20 in SHAM). rTMS was applied to the left dorsolateral prefrontal cortex (DLPFC). The order of applying or not applying rTMS (or SHAM) was counterbalanced and the second time of experiment was conducted two days later. rTMS stimuli contained 20 pulses per second (20 Hz) for 2.5 sec, with an inter-train interval of 28 sec. The pulse magnitude was adjusted to be 90% of the resting motor threshold. After standard preprocessing, mean fMRI timecourses were extracted using an anatomical atlas and FCN was generated based on the inter-regional correlation coefficients matrix. Two typical FCN properties: nodal betweeness and small-worldness were calculated using GTA. The former indicates the nodal role in FCN (hub node or not); the latter measures the network connectivity efficiency and cost. As compared to SHAM, rTMS significantly increased nodal betweeness in DLPFC, medial prefrontal cortex, hippocampus, and temporal cortex. 20 Hz rTMS is known to be excitatory, which may explain the nodal betweeness increase in regions receiving efferents from DLPFC. rTMS reduced small-worldness across all assessed FCN sparsity thresholds, which may be related to the nodal role change caused by focal rTMS. In summary, our results provide first evidence of focal neural stimulations induced reductions to the brain network topological properties. The network-wise alterations also provide new insights into the neural mechanisms of rTMS effects on the brain, which however still remains unclear though rTMS has been shown to be a promising tool to treat various brain diseases.

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Nanosymposium

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Presentation Number: 207.06

Topic: I.04. Physiological Methods

Support: Wellcome Trust Grant WMCR NG0355

Title: Taking control: polarity dependent modulation of the cognitive control network using transcranial direct current stimulation

Authors: *L. M. LI[1], I. R. VIOLANTE[1], E. ROSS[1], R. LEECH[1], A. LEECH[1], D. CARMICHAEL[2], D. J. SHARP[1];
[1]Divison of Brain Sciences, Imperial Col. London, Computational, Cognitive and Clin. Neuroimaging, London, United Kingdom; [2]Developmental Imaging and Biophysics Section, UCL Inst. of Child Hlth., University College London, United Kingdom

Abstract: INTRODUCTION: Transcranial direct current stimulation (TDCS) can affect cognitive control, although little is known about the physiological basis for this effect. The Stop Signal Task (SST) is widely used to assess response inhibition, an important component of cognitive control. During successful stopping, activation of the right inferior frontal gyrus (rIFG) and pre-supplementary motor area (Pre-SMA) occurs, with deactivation of the posterior cingulate cortex (PCC). We conducted a simultaneous functional MRI-TDCS study to investigate the effects of rIFG (F8) anodal (a-tDCS) and cathodal (c-tDCS) tDCS on brain activity during SST performance. We tested: (1) whether TDCS modulated activity in the network of regions involved in successful stopping; and (2) whether any effects were polarity dependent.
METHODS: 26 healthy subjects performed the Stop Signal Task (SST). This requires subjects to withhold an automatic response (pressing a left or right button) when an infrequent 'Stop' signal appears. Each participant performed the task under sham, a-tDCS or c-tDCS (2mA) in a counterbalanced order. An event related fMRI design was used and analysed using FSL (see Bonnelle et al; PNAS 12).
RESULTS: An expected pattern of neural activity was associated with successful response inhibition (StopCorrect>Go & StopCorrect>StopIncorrect contrasts). This included activation of the rIFG and preSMA as well as deactivation of the dorsal and ventral parts of the PCC. There were no effects of stimulation on the cortex directly beneath the stimulating electrode. In contrast, activity in regions remote from stimulation was modulated. Activity in a pre-SMA region involved in response inhibition was increased by c-tDCS, relative to sham and a-tDCS. This increase was seen for both StopCorrect>StopIncorrect and StopCorrect>Go. The ventral PCC deactivated during successful stopping. However, c-tDCS produced an increase in activity

in this region relative to a-tDCS. A strong positive correlation was seen between activity in dorsal PCC and stop signal reaction time (r=0.542, p=0.009), indicating worsening inhibitory control as activity increased. However, there were no significant behavioural effect of TDCS.
CONCLUSION:
Non-invasive electrical stimulation modulates brain network activity in a polarity dependent manner. Remote effects of c-tDCS were observed during response inhibition in the pre-SMA and PCC. The right IFG is thought to be involved in controlling activity in these areas as part of a distributed cognitive control network. Therefore, our results suggest that right IFG TDCS might be an effective way to modulate the function of cognitive control networks.

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Nanosymposium

207. Transcranial Stimulation and MRI Techniques

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Presentation Number: 207.07

Topic: I.04. Physiological Methods

Support: Pritzker Institute

Title: Predicting tms-induced activation in human neocortex derived from concurrent tms/pet and finite element analysis

Authors: ***D. MOGUL**[1], G. ARABKHERADMAND[1], T. D. KRIEG[1], F. S. SALINAS[2], P. T. FOX[2];
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Abstract: Transcranial magnetic stimulation (TMS) is a powerful technique to noninvasively activate neurons in the brain and is increasingly used in both clinical and research applications. Despite this growing interest, the relationship between TMS-generated electric fields and specific cortical electrophysiological responses is not well understood. The goal in this study was to investigate the relationship between induced electric fields and cortical activation measured by metabolic responses. For this purpose, we combined human subject-specific detailed finite element modeling (FEM) of the head to calculate induced cortical electric field (E-field) profiles and employed concurrent TMS application during positron emission tomography (PET) recordings as a measure of cortical activation. Using the precise coil position relative to each subject, the E-field vectors induced in each subject were calculated throughout the cortex. A

functional map of local circuit connections in and between neocortical columns was developed which was used to study the relationship between applied magnetic fields, hence induced E-fields, and activation in the neocortex. The theoretical model was fitted to experimental data in order to develop a predictive algorithm for TMS induced activation in the neocortex of humans. Previous research in our lab demonstrated that decomposing the E-field into orthogonal vectors based on cortical neuronal orientation differentiates the relative contribution of the E-fields that lead to activation. The sensitivities of activation of pyramidal neurons vs. interneurons to induced E-field vectors either normal (E_{norm}) or tangential (E_{tan}) to the cortical surface, respectively, were determined. Interneuronal sensitivity to induced E_{tan} was over twice as strong as pyramidal neurons to the induced E_{norm} vector that may help to explain why indirect activation (I-waves) of the cortex occurs at lower TMS amplitudes than direct activation of pyramidal neurons (D-waves). Furthermore, this study produced an algorithm for predicting the electrophysiological responses of neurons in human neocortex based on the effect of applied magnetic fields and columnar circuitry in human neocortex.

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Nanosymposium

282. Modeling Neuropsychiatric Disease

Location: SDCC 23A

Time: Monday, November 14, 2016, 8:00 AM - 11:30 AM

Presentation Number: 282.01

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grants

MSCRF

Title: Modeling Zika virus exposure with human iPSC-derived neural cells

Authors: *Z. WEN[1,2,3], H. TANG[5], C. HAMMACK[5], S. C. OGDEN[5], X. QIAN[7], Y. LI[4], B. YAO[4], M. XU[11], Y. CHENG[5], E. M. LEE[5], J. SHIN[7], F. ZHANG[4], W.-K. HUANG[7], J. TCW[12], K. M. CHRISTIAN[7,8], R. A. DIDIER[6], K. BRENNAND[12], W. ZHENG[11], P. JIN[4], H. SONG[7,8,9], G.-L. MING[7,8,9,10];
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of Med., Baltimore, MD; [11]Natl. Ctr. for Advancing Translational Sci., NIH, Bethesda, MD; [12]Dept. of Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Zika virus (ZIKV), a mosquito-borne flavivirus, is currently reported to be circulating in 26 countries and territories in Latin America and the Caribbean. While ZIKV infection has been linked to microcephaly in newborns and other brain abnormalities such as Guillain-Barré syndrome, how ZIKV impairs brain development and function is unknown. Here we show that two stains of ZIKV, Asian $ZIKV^{C}$ and African $ZIKV^{M}$, directly infects human induced pluripotent stem cell (hiPSC)-derived cortical neural progenitor cells (hNPCs) with high efficiency. Infected hNPCs further secrete infectious ZIKV particles. Importantly, ZIKV infection increases cell death and dysregulates cell cycle progression, resulting in attenuated hNPC growth. Gene expression analyses of infected hNPCs reveal transcriptional dysregulation, notably of cell cycle-related pathways. Our results identify human cortical neural precursors as a direct target of ZIKV infection. In addition, we establish a tractable experimental model system for investigating the impact and mechanism of ZIKV on human brain development and a platform for screening therapeutic compounds.

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Nanosymposium

282. Modeling Neuropsychiatric Disease

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Topic: A.03. Stem Cells and Reprogramming

Support: Grant, Academy of Finland

Grant, Finnish Brain Research Foundation

Grant, Aro ja Lea Ylppö Foundation

Title: Differentiation of human neural progenitors to glutamate-responsive cells in fragile X syndrome, a variant of autism

Authors: *M. L. CASTRÉN, V. S. ACHUTA;
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Abstract: Autism spectrum disorder (ASD) consists of a range of heterogeneous group of disorders. Fragile X syndrome (FXS) is a monogenic variant of ASD and the most common form of inherited intellectual disability. FXS is caused by the absence of FMR1 protein (FMRP) that is needed for normal neurogenesis and functional maturation of synapses and neuronal networks. The absence of FMRP results in alterations of neural progenitor fate determination and differentiation. Defects of both inhibitory and excitatory transmission impair functional connectivity and lead to hyperexitability in brain of FXS mouse. Maturation of glutamate receptor signaling is affected and an abnormally large proportion of NMDA-only neurons has been found at the close of the critical period in the somatosensory cortex of the developing FXS mouse brain. We have studied mechanisms underlying aberrances of FXS neurogenesis by investigating the differentiation and functional maturation of human neural progenitors lacking FMRP. We reprogrammed somatic cells of males diagnosed with FXS and healthy controls to induced pluripotent stem (iPS) cell lines. Transcriptional silencing of the *FMRI* gene was confirmed by real time PCR in FXS cell lines. Human iPS cells were differentiated to neuronal lineages using dual SMAD inhibition. Differentiation of neuronal progenitors was studied by immunocytochemistry and fura2-AM based intracellular calcium recordings. We exposed progenitors to specific ligands of glutamate receptors and observed abnormal differentiation of glutamate-responsive cell subpopulations from FXS progenitors. The FXS-specific differentiation defect was similar in human and mouse FXS neural progenitors but treatment responses to metabotropic glutamate receptor antagonist showed differences during differentiation of human and mouse progenitors. Our results demonstrate early defects during differentiation of patient-specific FXS neural progenitors to glutamatergic neurons. Human iPS-derived FXS progenitors provide a useful model to study novel treatment options for neurodevelopmental disorders.

Disclosures: **M.L. Castrén:** None. **V.S. Achuta:** None.

Nanosymposium

282. Modeling Neuropsychiatric Disease

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Time: Monday, November 14, 2016, 8:00 AM - 11:30 AM

Presentation Number: 282.03

Topic: A.03. Stem Cells and Reprogramming

Title: Modeling drug response in autism using pluripotent stem cells

Authors: *C. MARCHETTO, Y. KIM, R. SANTOS, A. D. MENDES, S. LINKER, F. GAGE; Salk Inst., La Jolla, CA

Abstract: Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that affects about 1% of children in the United States. ASD is characterized by deficits in verbal communication, impaired social interaction and limited and repetitive interests and behavior. The major impediment to testing hypotheses and potential therapeutic interventions for autism is the lack of relevant animal and cell models. The direct study of live brain tissue from ASD patients is unfeasible, and no suitable animal models can adequately reproduce the complicated structure, proper wiring and function of the human brain. Reprogramming of human somatic cells to a pluripotent state by over-expression of specific genes into induced pluripotent stem cells, or iPSCs (Takahashi et al., 2007) has provided an exciting opportunity to produce a relevant human cellular model for complex multigenetic disorders such as ASD. Here we use a new platform (Multielectrode Arrays-MEA) to perform functional field potential analysis of neuronal populations from ASD individuals and neurotypical controls during development and after treatment with drugs that are currently in clinical trials for ASD. Our results indicate that ASD neurons respond to drug treatment by increasing neuronal spiking and neuronal bursts. Additionally, we performed expression profile analysis on developing ASD neurons and neurotypical controls after drug treatment to uncover which pathways are potentially involved in the recovery of the neuronal activity observed. Studying biological basis of ASD and cellular drug responsiveness would likely lead to the development of clinically useful biomarkers of risk for this disorder, which may lead to the development of novel therapies.

Disclosures: C. Marchetto: None. **Y. Kim:** None. **R. Santos:** None. **A.D. Mendes:** None. **S. Linker:** None. **F. Gage:** None.

Nanosymposium

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Topic: A.03. Stem Cells and Reprogramming

Support: R01MH101454

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NYSCF

NARSAD

Title: Modeling network regulators of genetic predisposition to schizophrenia using stem cells

Authors: *K. BRENNAND[1], B. HARTLEY[1], S. ZHU[1], A. TOPOL[1], J. ENGLISH[2], M. HAUBERG[3], N. TRAN[1], C. RITTENHOUSE[1], A. SIMONE[4], D. RUDERFER[1], H. SHAH[1], G. CAGNEY[5], J. RAPOPORT[6], F. GAGE[4], P. SKLAR[1], M. MATTHEISEN[3], D. COTTER[2], G. FANG[1];
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Abstract: Schizophrenia (SZ) is a debilitating neurological disorder for which the molecular mechanisms underlying the disease state remain unclear. To address this, we reprogrammed fibroblasts from SZ patients into human induced pluripotent stem cells (hiPSCs) and subsequently differentiated these disorder-specific hiPSCs into neural progenitor cells (NPCs) and neurons; we previously reported aberrant migration, increased oxidative stress, abnormal WNT signaling and elevated global protein synthesis in SZ hiPSC NPCs, together with diminished neuronal connectivity, decreased neurite number, and impaired synaptic morphology in SZ hiPSC neurons. Now, we show that miRNA-9 (miR-9) was abundantly expressed in control NPCs, but also significantly down-regulated in a subset of SZ NPCs. We observed a strong correlation between miR-9 expression and miR-9 regulatory activity in NPCs as well as between miR-9 levels/activity, neural migration and diagnosis. Overexpression of miR-9 was sufficient to ameliorate a previously reported neural migration deficit in SZ NPCs, whereas knockdown partially phenocopied aberrant migration in control NPCs. Unexpectedly, proteomic- and RNAseq-based analysis revealed that these effects were mediated primarily by small changes in expression of indirect miR-9 targets, rather than large changes in direct miR-9 targets; these indirect targets are enriched for migration-associated genes. An expanded genome wide network-based analysis of aberrant mRNA and microRNA expression has now been completed in a replication cohort of hiPSCs derived from twelve patients with childhood-onset SZ (COS). Already, decreased miR-9 levels were confirmed in this cohort. Moreover, primary differential expression analysis revealed perturbed expression of members of WNT signaling, protocadherin and glutamate gene families. Such datasets, particularly when integrated with larger post-mortem patient datasets, will allow us to begin investigating links between genotype, gene expression and *in vitro* phenotype, to ascertain how SZ associated genetic variants maybe contributing to SZ predisposition at a cellular level.

Disclosures: K. Brennand: None. **B. Hartley:** None. **S. Zhu:** None. **A. Topol:** None. **J. English:** None. **M. Hauberg:** None. **N. Tran:** None. **C. Rittenhouse:** None. **A. Simone:** None. **D. Ruderfer:** None. **H. Shah:** None. **G. Cagney:** None. **J. Rapoport:** None. **F. Gage:** None. **P. Sklar:** None. **M. Mattheisen:** None. **D. Cotter:** None. **G. Fang:** None.

Nanosymposium

282. Modeling Neuropsychiatric Disease

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Topic: A.03. Stem Cells and Reprogramming

Support: Governor's Council for Medical Research and Treatment of Autism (PI: Millonig) CAUT13APS010

Governor's Council for Medical Research and Treatment of Autism (PI: DiCicco-Bloom) CAUT14APL031

Governor's Council for Medical Research and Treatment of Autism (PI: DiCicco-Bloom) CAUT15APL041

Title: Idiopathic autism patient-derived neural stem cells display defects in neurite outgrowth, cell migration, and cellular signaling pathways

Authors: *S. PREM[1], M. WILLIAMS[3], C. MCDERMOTT[4], X. ZHOU[1], P. YEUNG[2], C. LU[2], Z. PANG[2], L. BRZUSTOWICZ[5], P. MATTESON[1], J. MILLONIG[1], E. DICICCO-BLOOM[1]; [1]Rutgers Robert Wood Johnson Med. Sch., Piscataway, NJ; [2]Rutgers Robert Wood Johnson Med. Sch., New Brunswick, NJ; [3]Rutgers Grad. Sch. of Biomed. Sci., Piscataway, NJ; [4]Queens College, City Univ. of New York, Flushing, NY; [5]Dept. of Genet., Rutgers Univ., Piscataway, NJ

Abstract: Autism spectrum disorders (ASD) are neurodevelopmental disorders defined by impaired social interaction and repetitive restrictive behaviors. Our inability to directly study human neurons has thwarted discovery of cellular and molecular mechanisms of idiopathic ASD. Now, induced pluripotent stem cells (iPSCs) allow for generation and study of live human neural stem cells (NSCs) from affected individuals. To define developmental defects in idiopathic ASD, we are studying neurite outgrowth, cell migration, and signaling pathways in iPSC derived NSCs from 8 severely affected males (ASD) and their unaffected brothers (Sib). Our strategy employs extracellular factors (EFs) that challenge NSCs to reveal deficits not apparent at baseline and help identify dysfunctional signaling. To ensure observed phenotypes are not artifacts of viral reprograming involved in iPSC generation, we have assessed multiple NSC lines derived from 2 Sib iPSC clones and 3 ASD iPSC clones. Studies were conducted in control media or media with PACAP, NGF, or 5HT. To quantify neurite outgrowth, NSCs were plated at low density and analyzed at 48h for percent of cells with neurites. To examine migration, neurospheres were generated from NSCs in solution, and then plated onto Matrigel for 48 hours. Using phase images, cell migration is defined as total neurosphere area-inner cell mass area. To explore signaling, protein levels were assessed using western blot on NSCs treated with EFs. In 1 family,

Sib NSCs derived from multiple iPSC clones had higher baseline neurite outgrowth and showed increases in response to PACAP, NGF and 2 doses of 5-HT. In contrast, ASD NSCs displayed no increases except with 300 ug/mL 5-HT. Similarly, Sib neurospheres had increased migration with PACAP while ASD neurospheres were unresponsive. PACAP acts via the PKA-cAMP-P-CREB pathway and ASD NSCs, which did not respond to PACAP, exhibited 4x lower P-CREB levels compared to Sib NSCs. Conversely, increasing ASD P-CREB levels by using db-cAMP, restored neurite outgrowth to levels of PACAP-treated Sib NSCs. ASD NSCs also exhibited reduced levels of mTOR signals (3x lower P-AKT, 9x lower P-S6). In conclusion, ASD NSCs derived from multiple iPSCs showed impaired neurite outgrowth and migration at baseline and under EF stimulation. ASD NSCs simultaneously displayed impaired signaling molecules required to respond to EFs (PACAP-PCREB) and relevant to developmental disorders (mTOR: P-AKT, P-S6). While heterogeneity of ASD reduces the chances of identical phenotypes in all our patients, our studies indicate the value of using EFs to uncover impaired and patient-specific pathways which may lead to personalized ASD therapies.

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Nanosymposium

282. Modeling Neuropsychiatric Disease

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Topic: A.03. Stem Cells and Reprogramming

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Title: MicroRNA dysregulation in an induced pluripotent stem cell model of bipolar disorder.

Authors: ***M. BAME**[1], M. MCINNIS[2], S. O'SHEA[3];
[2]Psychiatry, [3]Cell & Developmental Biol., [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Bipolar disorder (BP) is a complex condition characterized by severe fluctuations in mood for which underlying pathological mechanisms remain largely unclear. Although family and twin studies have shown that there is a large genetic component to the disorder, a single causative gene has yet to be identified. Genome wide linkage studies have identified 16 potential regions spread over 13 different chromosomes to be associated with BP. Genome wide association studies have been marginally more successful at identifying specific loci associated with the disease, but the few variants identified in this manner are associated with only a negligible risk of developing BP. A potential mechanistic link to these findings is dysregulation of microRNA processing. MicroRNAs (miRNA) are small, non-coding RNAs approximately 20 nucleotides in length that are responsible for the post-translational regulation of multiple genes. These RNAs are believed to regulate 70-90% of human genes and have been shown to play important roles in neural development, as well as in the adult brain. Several miRNAs have been found to be dysregulated in postmortem studies of brain tissue isolated from bipolar patients, suggesting there may be miRNA processing defects. Because there are no cellular models of BP, we have taken advantage of the recent discovery that somatic cells can be reprogrammed to pluripotency then directed to form the full complement of neural cells. Using microarray analysis of BP and unaffected iPSC neurons derived from patient skin samples, we have identified 59 miRNAs involved in a wide range of cellular functions, including neuronal development, homeostasis, and signaling as significantly dysregulated in BP iPSC neurons. We have validated 5 miRNAs that were elevated (miR-382-5p, miR-128-3p, miR-138-2-3p, miR-487b-3p, and miR-195-5p) and 3 miRNAs that were lower in BP derived neurons (miR-874-5p, miR-10b-5p, and miR-10b-3p). GO analysis of miRNAs significantly elevated in BP derived neurons show that many of these miRNAs are involved in regulating a wide variety of cellular processes, including proliferation, cell junction and extracellular matrix organization, chromatin modification, mRNA processing, and carbohydrate metabolism. We are currently using a luciferase assay to validate a number of targets of these miRNAs, including CTNNB1 and WNT9a (Wnt pathway), NEUROD6 and PAX6 (neuronal differentiation and CNS patterning), GRM7 (glutamate signaling), and KIF2A and SNPH (primary cilia assembly and axonal mitochondrial transport). Validated targets will then be overexpressed in our iPSC derived cells to determine their effects on neuronal behavior.

Disclosures: **M. Bame:** None. **M. McInnis:** None. **S. O'Shea:** F. Consulting Fees (e.g., advisory boards); Genetech.

Nanosymposium

282. Modeling Neuropsychiatric Disease

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Topic: A.03. Stem Cells and Reprogramming

Support: The Rockefeller University

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Cure Alzheimer's Fund

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Title: A novel human iPSC-based model of Alzheimer's disease generated by knock-in of early-onset AD mutations displays disease-relevant, zygosity-dependent phenotypes

Authors: *D. PAQUET[1], D. KWART[1], A. CHEN[1], A. SPROUL[2], S. JACOB[3], S. TEO[1], K. M. OLSEN[1], A. GREGG[1], S. NOGGLE[3], M. TESSIER-LAVIGNE[1];
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Abstract: Our aging society is confronted with a dramatic increase in patients suffering from dementias, such as Alzheimer's disease (AD), for which no mechanism-based cures are available. Animal models, although useful for understanding aspects of AD pathology, do not capture key aspects of the disease and have limited use in developing treatments. A human AD model that reproducibly develops disease-relevant molecular pathology would enhance functional studies and improve screening for drug development. Recent advances in stem cell research allow reprogramming of mutant patient-derived cells to induced pluripotent stem cells (iPSCs) and subsequent differentiation into cortical neurons. Although first studies have shown promising results for AD, the field still lacks optimized technology for generating isogenic lines to confirm phenotype specificity. CRISPR/Cas9 has recently been developed into a versatile gene editing tool holding promise for generating models of human diseases, e.g. in iPSCs. Although CRISPR/Cas9 is used extensively to engineer gene knock-outs, editing cells by homology-directed repair (HDR) to introduce disease-associated mutations remains inefficient and inaccurate. Furthermore, targeted mutation introduction at single alleles, to model diseases caused by heterozygous mutations, such as early-onset Alzheimer's disease (EOAD), has not been reported. We developed a CRISPR/Cas9-based genome-editing framework that allows selective introduction of mono- and bi-allelic sequence changes with high efficiency, accuracy and predictable control of zygosity. Homozygous introduction requires using a guide RNA targeting close to the intended mutation, whereas heterozygous introduction can be accomplished by distance-dependent suboptimal mutation incorporation or by using mixed repair templates. Using this approach, we generated the first human induced pluripotent stem cells (iPSCs) with heterozygous and homozygous dominant EOAD mutations in amyloid precursor protein (APP^{Swe}) and presenilin 1 ($PSEN1^{M146V}$). We then differentiated mutant iPSCs into disease-relevant cortical neurons to study phenotypic changes in the neurons affected in the patients. In

doing so we found distinct genotype-dependent disease-associated phenotypes, particularly in the pathological amyloidogenic processing of APP and related molecular downstream changes. Taken together, our findings not only enable efficient introduction of disease-associated mutations with CRISPR/Cas9, but shed light onto the molecular mechanisms underlying human dementia.

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Nanosymposium

282. Modeling Neuropsychiatric Disease

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Presentation Number: 282.08

Topic: A.03. Stem Cells and Reprogramming

Support: NS095348

Title: Modeling for genetic risk for schizophrenia in iPSCs and mice reveals synaptic release deficits

Authors: ***N. KIM**[1], Z. WEN[2], J. LIU[3], K.-J. YOON[2], Y. ZHOU[4], Y.-T. LIN[5], Z. GUO[2], X. WANG[2], H. YU[2], K. M. CHRISTIAN[2], K.-S. HSU[5], W. LI[4], X.-Y. LU[3], H. SONG[2], G.-L. MING[2];
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Abstract: Dysregulated neurodevelopment with altered structural and functional connectivity of synapses is believed to underlie many neuropsychiatric disorders including schizophrenia and autism spectrum disorder. Genetic variations in risk genes such as *Disrupted in Schizophrenia 1* (*DISC1*) have been associated with increased susceptibility for neuropsychiatric disorders. Several mouse models have been generated with different *Disc1* mutations to study the functional consequences of mutant DISC1 expression, with variable results. To study DISC1 function in the context of specific mutations identified in patients, we generated model systems

based on a 4 base-pair (bp) frame-shift deletion at the C-terminus of *DISC1*, which was identified in an American family. In this family, Pedigree H, the 4 bp mutation co-segregates with major psychiatric disorders with high penetrance. First, we generated induced pluripotent stem cells (iPSCs) from members of Pedigree H with the 4 bp frame-shift deletion of *DISC1*. Forebrain neurons derived from these iPSC lines exhibited aberrent synaptic function, including impaired synaptic vesicle release, reduced synaptic transmission, and global transcriptomic changes, including dysregulation of many presynaptic genes. To investigate DISC1 dysfunction in vivo with intact neural circuitry at the behavioral level, we developed a novel knock-in (KI) line of mice carrying the analogous mutation to the human 4 bp deletion in *DISC1*. Consistent with the cellular phenotypes we observed in patient-derived human neurons, *Disc1* KI mice showed synaptic deficits and dysregulation of synaptic protein expression. Interestingly, *Disc1* KI mice exhibited abnormal behavioral phenotypes in a subset of assays related to schizophrenia-like behaviors. Next, based on molecular findings from both the iPSC and mouse model systems, we performed mechanism-guided pharmacological screening with human iPSC-derived forebrain neurons and found that pharmacological inhibition of phosphodiesterases rescued dysregulated synaptic function in human neurons and several phenotypes in the in vivo mouse model. In this study, we integrated information from disease-relevant models of human patient iPSC-derived neurons and novel transgenic mice and identified consistent phenotypes and a potential mechanistic target to ameliorate synaptic deficits associated with a genetic risk for psychiatric disorders.

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Nanosymposium

282. Modeling Neuropsychiatric Disease

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Topic: A.03. Stem Cells and Reprogramming

Support: Scottish Rite Charitable Foundation

Banting Foundation

Title: Investigation of GRIN2B dosage in developing neurons

Authors: ***G. MAUSSION**, S. TORRES-PLATAS, C. VASUTA, H. PENG, C. BOUDREAU-PINSONNEAULT, A. DIALLO, J.-F. THÉROUX, C. GIGEK, L. CRAPPER, G. TURECKI, E.

CHEN, K. ADAMS, N. MECHAWAR, T. WONG, C. ERNST;
Dept. of Psychiatry, Douglas Mental Hlth. Univ. Inst., Verdun, QC, Canada

Abstract: Genetic variations are found in a subset of patients with autism spectrum disorders (ASDs) or intellectual disabilities (IDs), however, the link between genetic variation and molecular or cellular processes underlying neuronal dysfunctions remain poorly understood. Using human neural progenitor cells (NPCs) derived from induced pluripotent stem cells (iPSC) from patients and genetically engineered controls may help to understand these mechanisms. *GRIN2B* mutations are associated with ASD and ID. The main objective of this study is to understand how mutation, deletion or disruption in *GRIN2B* leads to an altered neurodevelopmental program. Using RNA sequencing and qPCR, we investigate whole genome transcription profiles in four independent cellular models: (i) Neurons expressing a stable construct which silences *GRIN2B*, (ii) Neurons derived from patient iPSCs with a *GRIN2B* point mutation, (iii) Clonal neurons homozygous for an engineered *GRIN2B* loss of function mutation, and (iv) Clonal neurons heterozygous for the same loss of function mutation in *GRIN2B*. Functional annotation analysis shows significant enrichments of differentially expressed genes related to synapse functioning and ion transport. This dysregulation may compromise activity-dependent cell development and dendritic spine formation and may be crucial in the pathophysiology of ASD and ID.

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Nanosymposium

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Topic: A.03. Stem Cells and Reprogramming

Support: Swiss National Science Foundation, outgoing PD fellowship

Streims foundation

Title: Studying serotonergic neurotransmission using human pluripotent stem cell derived neurons *In vitro*

Authors: *K. C. VADODARIA, S. DAVE, C. FREDLENDER, L. FUNG, X. LI, F. GAGE; LOG-Gage, Salk Inst. For Biol. Sci., La Jolla, CA

Abstract: Serotonergic neurotransmission plays an important role in brain function and its dysfunction has been implicated in neuropsychiatric disorders including Major Depression. Human stem cell technology has revolutionized our capacity to generate and study human neurons in vitro. Human neuron-based disease modeling approaches have given novel insight into pathology of human neuropsychiatric disorders as well as hope for the development of in vitro assay platforms. As a first step for studying serotonergic neurotransmission in the context of neuropsychiatric disorders, recently, we have generated human serotonergic neurons in vitro from induced pluripotent stem cells (iPSCs) and fibroblasts (Vadodaria et al., 2016). Using human serotonergic- and pan-neuronal differentiation protocols, here we study serotonergic neurotransmission using multiple assay platforms including SSRI- and activity- responses of human neurons in vitro.

Disclosures: K.C. Vadodaria: None. **S. Dave:** None. **C. Fredlender:** None. **L. Fung:** None. **X. Li:** None. **F. Gage:** None.

Nanosymposium

282. Modeling Neuropsychiatric Disease

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Time: Monday, November 14, 2016, 8:00 AM - 11:30 AM

Presentation Number: 282.11

Topic: A.03. Stem Cells and Reprogramming

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Danish Research Council for Independent Research DFF-1335-00763

Title: Human ftd ipsc-derived neurons provide novel insights into imbalance of iron homeostasis and neurodegeneration

Authors: *Y. ZHANG[1], B. SCHMID[2], N. K. NIKOLAISEN[1], M. A. RASMUSSEN[2], B. I. ALDANA[1], K. CALLOE[1], T. C. STUMMANN[3], H. M. LARSEN[1], T. T. NIELSEN[4], J. HUANG[5], L. YE[5], F. XU[5], L. BOLUND[6], L. K. BAK[1], H. S. WAAGEPETERSEN[1], Y. LUO[6], J.

E. NIELSEN[4], B. HOLST[2], C. CLAUSEN[2], P. HYTTEL[1], K. K. FREUDE[1];
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Abstract: FTD is clinically, pathologically and genetically very heterogeneous young onset dementia. One unique subtype of FTD was previously described in a large Danish family, linked to chromosome 3 (FTD3). The underlying mutation in affected family members is located in a splice acceptor site generating a dominant negative protein with an altered c-terminus. This dominant gain of function mutation affects the functionality of the endosomal system, which is important for protein degradation pathway and cell surface receptor recycling. Here, I present a disease model based on human induced pluripotent stem cells (iPSC) from FTD3 patients and their isogenic controls generated via CRISPR/Cas9 gene editing. We were able to verify the endosomal dysfunction in the iPSC-derived neurons from FTD3 patients and identified additional mitochondrial abnormalities and subsequent increased oxidative stress in these neurons. All of these cellular phenotypes could be rescued in the gene-edited patient lines. We confirmed candidate genes and pathways involved in these pathogenic events through RNA-Seq analyses and intriguingly we identified several key enzymes and receptors mis-regulated, which are important for iron homeostasis. This crucial cellular function is linking endosomal and mitochondria dysfunction as well as oxidative stress and subsequently implying a central role for iron homeostasis in normal neuronal function. Since iron accumulations have been observed in several different forms of neurodegenerative diseases, such patient based iPSC models are promising platforms to further identify drugable targets to interfere and normalize iron homeostasis.

Disclosures: **Y. Zhang:** None. **B. Schmid:** None. **N. K. Nikolaisen:** None. **M. A. Rasmussen:** None. **B. I. Aldana:** None. **K. Calloe:** None. **T. C. Stummann:** None. **H. M. Larsen:** None. **T. T. Nielsen:** None. **J. Huang:** None. **L. Ye:** None. **F. Xu:** None. **L. Bolund:** None. **L. K. Bak:** None. **H. S. Waagepetersen:** None. **Y. Luo:** None. **J. E. Nielsen:** None. **B. Holst:** None. **C. Clausen:** None. **P. Hyttel:** None. **K. K. Freude:** None.

Nanosymposium

282. Modeling Neuropsychiatric Disease

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Presentation Number: 282.12

Topic: A.03. Stem Cells and Reprogramming

Support: Japan Agency for Medical Research and development, AMED

Title: Differential gene expression patterns and synaptic functions for each genetic class of Angelman syndrome patient-derived neurons

Authors: *M. ISHIKAWA[1], H. OKUNO[1], S. TANAKA[1], Y. NAKATAKE[2], H. KOMANO[1], W. AKAMATSU[3], M. KO[2], K. KOSAKI[4], S. SAITOH[5], H. OKANO[1];
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Abstract: Genomic imprinting is the epigenetic phenomenon by which certain genes are expressed in parent–of–origin-specific manner. Angelman syndrome (AS) is one of the most famous imprinted disorders which shows sever neurodevelopmental deficit, speech impairments, intellectual disability, epilepsy, prognathism, abnormal sleep patterns, and hyperactivity. In most cases, AS is caused by large deletion of maternally inherited human chromosome 15q11–q13, whose region contains UBE3A (ubiquitin protein ligase E3A) gene. In this study, using episomal plasmids, we established novel iPSCs from T lymphocytes of seven different AS individuals, including not only a large genomic deletion of maternal 15q11-13 region, but also uniparental disomy, or imprinting defect or epimutation in the imprinting center in 15q11-13 region, which can also cause AS pathogenesis. To investigate the AS–specific neuronal phenotypes, we performed the neuronal induction of iPSCs using a rapid single–step induction method and analyzed the expression levels of AS and other developmental disease-related gene in the neurons. All the AS–specific iPSC lines were successfully differentiated into mature neurons which expressed the marker genes of synapse and glutamatergic neurons. Interestingly, UBE3A gene expression level in neurons of AS with maternal 15q11-13 deletion was different from other class of AS patients and control iPSC-derived neurons. We are now investigating the functional differentiation with control and AS neurons using transcriptome analysis and Ca2+ imaging.

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Nanosymposium

282. Modeling Neuropsychiatric Disease

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Time: Monday, November 14, 2016, 8:00 AM - 11:30 AM

Presentation Number: 282.13

Topic: A.03. Stem Cells and Reprogramming

Support: NARSAD

MSCRF

SFARI

Title: Altered doses of psychiatric risk factor CYFIP1 lead to dysregulated protein and behavioral abnormalities in models of psychiatric disorders

Authors: *K.-J. YOON[1], F. R. RINGELING[2], Y. ZHOU[4], H. N. NGUYEN[1], S. J. TEMME[1], N.-S. KIM[1], Y.-T. LIN[6], B. XIAO[7], K.-S. HSU[6], S. CANZAR[8], W. LI[5], P. WORLEY[3], K. M. CHRISTIAN[1], H. SONG[1], G.-L. MING[1];
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Abstract: Copy number variations (CNVs) are caused by structural rearrangements of the genome such as deletions, duplications, inversions, and translocations, many of which are associated with neurodevelopmental disorders. Gene dosage imbalance caused by many different CNVs have been implicated in autism spectrum disorders (ASD) and schizophrenia (SZ). Interestingly, 15q11.2 duplications have been associated with ASD, whereas deletions of the same region have been identified as one of the three most frequent CNV risk factors for SZ. Moreover, a recent structural MRI imaging study showed that 15q11.2 deletion carriers have reciprocal structural changes in the same brain regions that are altered in duplication carriers, suggesting that 15q11.2 CNVs are prominent dosage-sensitive genetic risk factors for neuropsychiatric disorders. *CYFIP1* is one of four genes in 15q11.2 and encodes a protein that negatively regulates mRNA translation at synapses in an activity-dependent manner. Previous studies showed both increased and decreased levels of CYFIP1 lead to abnormalities in dendritic complexity and spine morphology, as well as synaptic plasticity. Thus, imbalance in CYFIP1 levels may lead to diametric alterations in common signaling pathways, which could underlie pathogenesis of 15q11.2 CNV-mediated risk for neuropsychiatric disorders. To address this question, we established both loss-of-function and gain-of-function mouse models of *Cyfip1*. RIP-seq analysis in hippocampus from these genetically modified mice identified a number of CYFIP1 targets. We further validated a subset of these targets in forebrain neurons derived from human iPSCs. Loss of *Cyfip1* resulted in SZ-like behavioral impairments, whereas the gain of *Cyfip1* mouse model showed ASD-related behavioral phenotypes. Our integrated analyses from multiple model systems provide insight into how 15q11.2 CNVs may contribute to divergent neuropsychiatric disorders.

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Nanosymposium

282. Modeling Neuropsychiatric Disease

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Topic: A.03. Stem Cells and Reprogramming

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NIH 1UH2TR000506-01

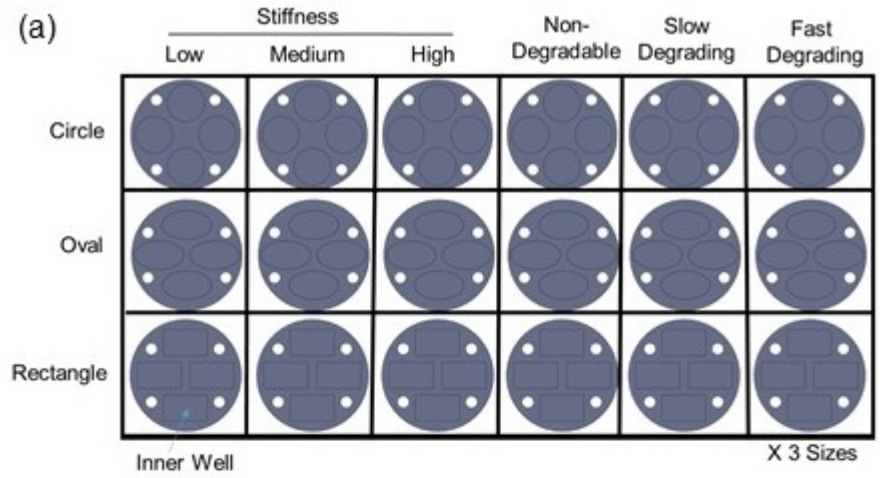
NIH R01EB10039

Title: The controlled formation of human vascularized neural assemblies using synthetic hydrogel technologies and the application of the assembled neural tissues in human neurodevelopmental disease modelling

Authors: *B. T. DALY[1,2], A. DIAS[2], C. SOREF[2], W. MURPHY[2,3];
[1]Human Models For Analysis of Pathways Ctr., Madison, WI; [2]Dept. of Orthopedic and Rehabil., Univ. of Wisconsin - Madison, Madison, WI; [3]Human Models for Analysis of Pathways Ctr., Madison, WI

Abstract: Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) have vastly enhanced the ability to capture the cellular diversity and native characteristics of human tissues. In particular, capturing the cellular diversity of the developing human brain is a major challenge in neural stem cell engineering. Here, we have generated neural progenitor cells, endothelial cells, and microglial precursors from iPSCs for inclusion in our neural assemblies (Fig.1a). Synthetic hydrogels have demonstrated the ability to form vascularized neural assemblies with high sample uniformity and is suitable alternative to Matrigel which is highly variable[1]. An enhanced throughput screen, consisting of chemically-defined synthetic hydrogels with variable stiffness, degradability and adhesion ligand presentation[2] with variable boundary conditions (geometry, size), was used to generate neural assemblies with defined cellular architectures (Fig.1b). The screen identified a subset of conditions that formed neural assemblies with controlled shapes and sizes (Fig.1c). These neuronal assemblies are being further characterized to understand how the identified conditions influence cortical layer formation within the construct. Conditions will be identified for the formation of a robust and reproducible neurodevelopmental model. Patient-derived iPSCs from microcephalic patients (from 3 different gene mutations) are currently being differentiated for incorporation into the optimized neuronal construct. From this a primary microcephalic disease model will be generated to understand the

etiology and progression of the neurodevelopmental disorder. References:1 Schwartz *et al. PNAS* 112, 12516-12521, (2015) 2 Murphy *et al. Nat Mater* 13, 547-557, (2014)



(b)

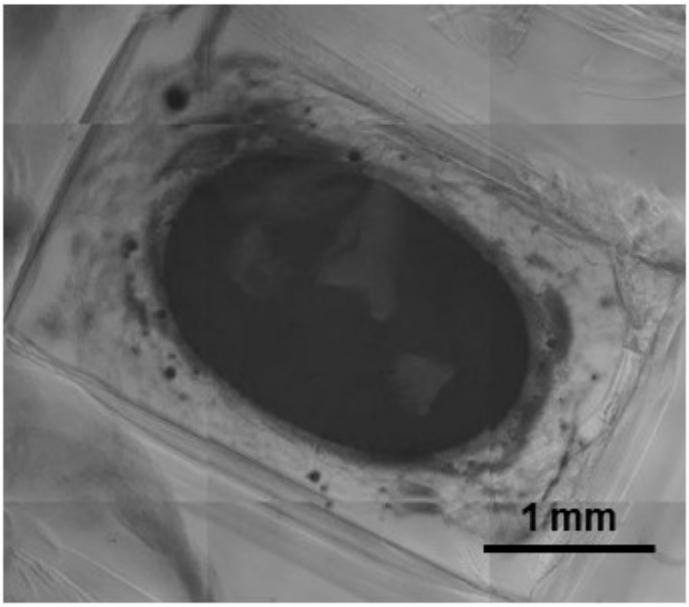


Fig.1 (a) Schematic illustrating a subsection of materials, sizes and geometries screened in the enhanced throughput screen to optimize neuronal architecture **(b)** Example of a neural assembly generated within the array.

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Nanosymposium

283. Epilepsy: Mechanisms

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Presentation Number: 283.01

Topic: B.11. Epilepsy

Support: NRF-2014R1A1A3049456

Title: Translational profiling of the dentate mature granule cells after pilocarpine-induced status epilepticus

Authors: *K. CHO[1], S. YUN[3], S. NAM[2], A. EISCH[3], J. HSIEH[3];
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Abstract: Newborn granule cells after epileptic seizures show various structural abnormalities. In contrast, seizure-experiencing mature granule cells have only been reported to exhibit a reduction in spine density, without other gross morphological changes. However, molecular signature of mature granule neurons after acute seizures is still unknown. To identify actively translated mRNAs in mature granule cells after status epilepticus (SE) compared to sham, we used neurotrophin-3 (NTF3) driven BAC-TRAP (bacterial artificial chromosome-translating ribosome affinity purification) transgenic mice. These mice express eGFP-tagged ribosomal protein L10a in mature dentate granule neurons, allowing mRNA isolation associated with polysomes by eGFP immunoprecipitation (IP). Seven-week-old NTF3 BAC-TRAP mice were administered scopolamine and terbutaline (i.p., 2 mg/kg), followed by pilocarpine injection (i.p., 200 mg/kg for males, 230 mg/kg for females). At 8 days after pilocarpine or saline injection, mice were sacrificed and allocated into RNA-seq or histology groups. For RNA-seq, 8 hippocampi isolated from 2 males and 2 females were pooled for further steps. After eGFP immunoprecipitation, purified mRNAs were subjected to the library generation for RNA-seq, which was sequenced with Illumina platform. For histology, immunohistochemistry was performed. We confirmed that eGFP was expressed in the granule cells of sham- and pilocarpine-injected mice, without a colocalization of eGFP and doublecortin, suggesting that eGFP was expressed only in embryo-generated mature granule cells in the dentate gyrus. Quantitative PCR analysis showed that IP fraction was enriched with eGFP and depleted with S100b, AIF1, PECAM1, and MBP, a representative astrocyte, microglia, endothelial cell, and oligodendrocyte gene, respectively, compared to unbound fraction. Based on this data indicating that isolated mRNAs are mainly derived from eGFP-positive mature granule neurons, we performed RNA-seq to identify differentially expressed genes in seizure-subjected granule cells compared to sham. Pathway analysis showed that reelin signaling in neurons, axonal guidance, mTOR signaling, actin cytoskeleton signaling, and growth hormone signaling were altered in

mature granule cells after acute seizures. In conclusion, we identified mature granule cell-specific transcripts after acute seizures using TRAP technology. We hope to address how mature granule cells after acute seizures contribute to the development of epilepsy and their critical molecular mechanisms to control epileptogenesis, which may provide new treatment options for preventing epilepsy.

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Nanosymposium

283. Epilepsy: Mechanisms

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Presentation Number: 283.02

Topic: B.11. Epilepsy

Support: NIH/NINDS R01 NS069861 (V.S)

Title: Dentate parvalbumin expressing chandelier cells show early reduction in excitability in experimental epilepsy

Authors: ***A. PRODDUTUR**, J. GUEVARRA, V. SANTHAKUMAR;
NEUROLOGY AND NEUROSCIENCES, Rutgers New Jersey Med. Sch., Newark, NJ

Abstract: Inhibitory neurons of the dentate gyrus are crucial in maintaining the functional "gate" that regulates spread of network excitability in the trisynaptic circuit. Molecular layer interneurons are positioned to provide feed-forward granule cell inhibition. Specifically, parvalbumin positive chandelier cells (PV-ChCs), frequently located in the inner molecular layer, regulate granule cell firing by providing synaptic inhibition to the axon initial segment. Circuit effects of PV-ChCs are likely distinct from PV-basket cells (PV-BCs) which underlie somatic feed-forward and feedback inhibition of granule cells. Here we examine if the intrinsic and synaptic physiology of PV-ChCs, which are morphologically and functionally distinct from PV-BCs, are altered in experimental epilepsy. Adult mice expressing YFP under control of Cre recombinase in parvalbumin-expressing neurons (Jackson labs: 008069) were treated with pilocarpine to induce status epilepticus (SE) for 2 hours. Whole-cell patch clamp recordings were obtained from YFP positive cells in the inner molecular layer in hippocampal sections prepared from one week (early) and one month (late) after SE, and in age-matched saline-injected controls. Cells were filled with biocytin during recordings and processed to reveal morphology. PV-ChCs were distinguished from PV-BCs by the presence of vertical axonal cartridges. One week after SE, PV-ChCs showed a significant decrease in firing rate in response to somatic current injection (400pA-800pA) compared to controls. The early post-SE decrease in

excitability recovered to control levels by one month. There was an early and persistent increase in membrane sag in response to hyperpolarizing current injection (-200pA), indicating an increase in hyperpolarization activated cation current (Ih) in post-SE PV-ChCs compared to controls. However, input resistance of PV-ChCs was not altered at either time point after SE. The post SE alterations in intrinsic physiology are unique to PV-ChCs and were not observed in PV-BCs in earlier studies (Yu et al., 2015). Consistent with findings in PV-BCs, the frequency and amplitude of spontaneous inhibitory synaptic currents in PV-ChCs were decreased early after SE. Together, our findings suggest that PV-ChCs undergo a robust early decrease in excitability after SE which could impair feed-forward inhibition of dentate granule cells and compromise the dentate gate early after seizures.

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Nanosymposium

283. Epilepsy: Mechanisms

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Topic: B.11. Epilepsy

Support: NIH NINDS

Title: Testing for correlation between dentate gyrus anatomic pathology and frequency of spontaneous seizures in a mouse model of temporal lobe epilepsy

Authors: *P. BUCKMASTER, E. ABRAMS;
Dept Comparative Med., Stanford Univ., Stanford, CA

Abstract: Mechanisms underlying temporal lobe epilepsy remain unclear. Loss of mossy cells and inhibitory interneurons, generation of ectopic hilar granule cells, and mossy fiber sprouting have been hypothesized to be epileptogenic. The mechanism that correlates best with seizure frequency would be a priority for further testing. Mice (n=128) were treated with pilocarpine to induce at least 2 h of status epilepticus when they were 58 ± 2 d old (mean ± sem). One month later, they were video-monitored for spontaneous convulsions 9 h/d for a month. After monitoring, their left hippocampus was isolated, straightened, and sectioned transversely. Mossy cells, ectopic hilar granule cells, and interneurons were visualized with GluR2- and Prox1-immunocytochemistry and in situ hybridization for glutamic acid decarboxylase (GAD), respectively. Mossy fiber sprouting was measured as the percentage of the granule cell and molecular layers labeled black by the Timm stain. The optical fractionator method was used to estimate numbers of cells per dentate gyrus. Control mice (n=10) had 3300 ± 250 mossy cells,

600 ± 30 ectopic hilar granule cells, 7780 ± 310 GAD neurons, and 2 ± 1% Timm staining. Averages of epileptic mice revealed mossy fiber sprouting (23X controls), generation of ectopic hilar granule cells (3.6X controls), loss of mossy cells (38% of controls), and loss of GAD neurons (75% of controls) (all $p<0.0001$). Severity of GAD neuron loss was greatest in the hilus (45% of controls), least in the molecular layer (103% of controls), and intermediate for neurons in or adjacent to the granule cell layer (80% of controls). Epileptic mice displayed 0.14 ± 0.02 seizures/h, range 0.01-0.47. Seizure frequency did not correlate with the extent of mossy fiber sprouting or generation of hilar ectopic granule cells. Seizure frequency did correlate with loss of mossy cells ($p=0.04$, $R=-0.18$) and total loss of GAD neurons ($p=0.01$, $R=-0.23$). Seizure frequency correlated with loss of GAD neurons in the granule cell layer ($p=0.005$, $R=-0.25$), but not in the hilus or molecular layer. Multiple linear regression analysis revealed that seizure frequency could be predicted by granule cell layer GAD neuron loss alone without mossy cell loss. Granule cell layer GAD neurons include those that express parvalbumin (PV). Control mice had 945 ± 72 PV neurons. Epileptic mice displayed PV neuron loss (69% of controls, $p<0.0001$), but it did not correlate with seizure frequency. Understanding mechanisms of temporal lobe epilepsy is important for developing better treatments. There are many competing hypotheses, but these findings prioritize loss of non-PV interneurons in the granule cell layer for further testing.

Disclosures: P. Buckmaster: None. **E. Abrams:** None.

Nanosymposium

283. Epilepsy: Mechanisms

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Support: NIH Grant NS073574

Epilepsy Foundation Pre-Doctoral Fellowship

T32 Synapse Neurobiology Training Program

Title: Hippocampal corticotropin-releasing hormone neurons potently modulate hippocampal function, excitability, and seizure susceptibility

Authors: *A. HOOPER[1], J. MAGUIRE[2];
[1]Neurosci., Tufts Univ., Boston, MA; [2]Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Hippocampal corticotropin-releasing hormone (CRH) neurons are a novel class of hippocampal interneurons with unique features, including a subset which back-project from CA1 to CA3. We recently characterized the connectivity and inhibitory identity of hippocampal CRH neurons, but their role in hippocampus-dependent behaviors and their capacity to influence hippocampal network excitability through GABAergic transmission remain unknown. Here, using the Cre-Lox system to selectively manipulate hippocampal CRH interneurons, we probe hippocampal excitability, hippocampus-dependent behaviors, and seizure susceptibility while stimulating, silencing, or ablating these neurons. We demonstrate that *in vitro* optical stimulation of hippocampal CRH neurons with Channelrhodopsin is sufficient to suppress electrically evoked postsynaptic responses in CA3. Furthermore, *in vivo* chemogenetic silencing with inhibitory Designer Receptors Exclusively Activated by Designer Drugs gives rise to locomotor hyperactivity, while ablation with Diphtheria Toxin A subunit causes deficits in spatial learning and memory. These hippocampal CRH neurons are ideally positioned to modulate the excitability of the hippocampal network. As such, susceptibility to kainic acid-induced seizures is decreased by optical activation of hippocampal CRH neurons. Conversely, seizure susceptibility is exacerbated by chemogenetic silencing or ablation of hippocampal CRH neurons, consistent with the predicted effects of respectively driving and suppressing GABAergic signaling. These data demonstrate that hippocampal CRH neurons exert an inhibitory influence on the hippocampal network as a potent modulator of hippocampal excitability.

Disclosures: **A. Hooper:** None. **J. Maguire:** None.

Nanosymposium

283. Epilepsy: Mechanisms

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Topic: B.11. Epilepsy

Support: Health Research Board HRA-POR-2013-325

Framework Programme 7 (Health; No. 602130)

Title: Systemic delivery of antagomir-134 produces anti-epileptogenic effects

Authors: ***C. R. RESCHKE**[1], V. R. VANGOOR[2], M. ROSSO[1], G. P. BRENNAN[1], L. F. A. SILVA[1], A. SANZ-RODRIGUES[1], A. BATOOL[1], E. JIMENEZ-MATEOS[1], M. CAMPBELL[3], J. PASTERKAMP[2], D. C. HENSHALL[1];
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Translational Neurosci., UMC Utrecht, Utrecht, Netherlands; [3]Smurfit Inst. of Genet., Trinity Col. Dublin, Dublin, Ireland

Abstract: Epilepsy is a serious neurological disease characterized by spontaneous recurrent seizures. Acquired epilepsy is associated with large-scale changes in gene expression which underlie the cell and network-level changes during epileptogenesis. Despite various efforts we still have no treatments to prevent the emergence of epilepsy following brain injury. MicroRNAs are a family of small non protein-coding RNAs that regulate gene expression. They play important roles in the pathogenesis of epilepsy controlling processes that are dysregulated in epileptogenesis, and have emerged as potential targets for epilepsy treatment. Recent work showed that miRNA-134 is overexpressed in the temporal lobe of patients with pharmacoresistant seizures and in experimental models of epilepsy. Silencing miR-134 using intracerebroventricular injections of antagomirs (Ant) potently suppressed evoked and spontaneous seizures in mice. In order to move this approach toward clinical translation we tested systemic delivery of these large macromolecules, timing delivery to coincide with blood-brain barrier (BBB) opening after *status epilepticus* (SE) in mice. Here, we also investigated the possible mechanism responsible for Ant-134 effects. SE was induced in C57BL/6 adult mice by an intra-amygdala injection of kainic acid. BBB permeability was assessed by dye injections and extravasation of serum protein into brain parenchyma, and confirmed by 7T magnetic resonance imaging (MRI). Presence of Ant-134 in the hippocampus was confirmed by *in situ* hybridization. Antagomirs were locked nucleic acid- and cholesterol-modified. Injections were then timed accordingly and mice subject to continuous long-term video-telemetry EEG recording. BBB opening in this model was apparent 2 h after SE. Systemic injection of Ant-134 at this time point did not alter the duration or severity of *status epilepticus* in mice but significantly reduced the number of spontaneous seizures recorded in mice compared with controls. These seizure-suppressive effects persisted up to 3 months after the SE. This phenotype could be reversed by genetic knockdown of Lim kinase-1, a known target of miR-134.Silencing miR-134 is increasingly and consistently found to produce anti-epileptogenic effects and these data indicate the option of systemic delivery which may have wider clinical use. Pre-clinical development of Ant-134 should now be considered.

Disclosures: C.R. Reschke: None. **V.R. Vangoor:** None. **M. Rosso:** None. **G.P. Brennan:** None. **L.F.A. Silva:** None. **A. Sanz-Rodrigues:** None. **A. Batool:** None. **E. Jimenez-Mateos:** None. **M. Campbell:** None. **J. Pasterkamp:** None. **D.C. Henshall:** None.

Nanosymposium

283. Epilepsy: Mechanisms

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Presentation Number: 283.06

Topic: B.11. Epilepsy

Support: CURE-Citizens United for Research in Epilepsy

National Health and Medical Research Council Australia

Title: Can a spider venom fix Dravet Syndrome?

Authors: *K. L.-A. RICHARDS[1], C. J. MILLIGAN*[1], V. HERZIG[2], R. J. RICHARDSON[1], M. GRUNNET[3], C. A. REID[1], G. F. KING*[2], S. PETROU*[1,4,5];
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Abstract: Purpose: Dravet syndrome (DS) is considered one of the most catastrophic epilepsy syndromes. Approximately 85% of DS cases result from heterozygous loss-of-function mutations in the *SCN1A* gene, which encodes the pore-forming α-subunit of the $Na_V1.1$ voltage-gated sodium channel. Deficiency of $Na_V1.1$ causes altered function of inhibitory interneurons resulting in seizures, movement disorders and other comorbidities seen in DS. We hypothesized that a drug specifically elevating $Na_V1.1$ activity in inhibitory interneurons could treat seizures in DS, restoring their ability to regulate brain excitability. **Method/Results:** Using planar patch-clamp technology, this study examined the selectivity of a synthetic peptide (GSD1), derived from spider venom, against a range of central, peripheral and cardiac Na_V channels. Electrophysiological recordings in HEK293T and CHO cells revealed that GSD1 (5 nM) is highly selective for human $Na_V1.1$ ($hNa_V1.1$); showed extreme specificity without significantly altering activity of closely related sodium channels including $Na_V1.2$ and $Na_V1.6$ in the brain and $Na_V1.5$ in the heart. Characterization of the biophysical effects of GSD1 on $hNa_V1.1$ channels revealed a significant hyperpolarizing shift in steady-state activation together with significant inhibition of fast inactivation producing a large increase in sustained current. Assessment using brain slice electrophysiology, from a DS mouse model that has an *SCN1A* gene mutation identical to that identified in DS patients, suggest GSD1 was able to rescue action potential firing loss in inhibitory interneurons thought to underlie epileptogenesis. *In vivo* analysis provided further evidence that peptide delivered via intra-cerebroventricular (ICV) infusion was able to significantly decrease interictal spikes and importantly, reduced whole brain hyper-excitability and thermogenic susceptibility in the DS model. **Conclusion:** These data demonstrate that enhancement of $Na_V1.1$ current is possible, paving the way for therapeutic application in DS. Exploiting venom peptides is one avenue for creating targeted therapeutics in genetic disorders, such as DS, which promise not only to control seizures, but to reduce associated co-morbidities.
*Authors contributed equally.

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Nanosymposium

283. Epilepsy: Mechanisms

Location: SDCC 25A

Time: Monday, November 14, 2016, 8:00 AM - 10:15 AM

Presentation Number: 283.07

Topic: B.11. Epilepsy

Support: NIH Grant 5R01NS079214

Title: *In vivo* drug discovery of novel therapeutics for Dravet Syndrome using zebrafish

Authors: *A. GRIFFIN, K. HAMLING, M. DINDAY, S. BARABAN;
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Abstract: Mutations in the voltage-gated sodium channel *SCN1A* result in Dravet Syndrome (DS), a catastrophic childhood epilepsy. Currently, antiepileptic drugs do not offer adequate seizure control for these patients. Zebrafish with a mutation in *scn1Lab* recapitulate spontaneous seizure activity and mimic the convulsive behavioral movements observed in DS. Importantly, *scn1Lab* mutant zebrafish also showed pharmacoresistance to several different antiepileptic drugs (Baraban et al. 2013), emulating the persistent drug resistant seizures observed in human patients. As zebrafish larvae are compatible with moderate- to high-throughput drug screening, we aimed to identify new antiepileptic compounds through our *in vivo* phenotypic discovery platform. Automated locomotion tracking in a 96-well plate format is used to monitor spontaneous behavioral seizures in *scn1Lab* mutants. By adding test compounds to the larval water drugs capable of decreasing the high-velocity movements corresponding to seizure-like convulsions can be rapidly identified. Electrophysiology recordings from the brain of intact treated *scn1Lab* larvae are used to confirm suppression of epileptic events. By using this two phase discovery platform we have screened over 2000 compounds. To date five compounds capable of suppressing epileptic activity have been identified. All of these compounds were subsequently tested in concentration-response studies. Two of these re-purposed compounds show novel antiepileptic activity. Overall, 2.3% of compounds were classified as 'false-positives' as they reduced swim activity in the behavioral assay but electrographic seizure events were still observed. Around 20% of compounds are classified as toxic as treated larvae have no visible heartbeat or movement in response to touch after a 90 min exposure. *In vivo* screening in zebrafish larvae also allows for cardiac activity monitoring after drug treatments to detect potential cardiac side effects of newly identified drugs. We conclude that recapitulation of the spontaneous seizures and pharmacological profile observed in DS make the *scn1Lab* mutants ideal for the rapid screening and discovery of novel antiepileptic drugs. Furthermore, our work demonstrates how zebrafish are becoming a valuable model system in the emerging area of precision medicine for epilepsy.

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Nanosymposium

283. Epilepsy: Mechanisms

Location: SDCC 25A

Time: Monday, November 14, 2016, 8:00 AM - 10:15 AM

Presentation Number: 283.08

Topic: B.11. Epilepsy

Support: W81XWH-13-1-0463

Title: The role of $K_v\beta2$ in modulating in virto seizure activity in mice treated with the ketogenic diet

Authors: *R. PARENT, G. FISHER, H. BURNS, A. SMARSH, G. MURPHY;
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Abstract: The ketogenic diet (KD) has been an effective antiepileptic treatment dating back to the mid-1920s. While a number of hypotheses have been advanced, the exact cellular mechanism that underlies the antiepileptic action of the KD remains unclear. Evidence has been accumulating that ketones can directly enhance voltage-gated potassium currents gated by potassium channel complexes that contain Kvβ2 auxiliary sub-units which themselves possess an aldo-keto reductase enzymatic core domain. *Therefore, we advance the novel hypothesis that ketones generated under ketogenic conditions directly interact with Kvβ auxiliary sub-units to increase voltage-gated potassium currents which in turn reduces neuronal excitability.*
At 8 weeks of age, wild-type (WT) and $Kv\beta2^{-/-}$ mice were separated into two groups, one of which was fed standard chow, and the other which was fed a KD for a minimum of 6 weeks. Ex vivo modified horizontal slices were prepared from each group. In virto extracellular field potential recordings were made from the hippocampus (HP), entorhinal cortex (EC) and lateral amygdala (LA), simultaneously in standard aCSF, with or without the addition of ketones. Epileptiform bursts were induced by removing Mg^{2+} and increasing K^+ concentrations in the aCSF. The percentage of slices that exhibited bursting, latency to continuous bursting, and inter-burst interval, were measured for at least one hour after perfusion with the modified aCSF.
In WT mice, acute application of ketones to slices from animals that were not maintained on a KD significantly reduces the induction of ictal events in the HP. This effect is HP specific and was not seen in $Kv\beta2^{-/-}$ mice or animals maintained on a KD. Neither acute application of ketones or maintenance on the KD affects the latency to the start of continuous bursting after induction of epileptiform bursts. The KD appears to increase excitability in the EC and LA, but not in the HP, regardless of diet and genotype, as measured by inter-burst interval. Deletion of $K_v\beta2$ itself had a differential impact on bursting activity in the amygdala in untreated mice, in which slices from $Kv\beta2^{-/-}$ mice are hyper excitable, and this observation was not reversed by the KD. This is consistent with previous data showing the LA is hyper-excitable in $Kv\beta2^{-/-}$ mice. Additionally, treatment with the KD appears to alter the functional connectivity in the brain,

regardless of genotype, as measured by the order in which the three areas (HP, EC, & LA) begin bursting after induction with modified aCSF. Our results suggest that the Kvβ2$^{-/-}$ mice may represent a novel mouse model for studying the KD and may also be useful for studying seizure phenotypes that originate in the amygdala.

Disclosures: R. Parent: None. **G. Fisher:** None. **H. Burns:** None. **A. Smarsh:** None. **G. Murphy:** None.

Nanosymposium

283. Epilepsy: Mechanisms

Location: SDCC 25A

Time: Monday, November 14, 2016, 8:00 AM - 10:15 AM

Presentation Number: 283.09

Topic: B.11. Epilepsy

Title: Electrophysiology-based HTS for positive allosteric modulators of N-Methyl-D-Aspartate receptors

Authors: N. B. FEDOROV[1], Y. A. KURYSHEV[1], J. FISHER[1], A. WRIGHT[1], C. WU[1], L. C. ARMSTRONG[1], *C. MATHES[1], M. ACKLEY[2];
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Abstract: N-methyl-D-aspartate glutamate receptors (NMDARs) are ionotropic receptors that may hold promise as new therapeutic targets for numerous indications including learning and memory, mood regulation, some forms of addiction and chronic pain. NMDARs form tetrameric complexes that consist of several homologous subunits. The subunit composition of NMDARs is flexible, resulting in a large number of receptor subtypes. Each receptor subtype has distinct biophysical, pharmacological and signaling properties, and carry out specific functions in the CNS in both normal and pathological conditions. These properties coupled with differential localization of NMDAR subunits suggests that drug candidates that display subunit selectivity will display diverse *in vivo* profiles. NMDARs positive allosteric modulators (PAMs) represent potential therapeutic strategies for refining the function of NMDARs. Here we report fully optimized conditions for identification and determination of the selectivity profile of PAMs on different subtypes of NMDARs. Optimized conditions include cell growth conditions, cell lifting and handling procedures, composition of internal and external physiological solutions and protocol of the experiments. These optimized conditions allowed us to achieve acceptable uniformity of responses with 4 replicates (multi-hole mode), which in turn translated into 12 compounds per plate (8 data point concentration response curve for each compound) and 8-10 plates a day. Feasibility of unified cell handling procedures for side by side comparison of different NMDARs subtypes on single plate will be discussed.

Disclosures: **N.B. Fedorov:** A. Employment/Salary (full or part-time): Charles River. **Y.A. Kuryshev:** A. Employment/Salary (full or part-time): Charles River. **J. Fisher:** A. Employment/Salary (full or part-time): Charles River. **A. Wright:** A. Employment/Salary (full or part-time): Charles River. **C. Wu:** A. Employment/Salary (full or part-time): Charles River. **L.C. Armstrong:** A. Employment/Salary (full or part-time): Charles River. **C. Mathes:** A. Employment/Salary (full or part-time): Charles River. **M. Ackley:** A. Employment/Salary (full or part-time): Ssage Therapeutics.

Nanosymposium

284. Brain Wellness and Aging: From Phenotypes to Mechanisms

Location: SDCC 33C

Time: Monday, November 14, 2016, 8:00 AM - 11:30 AM

Presentation Number: 284.01

Topic: C.01. Brain Wellness and Aging

Support: NIH / NIA (AG045571)

NIH / NIA Alzheimer's Center Grant (AG013854)

Title: The oldest-old with preserved cognition and the full range of Alzheimer pathology

Authors: A. REZVANIAN[1], D. T. OHM[1], L. KUKREJA[1], T. D. GEFEN[1], S. WEINTRAUB[1], E. ROGALSKI[1], R. KIM[2], C. AGUIRRE[2], M. CORRADA[2], *M.-M. MESULAM[1], C. KAWAS[2], C. GEULA[1];
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Abstract: Recent reports indicate the presence of amyloid plaques (AP) and neurofibrillary tangles (NFT) in brains of cognitively normal elderly with a density and distribution sufficient for pathologic diagnosis of Alzheimer’s disease (AD). The purpose of this study was to investigate whether full AD pathology is present in The 90+ Study participants with preserved cognitive function. Eight 90+ Study participants (95-100 years) were selected based on above average performance on tests of memory and preserved performance in other cognitive domains for their age. The thioflavin-S stain, and antibodies to phosphorylated tau (AT8) and amyloid-β (6E10), were used to assess the presence, density and morphology of AP and NFT. The Cresyl violet Nissl stain and antibodies to non-phosphorylated neurofilaments and microtubule associated protein 2 were employed to assess neuronal density. The hippocampus, parahippocampal gyrus, middle frontal gyrus and inferior parietal cortex were examined. Despite similarly preserved cognitive abilities, the eight cases displayed divergent patterns of AD pathology. One case showed very sparse NFT / Pre-NFT (Braak Stage [BS] I) and diffuse AP.

Another showed slightly higher densities of NFT / Pre-NFT (BS II) and a moderate density of diffuse AP. Three cases presented with greater density of NFT / Pre-NFT (BS III) and variable AP density. The remaining three cases were characterized by significantly higher densities and widespread distributions of NFT / Pre-NFT (BS IV, V and VI, respectively), cored / neuritic AP and neuropil threads, with two satisfying criteria for pathologic diagnosis of AD. Apparent density of neurons in the hippocampus and neocortex did not distinguish cases with divergent AD pathology. However, clinically confirmed AD cases, with density and distribution of AP and NFT comparable to 90+ Study brains with the most severe pathology, displayed clear neuronal loss in the hippocampus and neocortex. These results indicate presence of pathologically confirmed AD in the absence of cognitive impairment. It is possible that factors not yet identified mitigate the deleterious effects of AD pathology on neurons, and/or that certain individuals are in fact protected from processes that lead to AP / NFT formation.

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Nanosymposium

284. Brain Wellness and Aging: From Phenotypes to Mechanisms

Location: SDCC 33C

Time: Monday, November 14, 2016, 8:00 AM - 11:30 AM

Presentation Number: 284.02

Topic: C.01. Brain Wellness and Aging

Support: DBT Grant BT/HRD/35/01/04/2011

DIT Grant R&D/RDC/13(6)/2013

Title: Collateral information connectivity in brain maximizes around 7th decade in man to cognitively compensate for neurodegenerative changes during advanced ageing

Authors: *P. K. ROY;
Natl. Brain Res. Ctr., Manesar (NCR Delhi), India

Abstract: OBJECTIVE:
We study a critical but overlooked issue of brain's plasticity and coping with age. Similar reconstructive remodelling as coping mechanism is noted in neural networks in other electrically-excitable tissue like heart, under stress, e.g. ischaemia as in ageing [1].
METHODS:
We study the ageing process (age-related neurodegenerative change), using MRI-DTI scans of

about 400 individuals, 20-90 years age. Using our approach of whole brain tractography [2], we devise a novel network scaffold connectomics approach, taking care of the age-induced decline of neuronal fibre density. To develop a quantitative biophysics-based formulation of the neurodegenerative effect, we construct a biphasic cell-damage model under ageing-induced neurofibrillary tangle formation.

RESULTS:

We find that as ageing occurs, the brain's information flux, as estimated by centrality indices of network hub flow, undergoes full-scale re-modelling. The centralized nodal processing follows a quadratic inverted U-function, maximizing at the 6^{th}-7^{th} decade (mean 57 years: men; 64 years: women), declining thereafter; whilst after that peak, the peripheral distributed nodal processing increases, compensating for the aforesaid decline. We corroborate our formulation by (i) fMRI findings delineating that efficient cognitive processing in the aged increases the activation of distributed bilateral areas (ii) immunohistological or NMR-relaxometric findings, whereby oligodenroglia-induced neural myelination activity maximizes around 50-60 years. The quadratic function is quantitatively accounted by the biphasic damage process (promotion, induction) on oligodendrocytes by the tangle load.

CONCLUSION:

Under ageing, the information pathway redistribution from centralized to peripheral processing occurs, paralleling the centrifugal nature of tangle formation: starting in central limbic region, and gradually diffusing spatially to peripheral cortex. We here show spatial redistribution of information processing in senescence, which, with temporal redistribution of information processing under ageing [3], integrates into spatiotemporal information multiplexing behaviour, as a homeostatic strategy for cognitive reserve to cope against neurodegeneration. For ameliorating ageing-induced cognitive decline, our approach stresses the potentiality of agents that protect oligodendrocytes from neurodegeneration, e.g. azeliragon / glypromate.

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[1].G Billman, Amer J Physiol.-Heart, 297(4):H1171-H1193, 2009.
[2].W Otte et al, Neuroimage, 109:171-189, 2015.
[3]. R.Martins et al, Frontiers Human Neurosci. 9(221):1-17, 2015

Disclosures: P.K. Roy: None.

Nanosymposium

284. Brain Wellness and Aging: From Phenotypes to Mechanisms

Location: SDCC 33C

Time: Monday, November 14, 2016, 8:00 AM - 11:30 AM

Presentation Number: 284.03

Topic: F.03. Neuroendocrine Processes

Support: Alberta Innovates Health Solutions Postgraduate Fellowship

Title: Positive effect of aerobic exercise on the cortisol awakening response in healthy older adults: Results from the Brain in Motion Study

Authors: *L. L. DROGOS[1,2], K. L. WYNNE-EDWARDS[3], R. ZHOU[3], S. HALL[4], C. DUNCAN[1,2], M. J. POULIN[1,2,5,6,7];
[1]Pharmacol. & Physiol., [2]Hotchkiss Brain Inst., [3]Fac. of Vet. Med., [4]O'Brien Ctr. for the Bachelor of Hlth. Sci., [5]Libin Cardiovasc. Inst. of Alberta, [6]Cumming Sch. of Med., [7]Fac. of Kinesiology, Univ. of Calgary, Calgary, AB, Canada

Abstract: Evidence from both preclinical and clinical studies suggests aerobic exercise may dampen age related decline in cognitive performance. There is also evidence that alterations in the stress hormone, cortisol may be a mechanism by which aerobic exercise affects cognition and the brain. In healthy individuals, cortisol levels quickly increase and then taper across the morning hours). This cortisol awakening response (CAR) is used to assess functioning of the hypothalamic-pituitary-adrenal (HPA). We aimed to determine if aerobic exercise would affect the CAR in healthy older adults. We hypothesized the CAR would be more robust following the exercise intervention. This investigation was completed as an ancillary study of the larger Brain in Motion (BIM) study. Participants were generally healthy and screened for inclusion/exclusion criteria for the parent study. Thirty-eight participants were recruited (Mean age = 65.0 years; 60% female). Across the study, a total of six participants were lost, leaving a final longitudinal sample of 32 participants. Participants were asked to provide small (1ml) passive drool samples at the following points: waking, 15, 30, and 45 min post-waking and 3, 6, 9, and 12 hours post-waking. Saliva was quantified relative to a deuterated internal standard, by liquid chromatography coupled to tandem mass spectrometry, using an atmospheric pressure chemical ionization (APCI) source. To test for differences in cortisol across the exercise intervention, we utilized a Bayesian repeated measures analysis of covariance (ANCOVA) with 2 (Phase: Baseline, Post-Exercise) x 8 (Time: Wake, +15 Mins; +30 Mins, +45 Mins, +3Hrs, +6Hrs, +9Hrs, +12Hrs) design, controlling for age, and sex was considered a between-subjects factor. We saw a main effect of Time (BF_{10} = 2.929e +34; Posterior Probability > 99%) and Intervention (BF_{10} = 3.462; Posterior Probability = 77.59%). In contrast to previous studies, we did not observe a main effect of age, or sex. There were no significant interactions observed. As expected, there was a strong circadian effect on cortisol concentrations. Using Bayesian inference, there is a 77.59% likelihood of the effect of observing a change in the shape of the CAR of after the exercise intervention again. Utilizing our comprehensive set of salivary cortisol data in a small sample population, we observed a robust cortisol awakening response and increased CAR after exercise. These data provide support for the hypothesis that aerobic exercise is a potential mechanism for improving HPA axis function in otherwise healthy older adults.

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Nanosymposium

284. Brain Wellness and Aging: From Phenotypes to Mechanisms

Location: SDCC 33C

Time: Monday, November 14, 2016, 8:00 AM - 11:30 AM

Presentation Number: 284.04

Topic: C.01. Brain Wellness and Aging

Support: National Institute of Health award NIH (R21AG045766) to SKH

Australian NHMRC-ARC dementia training fellowship (APP1097397) to AES

University of South Australia Early Career Research Networking Award awarded to AES

Title: Physical activity modifies corticospinal excitability of the lower extremity in young and old adults

Authors: *A. E. SMITH[1], H. HASSANLOUEI[2], C. W. SUNDBERG[2], A. KUPLIC[2], S. K. HUNTER[2];
[1]Hlth. Sci., Univ. of South Australia, Adelaide, Australia; [2]Dept. of Physical Therapy, Marquette Univ., Milwaukee, WI

Abstract: Aging is associated with reduced neuromuscular function in the lower extremities, which may in part be due to changes in corticospinal excitability. Regular physical activity (PA) may slow or mitigate these declines. Surprisingly however, little is known about the relationship between aging, PA and excitability of the corticospinal networks. The purpose of this study was to determine the influence of age and PA on motor-cortical excitability by comparing the input-output curves of motor evoked potentials (MEPs) elicited in motor cortical areas and targeting the quadriceps muscles in young and old adults. Twenty-four young (23.0 ± 3.2 yrs, 12 women) and 39 old adults (70.2 ± 6.3 yrs, 21 women) participated in the study. The “motor hotspot” of the Vastus Lateralis (VL) was found with transcranial magnetic stimulation using a concave double cone coil. Active motor threshold (AMT) was defined as the minimum stimulator intensity for eliciting MEPs in the VL in at least 4 of 8 trials, while subjects performed intermittent isometric contractions at 10% maximal voluntary contraction (MVC) (~3 s contraction, ~7 s relaxation). Input-output curves were derived from MEPs (mean of 10 stimuli delivered during 10% MVC contractions) at 5% increments of the stimulator intensity ranging from 90% AMT until either the MEP plateau or maximal stimulator output was reached. Averaged MEPs at each intensity were normalized to the maximal compound muscle action potential of the VL (M_{max}) obtained via electrical stimulation of the femoral nerve. Input-output curves were fitted with a 4-parameter sigmoidal Boltzman curve and the slopes of the curve at the greatest rate of MEP change (S_{max}), 10% (S_{10}) and 25% (S_{25}) of maximal stimulator intensity were calculated. PA was assessed objectively with Actigraph GT-3X tri-axial accelerometry.

Subjects were grouped for analysis, into either high PA (>10,000 steps/day, n = 21) or low PA (<10,000 steps/day, n = 42). The low PA group had a steeper curve slope at S_{10} (0.3 ± 0.02, P= 0.01), S_{25} (0.7 ± 0.05, P= 0.01) and S_{max} (0.9 ± 0.06, P= 0.01) compared with high PA at S_{10} (0.2 ± 0.03), S_{25} (0.5 ± 0.05) and S_{max} (0.7 ± 0.07). Neither age nor sex influenced the motor cortical input-output curve characteristics. These findings provide the first evidence that habitual PA (>10,000 steps/day) influences the excitability of the motor-cortical projections to the lower extremities similarly, in young and old adults.

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Nanosymposium

284. Brain Wellness and Aging: From Phenotypes to Mechanisms

Location: SDCC 33C

Time: Monday, November 14, 2016, 8:00 AM - 11:30 AM

Presentation Number: 284.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG034531

NIH Grant NS092918

American Federation for Aging Research

Glenn Medical Foundation

Title: The X-chromosome confers resilience against Alzheimer's Disease toxicity

Authors: E. MINONES-MOYANO[1], L. BROESTL[1], A. ARNOLD[2], C. WHITE[3], D. BENNETT[4], P. DE JAGER[3], D. WANG[1], *D. B. DUBAL[1];
[1]Univ. of California San Francisco, San Francisco, CA; [2]UCLA, Los Angeles, CA; [3]Brigham and Women's Hospital, Harvard Med. School, and Broad Inst., Boston, MA; [4]Rush Univ., Chicago, IL

Abstract: Males die before females and this sexual dimorphism extends to neurodegenerative conditions such as Alzheimer’s disease (AD). We have shown that sex chromosomes mediate male disadvantage using human amyloid precursor protein (hAPP) mice that model aspects of AD. It remains unknown, however, what causes the sex chromosomal effects –and whether sex chromosomes modify human AD. We probed these questions with genetic, cognitive, and other experimental approaches in mice, primary neurons, and humans. To dissect causes of the sex chromosomal effects, we determined whether the presence of a Y or the lack of a second X

chromosome confers male disadvantage in AD-related toxicity *in vivo* and *in vitro* using the XY* model of sex chromosomal biology. Progeny of XY* males crossed with XX females include four sex genotypes roughly equivalent to: XX and XO mice with ovaries, and XY and XXY mice with testes. A sexual dimorphism that varies by the presence or absence of a Y is Y-chromosome-mediated; one that varies by the presence of 1 versus 2 X's, is X chromosome-mediated. We crossed XY* males with hAPP females to produce eight genotypes of mice exhibiting varying dosages of X and Y, with or without hAPP expression. After reproductive maturity, we gonadectomized mice to equate hormones and then assessed their survival and cognition. Male and female mice with one X (XY-hAPP and XO-hAPP) died faster and showed worse deficits than those with two X's (XX-hAPP and XXY-hAPP). Presence or absence of the Y did not govern mortality or deficits. Next, we tested whether the second X-chromosome confers neural resilience at the cellular level in primary cortical neurons from mice. In parallel with our *in vivo* findings, XY and XO neurons showed increased cell death following acute Aβ toxicity, compared to XX and XXY neurons. Thus, at the cellular and systems levels, adding an X effectively reversed male vulnerability to AD-related toxicity. To gain insight into X-factors that may contribute to brain resilience in humans, we evaluated expression of the X chromosome in the frontal cortex of elderly individuals from the Memory and Aging Project and Religious Orders Study. Preliminary results revealed X-encoded genes whose expression shows a positive correlation with cognition, suggesting a protective role for X factors against cognitive decline due to aging and dementia. Our study reveals a new role for the X chromosome in counteracting mortality, cognitive deficits, and cellular toxicity related to AD. Identification and therapeutic modulation of X-factors and/or X-mediated protective mechanisms may help increase longevity and resilience to neurodegenerative diseases in both men and women.

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Nanosymposium

284. Brain Wellness and Aging: From Phenotypes to Mechanisms

Location: SDCC 33C

Time: Monday, November 14, 2016, 8:00 AM - 11:30 AM

Presentation Number: 284.06

Topic: C.01. Brain Wellness and Aging

Title: Young human plasma as therapy for aging-associated cognitive disorders

Authors: *S. MINAMI, S. REGE, H. HACKBART, S. P. BRAITHWAITE;
Alkahest, Inc., San Carlos, CA

Abstract: As the population ages, dementia and age-related diseases, which result in a gradual to sharp decline in cognitive function, are becoming increasingly prevalent. Mounting evidence suggests that there are factors present in young blood that can counteract the effects of brain aging. The therapeutic potential of young blood has been demonstrated in studies where old mice receiving injections of young mouse plasma show increased neurogenesis and improved cognitive performance. An important step towards translating these findings to the clinic is to show that human plasma can drive similar effects. To assess the effects of human plasma on cognitive function, we performed systemic injections of plasma from 18-year-old human donors in aged immunodeficient mice. The young human plasma infusions reversed age-related cognitive deficits in hippocampus-dependent behavior tests. In addition, young human plasma was able to restore activity and motor deficits in old mice. Further analyses revealed histological and molecular correlates of aging-associated behavioral deficits. These findings implicate the potential of young plasma as a therapeutic and opens the door to possibilities for targeting specific plasma proteins for future therapies directed against age-related diseases.

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Nanosymposium

284. Brain Wellness and Aging: From Phenotypes to Mechanisms

Location: SDCC 33C

Time: Monday, November 14, 2016, 8:00 AM - 11:30 AM

Presentation Number: 284.07

Topic: C.01. Brain Wellness and Aging

Support: ADDF

Alzheimer's Assocation IIRG

NIA AG048615

Title: Identifying early mechanisms of Alzheimer's disease synaptic pathology for novel therapeutic strategies

Authors: *G. E. STUTZMANN[1], S. CHAKROBORTY[2], S. E. RILEY[4], A. LITTLEFIELD[2], C. A. BRIGGS[2], W. FROST[3], J. BUOLAMWINI[4];
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Abstract: Until recently, research on the etiology of Alzheimer's disease has focused largely on protein aggregates indicative of advanced pathology. Our research concentrates on proximal mechanisms that directly impinge upon synaptic plasticity and memory encoding. In particular, intracellular Ca^{2+} dysregulation is one of the earliest signaling deficits contributing to synaptic dysfunction as well as amyloid and tau histopathology and neurodegeneration. Here we describe further evidence of increased ryanodine receptor (RyR)-mediated Ca^{2+} release underlying early synaptic plasticity deficits, and demonstrate that our novel class of RyR-targeted compounds restores Ca^{2+} signaling and downstream processes in AD models.
Post-tetanic potentiation (PTP), a form of Ca^{2+}-dependent short term plasticity, is important for synaptic tagging and increasing synaptic strength, and reflects an increase in neurotransmitter vesicle release probability. Here, we combined whole cell patch recordings, field potential recordings, and 2-photon Ca^{2+} imaging in the hippocampus of 2-3 month old 3xTg-AD mice and NonTg controls, and measured PTP at the CA3-CA1 Schaffer collateral (SC) synapse, and the DG-CA3 mossy fiber (MF) synapse, after a high frequency tetanus (2x100 Hz, 10 s apart). At the single cell level, we found that SC PTP is significantly reduced in 3xTg-AD mice compared to controls (3.5 + 2.9% below baseline vs. 14.2 + 4.9% over baseline, respectively). Field potential recordings generated similar patterns with >40% decrease in AD mice. At the MF synapse, nearly identical results were obtained in 3xTg-AD mice. In all scenarios, the concurrent synaptically-evoked Ca^{2+} signals were significantly greater in AD mice. Interestingly, when early-LTP was measured (15-20 min post tetanus), the SC synapse remained impaired in the 3xTg-AD mice (>80% decrease vs NonTg control), whereas the MF synaptic response was not different, suggesting a selective compensation or protection mechanism.
A novel therapeutic approach to preserve normal Ca^{2+} and synaptic plasticity responses is to target the RyR with negative allosteric modulators (RyR-NAMs). To this end, we have developed and tested a series of RyR-NAMs using Ca^{2+} imaging, biochemical, and neurophysiological approaches in animal and cell models. We found that normalizing RyR function restores normal Ca^{2+} signaling, preserves synaptic plasticity, and reduces histopathology in AD models. RyR-stabilizing compounds may have broad therapeutic applications, ranging from protein aggregation disorders to network disruption of memory circuits.

Disclosures: **G.E. Stutzmann:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroLucent, Inc. **S. Chakroborty:** None. **S.E. Riley:** None. **A. Littlefield:** None. **C.A. Briggs:** None. **W. Frost:** None. **J. Buolamwini:** None.

Nanosymposium

284. Brain Wellness and Aging: From Phenotypes to Mechanisms

Location: SDCC 33C

Time: Monday, November 14, 2016, 8:00 AM - 11:30 AM

Presentation Number: 284.08

Topic: C.01. Brain Wellness and Aging

Support: NIA 1R03AG05087801 (Kimchi)

MGH Scholars' Fund (Cash)

Title: Aged rodents are behaviorally and neurophysiologically sensitive to combinations of inflammatory and anticholinergic manipulations related to delirium

Authors: *E. Y. KIMCHI[1], B. F. COUGHLIN[2], S. S. CASH[2];
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Abstract: Delirium is an acute disturbance of attention and awareness commonly seen in elderly patients. Epidemiologic studies have suggested that the most prominent predisposing risk factor for delirium is aging and that common precipitating risk factors include anticholinergic medications and inflammation. We have tested whether aged rats (22-26 months, n = 18) are specifically more sensitive to common delirium precipitating risk factors than young rats (3-6 months, n = 18). We simultaneously measured several behavioral and neurophysiological delirium-related phenotypes in rats following intraperitoneal injection of scopolamine (a muscarinic anatagonist) or lipopolysaccharide (an inflammatory stimulant). At higher doses of both scopolamine and lipopolysaccharide, both young and aged rats display delirium-related phenotypes. Neurophysiolgocally, rats exhibited increased EEG slowing, comprised of increased delta power and decreased gamma power in EEG recordings. Simultaneously, rats showed decreased accuracy on a flexible Go/Nogo auditory discrimination task as well as altered locomotor activity (increased locomotor activity with scopolamine, decreased locomotor activity with lipopolysaccharide). At intermediate doses, however, aged rats appeared to be both neurophysiologically and behaviorally more sensistive than young rats. Additionally, aged rats were particularly more neurophysiologically and behaviorally sensitive to combinations of these precipitants than young rats. Our results demonstrate that aging increases the predisposition to delirium related precipitants, in particular to multifactorial combinations. Our results demonstrate that aged rodents display the expected core translational features of delirium, providing evidence that rodents can serve as valuable models to understand acute neuropsychiatric and cognitive disorders associated with aging.

Disclosures: **E.Y. Kimchi:** None. **B.F. Coughlin:** None. **S.S. Cash:** None.

Nanosymposium

284. Brain Wellness and Aging: From Phenotypes to Mechanisms

Location: SDCC 33C

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Presentation Number: 284.09

Topic: C.01. Brain Wellness and Aging

Support: NSERC

OGS

CIHR

Title: Age-dependent increase in membrane lipid deregulation observed in brain regions vulnerable to age-related pathology

Authors: *S. CAUGHLIN[1], K. YEUNG[2], D. F. CECHETTO[1], S. N. WHITEHEAD[1];
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Abstract: Amyloid load is thought to be an important factor in the development of AD possibly through interactions with other factors that increase the brain's vulnerability to pathology. One possible point of interaction that has been largely ignored due to technological constraints is lipid deregulation, specifically, membrane lipids such as gangliosides. Shifts in ganglioside patterns have been observed in a number of brain-related pathologies but have also recently been reported in the natural aging process. For example, an increase in the ratio of 20 carbon ganglioside species compared to the predominant 18 carbon species was observed in the aging mouse hippocampus (Sugiura et al., 2008). Additionally, each ganglioside species was found to have a unique anatomical distribution across the rat hippocampus and cortex (Weishaupt et al., 2015). The significance of the spatial distribution of these lipid species and whether this phenomenon occurs in other brain regions vulnerable to age-related pathology remains unknown. MALDI Imaging Mass Spectrometry was used to visualize the distribution of A-series ganglioside species across the striatum, ventricles, hippocampus and substantia nigra, as well as to calculate the ratio of expression between different species in young (2-4 month), middle aged (12-15 month) and old (19-24 month) wild-type (WT) and transgenic (TG) APP rats. A trend of increased abundance of c20 ganglioside species was observed in an age-dependent manner in both the WT and TG rats. TG rats displayed a greater increase in 20:18 carbon ratio compared to the age-matched WT animals. The shift in the pattern of ganglioside abundance with age and its exacerbated deregulation in the presence of high levels of amyloid in the brain of TG rats suggests that abnormal ganglioside patterns contribute to brain vulnerability and may play a mechanistic role in the development of pathology in aging individuals.

Disclosures: **S. Caughlin:** None. **K. Yeung:** None. **D.F. Cechetto:** None. **S.N. Whitehead:** None.

Nanosymposium

284. Brain Wellness and Aging: From Phenotypes to Mechanisms

Location: SDCC 33C

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Presentation Number: 284.10

Topic: C.01. Brain Wellness and Aging

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Title: Store-operated calcium channel complex in postsynaptic spines as a new therapeutic target for Alzheimer's disease treatment

Authors: *I. BEZPROZVANNY[1], H. ZHANG[1], S. SUN[1], E. PCHITSKAYA[2], E. POPUGAEVA[2];
[1]UT Southwestern Med. Ctr., Dallas, TX; [2]St Petersburg State Polytechnical Univ., St Petersburg, Russian Federation

Abstract: Mushroom dendritic spine structures are essential for memory storage. In the previous studies we suggested that the loss of mushroom spines may explain memory defects in aging and Alzheimer's disease (AD). We further demonstrated that the stability of mushroom spines depends on STIM2-mediated neuronal store operated calcium (Ca^{2+}) influx (nSOC) pathway which is compromised in AD mouse models, in aging neurons and in sporadic AD patients (Sun at al, 2014, Neuron, vol 82, pp 79-93; Zhang at al, J. Neuroscience, vol 35, pp 13275-13286; Popugaeva at al, 2015, *Molecular Neurodegeneration*, 10:37). Our resuls suggested that spine nSOC pathway is acting by favoring activity of synaptic CaMKII, which stabilizes synaptic spines. The molecular identity of channel encoding SOC influx in spines remains unknown. We now demonstrate that TRPC6 and Orai2 channels form a STIM2-regulated nSOC Ca^{2+} channel complex in hippocampal mushroom spines. We further demonstrate that a known TRPC6 activator hyperforin and a novel nSOC positive modulator NSN21778 can stimulate activity of nSOC pathway in the spines and rescue mushroom spine loss in both presenilin and APP knock-in mouse models of AD. We further show that NSN21778 rescues hippocampal long-term potentiation impairment in APP knock-in mouse model. We conclude that STIM2-regulated TRPC6/Orai2 nSOC channel complex in dendritic mushroom spines is a new therapeutic target for treatment of memory loss in aging and AD and that NSN21778 is a potential candidate molecule for therapeutic intervention in brain aging and AD. Activation of this pathway favors activity of synaptic CaMKII and prevents synaptic spine and memory loss in aging and AD

Disclosures: **I. Bezprozvanny:** None. **H. Zhang:** None. **S. Sun:** None. **E. Pchitskaya:** None. **E. Popugaeva:** None.

Nanosymposium

284. Brain Wellness and Aging: From Phenotypes to Mechanisms

Location: SDCC 33C

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Presentation Number: 284.11

Topic: C.01. Brain Wellness and Aging

Support: NIH P01HL088594

Title: Pin1 regulates dendritic spines in Aβ42 treated neurons

Authors: ***J. S. MALTER**[1], N. STALLINGS[1], M. O'NEAL[1], J. HU[1], I. BEZPROZVANNY[2]; [1]Pathology, [2]Physiol., UT Southwestern, Dallas, TX

Abstract: Dendritic spine loss is a prominent feature of early stage Alzheimer's Disease (AD). Aβ42 causes synaptic pathology by affecting Ca2+ homeostasis, altering CamKII and calcineurin (CN) pathways. FK506 rescued spines in neurons treated with Aβ42 suggesting a CN target modulated spine losses after Aβ42. We now show that the cis-trans prolyl isomerase Pin1 is a target of Aβ42 and CN signaling. Pin1 binds to and isomerizes peptide bonds between phospho-Ser (pS) or phospho-Thr (pT) and Pro (pS/pT-P). We show that Pin1 is activated by CamKII mediated phosphorylation, driven by SOCE signaling and suppressed by CN mediated dephosphorylation, activated by Aβ42 signaling. Pin1 KO neurons or WT cells treated with Aβ42 showed ~35% fewer mature and total spines than untreated WT cells, suggesting a common mechanism. As exogenous Pin1 rescued spine counts of both Pin1 KO or Aβ42 treated WT neurons, Pin1 loss-of-function likely caused spine loss after Aβ42. Finally, we demonstrate that FK506 had no effect on spines in Pin1 KO neurons irrespective of Aβ42 treatment. Therefore, we propose that Pin1 is a novel target for inhibitory Aβ42 and CN signaling. We hypothesize that partial CN inhibition in vivo with FK506 will preserve Pin1 activity and protect against Aβ42-mediated synaptic pathology. Conversely, we further propose that the beneficial effects FK506 in AD models will be abolished in the absence of Pin1.

Disclosures: **J.S. Malter:** None. **N. Stallings:** None. **M. O'Neal:** None. **J. Hu:** None. **I. Bezprozvanny:** None.

Nanosymposium

284. Brain Wellness and Aging: From Phenotypes to Mechanisms

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Presentation Number: 284.12

Topic: C.01. Brain Wellness and Aging

Support: RO1NS075222

T32 GM007367

Title: Role of Calcium in mediating MPP^+ toxicity in SN and VTA dopaminergic neurons.

Authors: *O. LIEBERMAN, S. CHOI, E. KANTER, D. SULZER, E. MOSHAROV; Columbia Univ., New York, NY

Abstract: Systemic administration of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that was first identified to induce parkinsonism in humans, selectively kills dopaminergic neurons of substantia nigra (SN), while leaving the neighboring ventral tigmentum area (VTA) neurons relatively intact, closely mimicking the difference in vulnerability of these neuronal populations in Parkinson disease. It is, however, controversial whether higher sensitivity of SN neurons to MPTP results from extra-neuronal toxin metabolism and transport or from the intrinsic properties of these cells that predispose them to neurodegeneration. We compared metabolic changes induced by MPP^+ in cultured mouse SN and VTA neurons, a system that excludes extra-neuronal metabolism and transport of MPTP and MPP^+. We found significantly higher concentrations of NO and cytosolic DA in toxin-treated SN neurons, which resulted in higher mitochondria oxidation and neurotoxicity. Furthermore, the contribution of Complex I inhibition and DA production to MPP^+-induced toxicity was different between the two neuronal populations with a larger role played by disrupted DA homeostasis in SN neurons and energy depletion in VTA neurons. DAT activity was higher in SN compared to VTA neurons, although this was not sufficient to fully explain the difference in toxicity. Importantly, we observed a large increase in cytoplasmic Ca^{2+} in SN but not VTA neurons exposed to the toxin, which was dependent on L-type Ca^{2+} channels, NMDA receptors and ryanodine receptors activity. Pharmacological blockade of any of these channels normalized MPP^+-induced alterations in NO and DA in SN neurons, also decreasing mitochondria oxidation and neurotoxicity. Deletions of alpha-synuclein, which is protective against MPP^+ toxicity both *in vivo* and *in vitro*, prevented the buildup of Ca^{2+}, NO and DA, providing a novel mechanism for the normal function of the protein. Overall, our data suggest that higher sensitivity of SN neurons to MPP^+ reflects general susceptibility of these cells to some types of insults, a large portion of which is dictated by the presence of L-type Ca^{2+} channels and downstream upregulation of NO and DA production.

Disclosures: **O. Lieberman:** None. **S. Choi:** None. **E. Kanter:** None. **D. Sulzer:** None. **E. Mosharov:** None.

Nanosymposium

284. Brain Wellness and Aging: From Phenotypes to Mechanisms

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Presentation Number: 284.13

Topic: C.01. Brain Wellness and Aging

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Institut de Recherche Servier

Title: Genetic impairment of the store-operated Ca^{2+} signaling triggers autophagic dysfunction in iPSC-derived neurons from PARK14/PLA2g6ex2^{KO} and Orai1^{KO} mouse models

Authors: ***A. YEN**[1], G. MOSTOSLAVSKY[2], V. BOLOTINA[1];
[2]Ctr. for Regenerative Med., [1]Boston Univ. Sch. of Med., Boston, MA

Abstract: We have recently discovered that genetic impairment of PARK14-dependent store-operated Ca^{2+} signaling is associated with Parkinson's disease (PD) (Zhou et al, 2016). The goal of this study was to test if genetic loss of Orai1 channel can mimic PARK14-dependent defect in store-operated Ca^{2+} entry (SOCE), loss of Ca^{2+} in endoplasmic reticulum (ER) stores and autophagic dysfunction, and if such defects can be detected in iPSC-derived DA neurons. Advanced live cell imaging was used to analyze and compare these functions in mouse embryonic fibroblasts (MEFs), induced pluripotent stem cells (iPSC), and iPSC-derived A9 midbrain dopaminergic (DA) neurons from Orai1^{KO} and PLA2g6ex2^{KO} mouse models. We found that constitutive knockout of either Orai1 or PLA2g6ex2 results in a significant loss of SOCE and depletion of ER stores in primary MEFs: 78±3% and 40±3% of SOCE, and 61±4% and 72±4% of Ca^{2+} in the stores was lost in PLA2g6 ex2^{KO} and Orai1^{KO} respectively. These deficiencies were preserved after MEF reprogramming into iPSC and terminal differentiation into A9 DA neurons. Importantly, depletion of ER Ca^{2+} stores in MEFs and iPSC-derived neurons from Orai1^{KO} and PLA2g6ex2^{KO} mice resulted in significant autophagic dysfunction, which was similar to that triggered by depletion of ER caused by TG-induced inhibition of SERCA. Thus, genetic manipulations with either Orai1 or PLA2g6 result in impairment of store-operated Ca^{2+} signaling and autophagic dysfunction, which is sustained and can be detected throughout the MEF/iPSC/neurons lineage. These results suggest that defects in store-operated

Ca^{2+} signaling caused by genetic disruption of different components of SOCE mechanism can trigger autophagic dysfunction in DA neurons, which may increase their vulnerability and cause premature demise associated with Parkinson's Disease.

Disclosures: **A. Yen:** None. **G. Mostoslavsky:** None. **V. Bolotina:** None.

Nanosymposium

284. Brain Wellness and Aging: From Phenotypes to Mechanisms

Location: SDCC 33C

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Topic: C.01. Brain Wellness and Aging

Support: MJFF grant

Title: Causal relationship between store-operated Ca^{2+} signaling, autophagy, UPR and viability of DA neurons, and its role in Parkinson's disease

Authors: *V. M. BOLOTINA, A. YEN, Q. ZHOU;
Med., Boston Univ. Sch. of Med., Boston, MA

Abstract: Recently we described a previously unknown sequence of pathological events triggered by genetic or idiopathic defects in PARK14-dependent store-operated Ca^{2+} signaling, which leads to the loss of dopaminergic (DA) neurons and PD-like motor dysfunction in PLA2g6ex2^{KO} mouse model (Zhou et al, 2016). Here we present new evidence for causal relationships between the store-operated Ca^{2+} entry (SOCE), filling state of endoplasmic reticulum (ER) Ca^{2+} stores, autophagy, unfolded protein response (UPR) and cell viability, which may determine whether DA neurons will live or die. Advanced live cell imaging and other techniques were used for the analysis of these processes in mouse embryonic fibroblasts (MEFs) and iPSC-derived neurons from Orai1^{KO} and PLA2g6ex2^{KO} mice, as well as in a model DA neuronal cell line. We found that significant autophagic dysfunction can be triggered even by a moderate (30-60%) loss of Ca^{2+} in ER stores caused by either impairment of endogenous SOCE, or direct attenuation of SERCA-induced Ca^{2+} refilling in ER. In contrast, significant UPR (reflected by increase in CHOP expression) starts to develop only when 80-90% of the ER Ca^{2+} was constitutively depleted: titration of sustained ER Ca^{2+} levels following prolonged (24 hours) treatment of the cells with 1-100nM thapsigargin (TG) revealed a 30 fold difference in TG needed to achieve IC_{50} for Ca^{2+} depletion and EC_{50} for CHOP activation (1nM and 30nM, respectively). Progressive loss of cell viability closely followed UPR, rather than the loss of Ca^{2+} in ER. Autophagic dysfunction showed no correlation with CHOP activation. Notably, direct activation of UPR by tunicamycin (leading to a 10 fold increase in CHOP) by itself did not affect

the level of Ca^{2+} in ER. We also found that pathological depletion of ER stores, rather than the loss of SOCE, is the cause of autophagic dysfunction and UPR: molecular up-regulation of SOCE by overexpression of Orai1 channels significantly improves autophagy and reduces UPR. Our results suggest that refilling of Ca^{2+} in the ER is essential for prevention of autophagic dysfunction, as well as UPR. Impairment of PARK14/PLA2g6-dependent refilling of ER Ca^{2+} stores can increase vulnerability of DA neurons, and play important role in their age-dependent demise leading to Parkinson's disease.

Disclosures: **V.M. Bolotina:** None. **A. Yen:** None. **Q. Zhou:** None.

Nanosymposium

285. Development of Novel Therapeutics for Alzheimer's Disease: In Vitro Studies

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Presentation Number: 285.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA R01 AG033036

Title: Functional brain activation patterns in cognitively normal older adults are differentially associated with Alzheimer pathology and white matter hyperintensities

Authors: *B. T. GOLD, C. BROWN, J. HAKUN, Y. JIANG, C. SMITH;
Univ. of Kentucky Chandler Med. Ctr., Lexington, KY

Abstract: Background: The preclinical stage of Alzheimer's disease (AD) appears to be associated with altered functional brain activation patterns. However, relatively little is known about these functional brain alterations may be associated with distinct AD risk factors such as pathology (Aβ and tau protein levels) and white matter hyperintensities (WMH). Here we explored these potential relationships in cognitively normal older adults. **Methods:** 35 cognitively normal older adults (65-93 years) and 29 younger adults (18-34 years) completed a working memory task while fMRI was performed using a 3T Siemens TIM scanner. Magnitude of BOLD activation in ROIs were computed by averaging over all trials in each task epoch relative to baseline for each participant. In addition, functional connectivity (fC) values were computed via a modified beta-series analysis. Connectivity between the left dorsolateral prefrontal cortex (DLPFC) and left and right lateral occipital cortex (LOC) was then averaged to create a single fronto-occipital fC estimate per task phase for each participant. Principle components analysis (PCA) was conducted using the BOLD magnitude and fC values. Lumbar CSF was drawn and $A\beta_{1\text{-}42}$, total tau (t-tau) and phosphorylated-tau_{181} (p-tau_{181}) were measured according to the standard ADNI protocol. WMHs were computed using standard procedures.

Partial correlation analyses were performed, controlling for age and sex. **Results:** PC_1 reflected a pattern of high BOLD magnitude and low fC, while PC_2 reflected a pattern of high BOLD magnitude and high fC. In older adults, PC_1 was negatively correlated with $A\beta_{42}$ (r = -0.48, p = 0.005), and positively correlated with the t-tau/$A\beta_{42}$ ratio (r = 0.51, p = 0.003) but was not associated with WMH volume in any of the four lobes (p's ≥ 0.11). In contrast, in older adults PC_2 was positively correlated with WMH volume in the frontal lobes (r = 0.51, p = 0.004) parietal lobes (r = 0.37, p = 0.052) and temporal lobes (r = 0.42, p 0.02) and was also associated with $A\beta_{42}$ (r = -0.37, p = 0.048). **Conclusions:** Our results suggest that the expression of specific forms of attempted functional compensation may depend in part upon the type of underlying pathology. Increased BOLD magnitude in frontal cortex may represent attempted compensation for preclinical stages of AD pathology whereas increases in both BOLD magnitude and functional connectivity and may represent attempted compensation for gross vascular lesions.

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Nanosymposium

285. Development of Novel Therapeutics for Alzheimer's Disease: In Vitro Studies

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Title: Environmental enrichment changes hippocampal network activity and modifies its sensitivity to amyloid-β *In vitro*.

Authors: *A. GONZALEZ ISLA[1], F. VAZQUEZ-CUEVAS[2], F. PENA-ORTEGA[2]; [1]Univ. Nacional Autónoma De México, Queretaro, Mexico; [2]Univ. Nacional Autónoma de Mexico, Queretaro, Mexico

Abstract: Environmental enrichment (EE) can produce neuroprotective changes in different neural networks, including the hippocampus, and can prevent amyloid beta (Aβ) accumulation and cognitive deficits in transgenic models of Alzheimer disease. However, it is still not known whether EE changes hippocampal activity as well as its sensitivity to Aβ. Here, we tested these possibilities by exposing mice to an enriched environment and by measuring hippocampal network activity through local field potential recordings in brain slices. We found that hippocampal slices obtained from animals that underwent EE for 4 weeks exhibited an increased hippocampal spontaneous network activity, recorded *in vitro*, compared to control slices obtained from mice housed under standard conditions. The increased in power observed in slices from EE animals was observed in almost all frequency components faster than 3.9 Hz. Aβ application reduced the hippocampal spontaneous network activity in a similar proportion in slices obtained from both control and EE groups. However, Aβ inhibited the activity in different frequency bands in each group. Furthermore, the power of the hippocampal activity in the presence of Aβ of slices obtained from EE animals was not different from the one recorded in slices obtained from control animals under basal conditions (i,e, before Aβ application). Thus, whereas EE does not prevent Aβ-induced hippocampal activity inhibition, it protects the hippocampal network from reaching the hypoactive levels observed in control hippocampus in the presence of Aβ. Whether this effect is enough to sustain normal hippocampal-dependent computations, need to be determined.

Disclosures: **A. Gonzalez Isla:** None. **F. Vazquez-Cuevas:** None. **F. Pena-Ortega:** None.

Nanosymposium

285. Development of Novel Therapeutics for Alzheimer's Disease: In Vitro Studies

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Presentation Number: 285.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Department of Biotechnolgy (PKM)

Tata Innovation Fellowship (PKM)

Title: A study of brain glutathione levels in anterior and posterior cingulate in mild cognitive impairment and Alzheimer's disease

Authors: S. SAHARAN[1], S. MORE[1], S. A. KHAN[1], M. TRIPATHI[2], *P. K. MANDAL[1,3];
[1]Neuroimaging and Neurospectroscopy lab, Natl. Brain Res. Ctr., Gurgaon, India; [2]AIIMS, Delhi, India; [3]Radiology, Radiology, Johns Hopkins Univ., Baltimore, MD

Abstract: Accruing evidence suggests oxidative stress (OS) to be a major determinant of Alzheimer's disease (AD) onset and its ineluctable progression. The predominant brain antioxidant, Glutathione (GSH), acts to neutralize oxidizing products and maintain redox homeostasis in the brain. We have previously shown GSH levels in hippocampi and frontal cortices to be significantly reduced along AD progression and accurately differentiate patients with AD, as well as its prodrome mild cognitive impairment (MCI), from healthy control (HC) subjects. In this study, we aimed to measure GSH in the anterior cingulate (AC) and posterior cingulate (PC) cortices. Both AC and PC have been shown to be affected by AD pathology in volumetric, functional, and neuropathological studies. However, only one study has thus far assessed GSH alterations in these regions in MCI; the study found an increase in the ratio of GSH to Creatine (Cr) in both AC and PC in MCI compared to HC. In this study, we used proton magnetic resonance spectroscopy (MRS) with MEGA-PRESS sequence to measure *in vivo* absolute concentrations of GSH in AC and PC of 21 AD, 20 MCI, and 20 age- matched HC subjects. Data were analyzed with a mixed-effects model and the main effects of the factors GROUP (HC, MCI, AD) and REGION (AC, PC) were assessed. There was significant effect of the factor GROUP ($df = 55.470$, $F = 8.591$, $p = 0.001$), with reduced GSH levels in AD compared to both HC ($p = 0.001$) and MCI ($p = 0.003$). There was also a significant effect of REGION ($df = 46.404$, $F = 21.022$, $p < 0.001$), indicating that GSH concentrations are significantly different in AC and PC regions. Using receiver operating characteristics analysis, we showed that the combined estimation of GSH in AC and PC differentiated AD from HC with 78.6% diagnostic accuracy, 75% sensitivity and 81.3% specificity. Further, cingulate GSH levels also reliably differentiated patients with AD from those with MCI with 82.6% accuracy, 66.7% sensitivity and 100% specificity. Additionally, we found a significant positive correlation between GSH concentrations and cognitive performance (as assessed with MMSE) in both AC ($r = 0.564$, $p = 0.001$) and PC ($r = 0.349$, $p = 0.030$). The present study offers insight into the molecular underpinnings of AD pathology and convincingly evidences GSH levels alterations in the cingulate cortices. The results of our study are indicative of the potential of cingulate GSH levels as a diagnostic biomarker for conversion of MCI to AD.

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Nanosymposium

285. Development of Novel Therapeutics for Alzheimer's Disease: In Vitro Studies

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Blas Frangione Foundation

Title: Lack of neuronal uptake of tau antibodies impairs their efficacy in preventing tau pathology and related toxicity

Authors: *E. E. CONGDON[1], D. UJLA[1], D. SHAMIR[1], H. B. R. SAIT[1], E. M. SIGURDSSON[1,2];
[1]Neurosci. and Physiol., [2]Psychiatry, New York Univ. Sch. of Med., New York, NY

Abstract: Our previous findings show two ways in which antibodies can target tau pathology, via intracellular clearance or extracellular blockage. The relative prevalence of these mechanisms, as well as antibody efficacy likely depends on multiple factors, including isoelectric point (IEP). To test how IEP influences uptake and efficacy we have used multiple antibodies with a range of IEPs in primary JNPL3 neurons alone or in the presence of Alzheimer's-derived paired helical filament (PHF) tau.
For these experiments three different tau antibodies were chosen: 1B9 (P-Thr 212/P-Ser214), 2C11 (P-Ser262) and Tau-5 (210-244). To examine the efficacy of these antibodies, primary neurons were exposed to 10 μg/ml PHF and 1 μg/ml of the antibody in one of two ways for up to seven days. Either PHF and antibody were added together (PHF+Ab) or PHF was added followed 24 h later by the antibody (PHF→Ab).
Exposure to PHF resulted in toxicity as measured by LDH and NeuN levels (85% increase and 94% decrease respectively, $p<0.05$, 0.001). Under PHF+Ab conditions, both 2C11 and Tau-5 had significantly lower LDH ($p<0.001$, 0.01) and significantly higher NeuN ($p<0.0001$ for both), compared to PHF alone, and did not differ from untreated controls.
Likewise, PHF incubation increased both total and phospho-tau levels (6.7 fold and 5.1 fold control, respectively, when normalized for NeuN levels). All three Ab, 1B9, 2C11 and Tau-5 reduced/prevented PHF-induced increases in total tau levels (2.8, 1.6 and 0.87 fold control, $p<0.05$, 0.0001, 0.0001, respectively) under the PHF+Ab condition. In this paradigm, all three

also led to significantly lower levels of phospho-tau when compared to PHF alone samples (0.98, 0.78, 0.94 fold control for 1B9, 2C11 and Tau-5, respectively, $p<0.01$ for all).
Notably, all antibodies were ineffective in preventing PHF toxicity or increases in tau/phospho-tau under the PHFAb condition as their neuronal uptake is limited. Lack of neuronal uptake may be explained by their IEPs, with 1B9 and 2C11 being basic (8.0) and Tau-5 acidic (5.0). In contrast, 4E6 (IEP 6.5; P-Ser396, 404 epitope), an antibody we have used successfully in cell and animal models, is readily taken up by neurons and effective under both PHF+Ab and PHF→Ab conditions.
These results indicate that neuronal uptake of tau antibodies improves their efficacy in clearing pathological tau and preventing its seeding/toxicity. Antibodies that are not internalized can block uptake and toxicity of extracellular tau, but are ineffective against intracellular tau pathology, which is the major pool to target.

Disclosures: **E.E. Congdon:** None. **D. Ujla:** None. **D. Shamir:** None. **H.B.R. Sait:** None. **E.M. Sigurdsson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); H Lundbeck A/S.

Nanosymposium

285. Development of Novel Therapeutics for Alzheimer's Disease: In Vitro Studies

Location: SDCC 32B

Time: Monday, November 14, 2016, 8:00 AM - 10:00 AM

Presentation Number: 285.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: a Grants-in-Aid for Scientific Research (C) (23500439) from the Ministry of Education, Culture, Sports, Science and Technology of Japan

a Grant-in-Aid for the Cooperative Research Project from the Institute of Natural Medicine at the University of Toyama in 2014 and 2015

Discretionary Funds of the President of University of Toyama

Title: Kamikihito regulates axonal growth via cytosolic aspartate aminotransferase activation

Authors: *R. KOBAYASHI[1], H. WATARI[1,2], Y. SHIMADA[2], C. TOHDA[1];
[1]Inst. of Natural Med., Univ. of Toyama, Toyama, Japan; [2]Dept. of Japanese Oriental Medicine, Grad. Sch. of Med. and Pharmaceut. Sciences, Univ. of Toyama, Toyama, Japan

Abstract: Alzheimer’s disease (AD) is the most common cause of dementia. We have investigated anti-AD drugs from traditional Japanese Kampo medicine, especially kamikihito (KKT). We previously reported that KKT improved memory and restored axonal degeneration in

a mouse model AD, 5XFAD. Hyperphosphorylation of tau was reduced by KKT via PP2A activation. To identify the direct binding proteins of KKT components, we used the drug affinity responsive target stability (DARTS) method, and cytosolic aspartate aminotransferase (cAST) was identified. There were no reports showing that cAST related to axonal growth and memory enhancement. In this study, we investigated the effects of cAST on KKT functions. Primary cultured cortical neurons were treated with amyloid beta partial peptide (25-35) (Aβ (25-35)), and the cAST expression and activity were evaluated by western blotting and an AST activity assay, respectively. cAST in Aβ (25-35)-treated neurons showed no change in the expression level but low activity. In contrast, treatment with KKT recovered the cAST activity to control level. To confirm *in vivo,* KKT (200 mg/kg/day) was administered to 5XFAD mice once a day for 15 days. Although the activity of cAST in the cerebral cortex of 5XFAD mice was slightly low compared with wild-type mice, oral administration of KKT reversed the cAST activity. To investigate the effect of the inhibition and knockdown of cAST on the KKT effect, cortical neurons were treated with *O*-(carboxymethyl) hydroxylamine hemihydrochloride (OCHH; an AST inhibitor) or transfected with siRNA for cAST, and the degree of axonal atrophy was evaluated under those conditions. KKT treatment restored the axonal density, whereas the KKT-induced increase in axonal density was diminished by OCHH treatment or knockdown of cAST. To investigate the effect of cAST on axonal growth in normal neurons, OCHH treatment or knockdown of cAST was provided to normal neurons. As a result, the down regulation of cAST was not related to axonal damage. Regulation of axonal growth mediated by cAST may be limited under Aβ existence. In conclusion, KKT up-regulates the cAST activity probably via direct binding to cAST, resulting in axonal growth. The intracellular events caused by KKT-elicited cAST activation are under investigation.

Disclosures: **R. Kobayashi:** None. **H. Watari:** None. **Y. Shimada:** None. **C. Tohda:** None.

Nanosymposium

285. Development of Novel Therapeutics for Alzheimer's Disease: In Vitro Studies

Location: SDCC 32B

Time: Monday, November 14, 2016, 8:00 AM - 10:00 AM

Presentation Number: 285.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DST-SERB EMR/2014/000336

Center for Biomedical Engineering, IITGandhinagar

Title: Controlling post translational modifications to modulate tau aggregation: a toolbox approach

Authors: *S. GUPTA, G. K. VISWANATHAN, K. RALHAN;
Biol. Engin., Indian Inst. of Technol. Gandhinagar, Gandhinagar, India

Abstract: Microtubule Associated Protein Tau (MAPT) undergoes several post translational modifications (PTMs) e.g. phosphorylation, glycosylation, glycation and nitration which are vital for effective functioning and binding to the microtubules. However, tau is abnormally modified in Alzheimer's disease affected brain which destabilizes tau's binding with microtubule and promotes self-aggregation resulting in Paired Helical Filaments (PHFs) and NeuroFibrillary Tangles (NFTs) formation. While certain PTMs can be protective, some may be deleterious. A single amino acid target can compete for more than one PTM e.g. phosphorylation and glycosylation competes for -OH of Ser and Thr further complicating the in vitro study of PTMs. We study combinatorial effects of PTMs by forcing tau undergo more than one modification simultaneously and have developed a tool box of chemical methodologies for PTMs (e.g. Phosphorylation and glycation) which are compatible with each other. Though less site-specific than enzymatic, chemical based methods were preferred as these can be performed at large scale. We have further studied aggregation propensities of modified tau and observed that while phosphorylated tau self-aggregates without an inducer, glycated tau does not. However, adding phosphorylated tau can induce aggregation of glycated tau. We also observed that a library of short peptides which inhibits heparin based tau aggregation behaves differently for chemically modified tau aggregation with some peptides showing enhanced inhibition while others having no effect. We are further trying to correlate these observations with the presence of charged moieties due to chemical modification of tau protein. Inhibitory peptides are expected to be highly target specific and a further understanding about their interaction with modified tau shall aid in improving inhibitors' efficacy and hasten the quest for AD therapeutics.

Disclosures: S. Gupta: None. **G.K. Viswanathan:** None. **K. Ralhan:** None.

Nanosymposium

285. Development of Novel Therapeutics for Alzheimer's Disease: In Vitro Studies

Location: SDCC 32B

Time: Monday, November 14, 2016, 8:00 AM - 10:00 AM

Presentation Number: 285.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH E2523

Title: Tissue-specific ABCA1 agonist in Alzheimer's

Authors: *M. BEN AISSA[1], M. LADU[2], G. R. J. THATCHER[1];
[1]Col. of Pharmacy, UIC, Chicago, IL; [2]Anat. and cell biology, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Therapeutic strategies targeting of amyloid-β peptide (Aβ) have not yet been proven and carriers of *APOE4* the greatest AD risk factor, are often omitted from clinical trials as they fail to respond. Thus, it is critical to develop novel therapeutic candidates that target mechanisms of APOE4-induced AD risk. Recent evidences has established a critical role for Apolipoprotein E (apoE) in Aβ clearance in the brain and the less lipidated apoE4 was associated with poor Aβ processing. Accordingly, improving apoE function by identifying novel compound(s) that increase lipidation of apoE4 would be a promising approach to AD. Importantly, recent evidence has established a critical role for RXR/LXR heterodimers on ATP-binding cassette transporter A1 (ABCA1)-induced apoE lipidation, Aβ clearance and memory improvement in AD mice. Therefore these receptors have emerged as attractive targets for the treatment of Alzheimer's disease (AD). However, LXR/RXR regulates many genes involved in lipid metabolism, causing unacceptable side effects in the periphery, including liver steatosis. **The objective is to activate ABCA1 in glia/astrocytes in the absence of adverse effects caused by activation of lipogenic genes in the liver, notably SREBP-1c.** 1) HTS was conducted to screen for activation of ABCA1 in a cell line mimicking astrocytes (CCF-STTG1); 2) counterscreen for SREBP-1c activation in a cell line mimicking hepatocytes (HepG2); expressing LXR responsive element (LXRE)-Luciferase respectively at the ABCA1 and the SREBP1c- promoters. Screening conditions have been validated and optimized to obtain an average Z' factors exceeding 0.7. The scalability of the assay was confirmed by conducting a preliminary screen of the Enzo® Nuclear Receptor (NR) ligand library and a novel RXR ligand library. Based on these preliminary data, > 10,000 compounds were screened and >550 compound were retained for counter-screen. Following hit validation, promising compounds were verified using a secondary assay, gaining insight into mechanistic pathways. In Conclusion, the further examination of discovered hits gives us a) combinatorial therapeutic options to potentiate ABCA1 effects in CNS and therefore improve apoE functions, b) mechanistic insight into the roles of hit compounds. This HTS screening can serve as a robust and accurate method for target discovery in AD.

Disclosures: **M. Ben Aissa:** None. **M. LaDu:** None. **G.R.J. Thatcher:** None.

Nanosymposium

285. Development of Novel Therapeutics for Alzheimer's Disease: In Vitro Studies

Location: SDCC 32B

Time: Monday, November 14, 2016, 8:00 AM - 10:00 AM

Presentation Number: 285.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer Society of Canada

FRQ-S

CIHR

Title: A small-molecule peptide inhibitor of Caspase-6 prevents neuronal degeneration in human primary neurons and reverses Caspase-6-dependent cognitive impairment in mice

Authors: *P. PAKAVATHKUMAR[1,2], A. NOËL[1,2], J.-E. AHLFORS[3], A. C. LEBLANC[1,2]; [1]Lady Davis Inst., Jewish Gen. Hosp., Montréal, QC, Canada; [2]Integrated Program in Neurosci., McGill Univ., Montréal, QC, Canada; [3]New World Labs. Inc., Laval, QC, Canada

Abstract: Caspase-6 (Casp6), a member of the aspartate-specific cysteinyl proteases, has been implicated in early Alzheimer Disease pathology. Low levels of Casp6 activity are detected in the brain and cerebrospinal fluid of non-cognitively impaired individuals where it correlates negatively with cognitive function. Moreover, overexpression of human Casp6 in the CA1 region of the mouse hippocampus results in age-dependent cognitive deficits. Thus, Casp6 activation is a novel target for therapeutic intervention. New World Laboratories Inc. developed potent, non-toxic, blood-brain permeable, competitive, and irreversible Casp6 inhibitors, NWL-117 and -154. Both compounds showed half-maximal inhibitory concentrations (IC_{50}) of 0.6 μM and 0.2 μM against recombinant active Casp6, respectively. Human colon carcinoma cells (HCT116) transfected with a self-activating form of Casp6 and serum-deprived human primary neurons were used to assess the inhibitory activity of NWL-compounds in cells. Non-toxic concentrations of NWL-compounds (100 μM, 2 hours) significantly inhibited Casp6 activity in HCT116 cells as measured by an *in vitro* fluorogenic assay and by western blot analysis for α-tubulin-cleaved by Casp6 (TubΔCasp6). In time course experiments, NWL-117 and -154 significantly inhibited Casp6 activity within 15 minutes of treatment. In contrast, 2 hours after removing the compounds, cells-treated with NWL-117 and NWL-154 recovered 85% and 50% of their original Casp6 activity, respectively. In serum-deprived human primary neurons, both compounds significantly inhibited endogenous Casp6-mediated fluorogenic activity and decreased TubΔCasp6 levels. Immunofluorescence experiments showed significant decreases TubΔCasp6 within axons of serum-deprived neurons following treatment with NWL-117 and NWL-154. Neuroprotection was also observed by time-lapse live cell imaging showing a significant reduction of neuritic beading in the presence of NWL-117. Mice that acquire Casp6-mediated age-dependent memory impairment were treated acutely with NWL-117 and tested using the Novel Object Recognition task. Treatment with NWL-117 significantly reversed novel object recognition deficits compared to saline-treated mice. This was paralleled with a decreasing trend in the levels of TubΔCasp6 and inflammation-associated Iba1 microglial staining in the hippocampi of treated mice. These results indicate that pharmacological inhibition of Casp6 may remedy Casp6-dependent neurodegeneration and cognitive impairments. Further development of potent, non-toxic, selective, and blood-brain permeable inhibitors of Casp6 is necessary.

Disclosures: **P. Pakavathkumar:** None. **A. Noël:** None. **J. Ahlfors:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); New World Laboratories Inc. **A.C. LeBlanc:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NEW WORLD LABORATORIES INC..

Nanosymposium

286. Structural Changes, Connectivity, and Deep Brain Stimulation Treatment in Parkinson's Disease

Location: SDCC 30B

Time: Monday, November 14, 2016, 8:00 AM - 10:30 AM

Presentation Number: 286.01

Topic: C.03. Parkinson's Disease

Support: ERC SPEED 313481

Title: Structural changes in the Substantia Nigra in Parkinson's disease

Authors: ***M. C. KEUKEN**, B. R. ISAACS, R. BALESAR, A. ALKEMADE, B. U. FORSTMANN;
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Abstract: Parkinson's disease (PD) is a chronic and progressive neurodegenerative disease characterised by the loss of dopaminergic (DA) neurons in the substantia nigra (SN) (Shulman et al., 2011). Previous magnetic resonance imaging (MRI) studies investigating the volumetric changes of the SN associated with PD have failed to produce a consensus. Studies on volumetric changes of the SN in PD patients report either no alterations (Oikawa et al., 2002; Peran et al., 2010), reductions (Hutchinson et al., 2003; Minati et al., 2007; Menke et al., 2009), or, counter-intuitively, increases (Cho et al., 2011; Kwon et al., 2012). A potential mechanism for the increase of SN volume has been speculated to be an accumulation of iron (Kwon et al., 2012). It remains unclear what the effects of PD on the SN are, and what underlying mechanism drives this potential change. In the present study, 7T structural T2*-weighted MRI scans were acquired for 12 PD patients (mean age: 68, SD: 6.9, 6 females), and 12 age-matched controls (mean age: 65, SD: 7.9, 6 females). The SN was manually segmented by two raters who were blind to the clinical diagnosis. In line with previous 7T MRI studies, an increase in PD SN volume estimates was found (Cho et al., 2011; Kwon et al., 2012). Additionally, Quantitative Susceptibility Mapping (QSM) was performed to assess the presence of nigral iron. No difference in iron was found between the PD group and healthy controls. Therefore, the reported increase in SN volume for PD patients could not be attributed to an increase in pathological iron load. We assessed the state of microglia activation as a proxy for nigral inflammation to investigate an alternative

explanation for the increase in volume. Previous histological studies have speculated that the enlargement of the grey matter structures in PD patients may be due to compensatory mechanisms of chronic neuroinflammation (Depino, Earl, Kaczmarczyk., 2003; Hirsch & Hunot, 2009; Lin et al. 2013; McGeer, Itagaki, Boyes., 1988; Yamada, McGeer, McGeer., 1991). We tested this mechanism by staining a central SN slice for Iba1, a marker for microglial activation, in post mortem tissue specimens of seven PD patients and seven age-matched controls. The histochemical results showed an increased number of Iba1 positive structures in close association with neuromelanin positive DA neurons, supporting an increased state of inflammation in the SN of PD patients. It is therefore possible that the PD SN volumetric changes observed in MRI reflect inflammatory responses to dopaminergic cell death, rather than pathological iron accumulation.

Disclosures: **M.C. Keuken:** None. **B.R. Isaacs:** None. **R. Balesar:** None. **A. Alkemade:** None. **B.U. Forstmann:** None.

Nanosymposium

286. Structural Changes, Connectivity, and Deep Brain Stimulation Treatment in Parkinson's Disease

Location: SDCC 30B

Time: Monday, November 14, 2016, 8:00 AM - 10:30 AM

Presentation Number: 286.02

Topic: C.03. Parkinson’s Disease

Support: Biomarkers Across Neurodegenerative Diseases grant 9680

the Michael J. Fox Foundation for Parkinson’s Research (MJFF)

W. Garfield Weston Foundation

NIH ENIGMA Center grant U54 EB020403

Big Data to Knowledge (BD2K) Centers of Excellence program

Title: White matter structural changes in parkinson’s disease

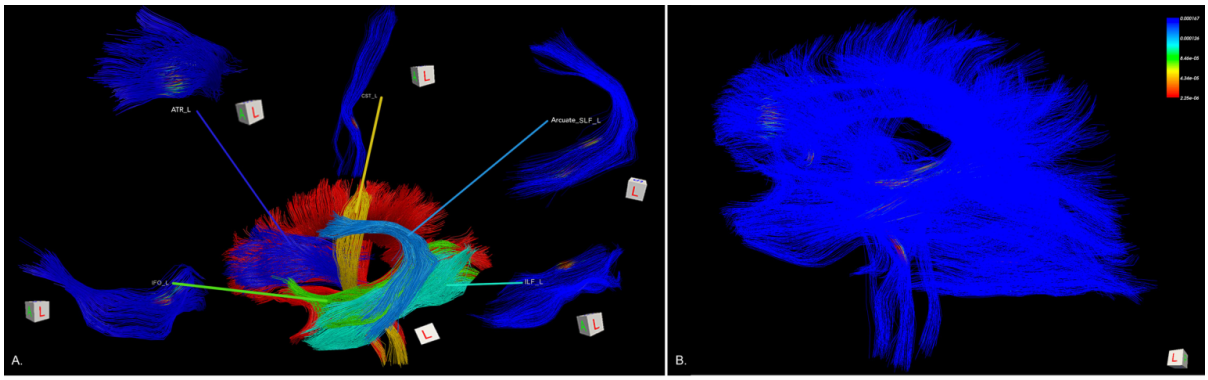
Authors: *A. RAGOTHAMAN, E. L. DENNIS, M. DAIANU, J. GALVIS, Y. JIN, G. PRASAD, P. M. THOMPSON;
USC, Los Angeles, CA

Abstract: Identifying structural brain changes in Parkinson’s disease (PD) is a challenging task. PD affects both motor and non-motor regions of the brain. Diffusion MRI (dMRI) can identify

changes in white matter microstructure, and patients show lower fractional anisotropy (FA) values in various brain regions. To better understand the profile of effects, here we analyzed dMRI scans from 127 PD patients (81M/46F) and 59 age-matched healthy controls (38M/21F), scanned as part of the Parkinson's Progressive Markers Initiative (PPMI). Diffusion images were pre-processed using FSL and probabilistic tractography was computed using Camino. 19 major white matter (WM) fiber tracts were re-constructed using a label fusion and fiber clustering method we developed, called autoMATE (automated multi-atlas tract extraction). The method extracts anatomically meaningful WM tracts using a multi-atlas framework, adapting to individual variability in tract shapes. We performed mass univariate tests to detect group differences in the WM tracts between PD patients and controls, while covarying for age and sex and correcting for multiple comparisons using the false discovery rate method (FDR). PD patients showed significant decreases in FA and increases in radial diffusivity (RD) in the following WM tracts which are shown in figure: the left anterior thalamic radiation, left inferior longitudinal fasciculus, left inferior fronto-occipital fasciculus, left arcuate fasciculus, and part of the superior longitudinal fasciculus.

The 19 fiber tracts were also used to classify PD patients and healthy controls using a feedforward backpropagation-learning algorithm - implemented as a neural network, with 25 nodes in a single hidden layer. This resulted in a classification accuracy of ~68%, based on 10-fold cross-validation.

The regions that assisted classification are implicated in motor tasks. These results suggest that diffusion MRI can be used to find biologically meaningful areas of alterations in Parkinson's disease, which may serve as biomarkers of the disease.



A. is a representation of the white matter tracts to show their location in the brain as well as the isolated tracts indicating significant P-Value's.
B. shows the P-map of the major white matter tracts which show group differences between PD patients and healthy controls with FDR Threshold 0.000167. Red shows the regions of reduced FA in patients; Blue shows the regions with no significant difference.
ATR_L - left anterior thalamic radiation; CST_L - left corticospinal tract; IFO_L - left inferior fronto-occipital fasciculus; ILF_L - left inferior longitudinal fasciculus; SLF_L - left arcuate fasciculus (part of superior longitudinal fasciculus);

Disclosures: **A. Ragothaman:** None. **E.L. Dennis:** None. **M. Daianu:** None. **J. Galvis:** None. **Y. Jin:** None. **G. Prasad:** None. **P.M. Thompson:** None.

Nanosymposium

286. Structural Changes, Connectivity, and Deep Brain Stimulation Treatment in Parkinson's Disease

Location: SDCC 30B

Time: Monday, November 14, 2016, 8:00 AM - 10:30 AM

Presentation Number: 286.03

Topic: C.03. Parkinson's Disease

Title: An exploratory whole-brain cohort study of structural connectivity of Parkinson disease progression

Authors: *A. KAMALIAN[1,2], F. RAHMANI[3], M. DOLATSHAHI[3], A. ANJOMSHOA[3], N. HOSSEINI[2], M. AARABI[4];
[1]MEDICAL SCIENCES, TEHRAN UNIVERSITY, SHIRAZ, Iran, Islamic Republic of; [2]Student Scientific Res. Ctr., Tehran, Iran, Islamic Republic of; [3]Tehran Univ. of Med. Sci., Tehran, Iran, Islamic Republic of; [4]Basir Eye Hlth. Res. Ctr., Tehran, Iran, Islamic Republic of

Abstract: Background: Considered a progressive neurodegenerative disorder, scientific efforts have been concentrated on discovering the areas involved and the pattern of Parkinson Disease (PD) progression.
Objective: To compare brain structural connectivity of PD cases with progressive motor symptoms to unprogressive PD cases and monitoring their structural progression pattern
Methods: Data used in this paper was obtained from Parkinson's Progression Markers Initiative (PPMI) database (www.ppmi-info.org). Baseline Diffusion-Weighted Images (DWI) of 22 PD cases (mean age 64.81) showing more than 0.05 increase in their UPDRSIII scores in a two-year follow up were compared to 12 PD cases (mean age 62.67) showing either insignificant increase or a decrease in their scores (cases unmedicated at the time of assessments). The DWI were acquired on a SIEMENS TrioTim (TE=88 ms, TR=700 ms) and corrected for subject motion and eddy-current induced geometrical distortion, then, included in the connectometry database in dsi-studio.
Diffusion MRI connectometry was conducted to compare group differences between baseline images of progressive PD and unprogressive PD cases (length threshold=50 mm).
Then diffusion MRI connectometry was conducted to obtain paired group differences between baseline images of progressive PD cases with their follow up session images 2 years later. The same connectometry parameters were used.
Results: The analysis results showed tracks with increased quantitative anisotropy (QA) in progressive PD cases (False Discovery Rate [FDR] =0.04) in comparison with unprogressive PD cases.
The paired group difference analysis demonstrates increased QA (FDR= 0.053) in baseline progressive PD compared to their images 2 years later, Figure 1.
Conclusion: Results indicate an increased QA in the genu of corpus callosum and cingulate

tracts of unprogressive PD brains compared to progressive PD.
Decreased QA in right corticospinal tract, inferior longitudinal fasciculus, and corpus callosum in 2 years, indicates possible areas of degeneration during PD progression process.

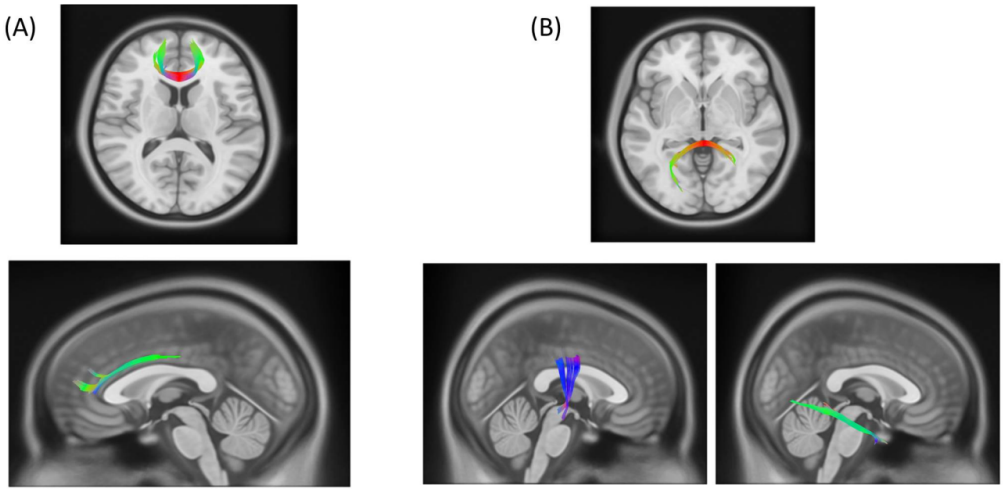


Figure. (A) tracts with significant increase in QA in unprogressive PD cases compared to progressive cases: genu of CC, and anterior part of cingulum. **(B)** tracts with significant decrease in QA after 2 yrs in progressive PD cases: splenium of CC, CST, and ILF.

Disclosures: A. Kamalian: None. **F. Rahmani:** None. **M. Dolatshahi:** None. **A. Anjomshoa:** None. **N. hosseini:** None. **M. Aarabi:** None.

Nanosymposium

286. Structural Changes, Connectivity, and Deep Brain Stimulation Treatment in Parkinson's Disease

Location: SDCC 30B

Time: Monday, November 14, 2016, 8:00 AM - 10:30 AM

Presentation Number: 286.04

Topic: C.03. Parkinson's Disease

Title: Whole plasma associates with brain structural changes in early Parkinson disease: a DTI study

Authors: *F. RAHMANI[1], A. KAMALIAN[2], N. HOSSEINI[2], M. DOLATSHAHI[2], A. ANJOMSHOJA[2], M. AARABI[2];

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Abstract: Objective: Studies show that high HDL levels is essential for preservation of cognitive function and delays onset of Parkinson disease (PD). Higher total cholesterol and LDL cholesterol levels almost reduce PD risk and severity of motor disturbances. Herein we investigated microstructural correlates of white matter regions with plasma lipid profile in drug-naïve PD patients, based on diffusion tensor imaging.
Methods: We used Parkinson's Progression Markers Initiative (PPMI). DWI data were analyzed using the diffusion MR toolbox 'ExploreDTI', in following steps: (i) correction for subject motion and eddy current induced distortions; (ii) tensor estimation using the REKINDLE approach for outlier detection; and (iii) automated atlas based analysis with the Johns Hopkins University's Mori white matter atlas using affine and elastic registration based on 'elastix'. FA, AD, RD, and MD values were then calculated in the 40 brain regions of the Mori atlas. Average values partially correlated against whole plasma profile.
Results and Discussion: Table 1 shows Results of significant ROI correlations between the Plasma profiles and DTI parameters. HDL and Apo-A1 share the same correlates with brain regions both having significant negative correlation with retrolenticular part of the corpus callosum (RLIC) and with cingulum. Diffusivity along the main axis of RLIC is inversely correlated with levels of Apo-A1, HDL and total cholesterol levels. Cholesterol and LDL levels both had significant correlation with right CST diffusometric parameters in terms of MD, FA and RD. Axial and mean diffusivity of stria terminalis and fornix diffusometric measures were inversely correlated with LDL levels while higher LDL levels increased diffusivity perpendicular to the main axis of the fiber (RD). Increase in mean diffusivity of several white matter tracts is a key feature of early PD more specifically the corpus callosum, cingulum and the inferior longitudinal fasciculus (ILF) and CST exhibit increased MD which indicates axonal degeneration and demyelination in these areas in earliest stages of PD.

Table 1 – Correlation between Mori-Atlas regions with PD and plasma lipid profile indicators

	FA	MD	AD	CP
Apo_A1			Right RLIC*(-0.322) Left CG*(0.383) Right CG*(-0.401)	Genu of CC*(-0.408) Left anterior CR *(-0.367) Left CG *(0.352) Right CG*(0.355) Left hippocampus*(0.352)
Total Cholesterol	Right CST*(-0.36)	Right CST **(0.467)	Right CST*(0.425) RLIC*(0.388)	Fornix (column and body)*(0.368) Left RLIC*(0.329) Left CG*(0.371) Right CG*(0.349)
LDL Cholesterol		Right CST *(0.388) Left ST of fornix *(-0.353)	Right CST*(0.375) Left RLIC*(-0.39) Left ST of fornix*(-0.329)	Fornix (column and body)*(0.346) Left ML*(0.339) Right ML*(0.359) Right posterior CR*(0.35)
HDL Cholesterol		Left PLIC *(-0.372)	Right RLIC *(-0.387) Left CG **(-0.529) Right CG*(-0.479)	Left CST*(0.335) Left EC*(0.431) Left CG*(0.386) Left hippocampus*(0.352)
Non-HDL cholesterol	Right CST*(0.345)	Left CST**(0.468) Right CST*(0.359)	Right CST*(0.466)	Fornix (column and body)*(0.418) Left EC*(0.396) Left ML*(0.34) Right ML*(0.395)

1-FA=Fractional Anisotropy, MD= Mean Diffusivity, AD= Axial Diffusivity, CP=Planar Anisotropy, CST=Corticospinal Tract, PLIC=Posterior limb of Internal Capsule, ST=Stria Terminalis, RLIC=Retrolenticular part of Internal Capsule, CC=Corpus Callosum, CG= Cingulate gyrus, ML=Medial Lemniscus, EC=External Capsule

2- * indicates a significant level of 0.05 and ** of 0.01. Value in parenthesis show Pearson Coefficient for each pair.

Disclosures: F. Rahmani: None. **A. Kamalian:** None. **N. Hosseini:** None. **M. Dolatshahi:** None. **A. Anjomshoja:** None. **M. Aarabi:** None.

Nanosymposium

286. Structural Changes, Connectivity, and Deep Brain Stimulation Treatment in Parkinson's Disease

Location: SDCC 30B

Time: Monday, November 14, 2016, 8:00 AM - 10:30 AM

Presentation Number: 286.05

Topic: E.02. Cerebellum

Support: MH091657

Title: A quantification of normative grey-matter structural variability, covariance, and heritability in the human cerebellum

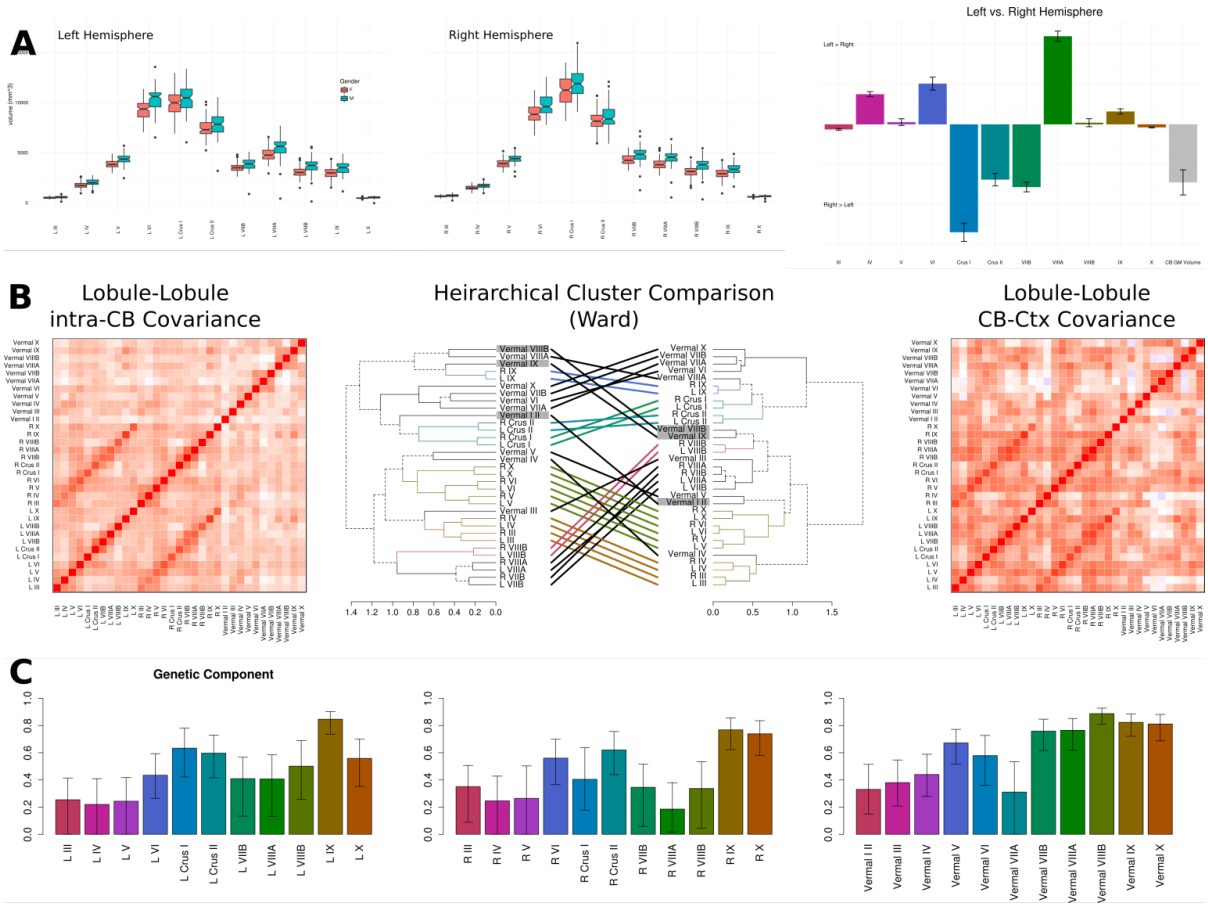
Authors: *C. J. STEELE[1,2], S. PATEL[3,4], G. DEVENYI[1,5], J. KNIGHT[3,4], B. MISIC[5,6], M. CHAKRAVARTY[1];
[1]Psychiatry, Cerebral Imaging Centre, Douglas Mental Hlth. Uni, Montreal, QC, Canada; [2]Max Planck Inst. for Human Cognitive and Brain Sciences,, Leipzig, Germany; [3]Campbell Family Mental Hlth. Res. Institute, Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [4]Inst. of

Med. Sci., Univ. of Toronto, Toronto, ON, Canada; [5]Montreal Neurolog. Inst., [6]Dept. of Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: The cerebellum (CB) contains ~50% of the neurons in the human brain yet we know surprisingly little about its structural variance, relationship with the cortex, or heritability. Growing evidence that the CB plays an important role in diseases such as Alzheimer's, dementia, Parkinson's, and Multiple Sclerosis underscores the need for more accurate quantification of CB structure and its genetic basis. Therefore, we quantified the lobule-specific variability, intra-CB structural covariance, CB-cortical structural covariance, and lobule-specific heritability to provide the foundation for its potential use as a biomarker of disease.

MAGeTbrain was used to segment the CB lobules of individuals from the Human Connectome Project. 212 unrelated datasets were used to determine lobule-specific mean and variance, and to compute correlation matrices to quantify intra-CB and CB-cortical structural covariance. Lobule-specific heritability was assessed in a related sample (100 MZ, 86 DZ, 78 sibs) with structural equation modeling in OpenMx.

Lobular volume followed an approximately logarithmic increase from the smallest lobules to Crus I/II in both the superior and inferior CB. Volume of the right hemisphere was significantly greater than that of the left, an effect primarily driven by Crus I, II, and VIIb (Fig1A). Intra-CB and CB-cortical correlations were similarly structured with two main clusters delineating motor and non-motor connected lobules (Fig1B). However, vermal I II, VIIIB, and IX showed higher similarity with the “motor” cluster when CB-cortical connectivity was considered, suggesting that established motor/non-motor distinction may be overly simplistic. The heritability of most lobular volumes ranged from ~20 - 80%, with each hemisphere exhibiting a similar pattern and the vermal regions exhibiting higher heritability over all (Fig1C). We provide the first population quantification of CB anatomy, structural covariance, and heritability - and provide initial evidence for the structural covariance that may underlie CB-cortical functional connectivity.



Disclosures: **C.J. Steele:** None. **S. Patel:** None. **G. Devenyi:** None. **J. Knight:** None. **B. Misic:** None. **M. Chakravarty:** None.

Nanosymposium

286. Structural Changes, Connectivity, and Deep Brain Stimulation Treatment in Parkinson's Disease

Location: SDCC 30B

Time: Monday, November 14, 2016, 8:00 AM - 10:30 AM

Presentation Number: 286.06

Topic: E.03. Basal Ganglia

Support: NIH Grant DA024689

NIH Grant DA033554

INSERM Grant

Title: Dopamine d2 receptors modulation of the striatal circuitry

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Abstract: Dopamine signaling in the striatum is critical for the control of motor behavior. The striatum has one type of output neurons, the medium spiny neurons (MSN). These neurons are divided into two subpopulations: $D1R^+$-MSNs expressing the dopamine D1 receptors and $D2R^+$-MSNs expressing the dopamine D2 receptors. $D1R^+$-MSNs and $D2R^+$-MSNs form the direct and indirect output pathways, respectively. Dopamine in the striatum is also important in the control of cholinergic interneurons; the activity of these interneurons strongly affects responses of the direct and indirect pathway neurons. Cholinergic interneurons express both D2R and D5R, which when activated regulate the activity of these neurons and acetylcholine release. The knockout of the dopamine D2 receptors in mice result into a Parkinsonian phenotype, which indicates that D2R signaling is fundamental for the control of motor activity. We have recently generated cell-specific D2R mutants that allow establishing the mechanisms by which D2R regulates motor functions. Interestingly, the knockout of D2R in MSNs is the one that impairs motor function in basal condition. We show that knockout of D2R in dopaminergic neurons or in cholinergic interneurons does not affect motor activity in basal conditions but it does in specific behavioral conditions as well as in response to dopamine agonists and antagonists. These results suggest that a finely D2R-regulated dopamine/acetylcholine balance controls the activity of the striatal MSNs and is required for normal motor activity and coordination of movements.

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Nanosymposium

286. Structural Changes, Connectivity, and Deep Brain Stimulation Treatment in Parkinson's Disease

Location: SDCC 30B

Time: Monday, November 14, 2016, 8:00 AM - 10:30 AM

Presentation Number: 286.07

Topic: C.03. Parkinson’s Disease

Support: UC Berkeley, EECS Departmental fellowship

NIH R01 NS069779

Title: Intraoperative real-time ecog spectrogram for movement induced spectral change in patients with parkinson's disease (pd)

Authors: *N. TIAN[1], S. MIOCINOVIC[2], C. CORREA[1], A. MILLER[2], R. MOAZZEZI[1], C. DE HEMPTINNE[2], P. A. STARR[2], K. GOLDBERG[1];
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Abstract: Objectives
Movement-induced beta (13-30Hz) and gamma (>50Hz) changes are normal features of motor cortex physiology and are detectable by electrocorticography (ECog). Sensing electrodes placed over primary motor area (M1) show promise as detectors of physiological biomarkers of motor dysfunction in Parkinson's disease (PD). Movement related gamma changes can be used to confirm or modify correct localization of ECoG electrodes, but the time required for collection and analysis of movement related changes may impede efficient intraoperative localization.We present a prototype intraoperative system that updates six spectrograms ~3 times per second for signals sampled at 11kHz with spectral resolution of 3Hz/bin (536 bins) and 250 msec sliding window.
Methods
The prototype uses NeuroOmega's Matlab interface, and has an interactive graphical user interface (GUI). With IRB approval and informed consent, we tested this system intraoperatively in two patients undergoing implantation of deep brain stimulators in the awake state. A six-contact ECoG array was temporarily implanted over M1 via a frontal burr hole. Arm movements were monitored using EMG electrodes on arm muscles and a wrist accelerometer. Patients performed repetitive elbow flexion extension movements. Each real-time spectrogram display session includes: 1) 10 sec resting baseline, 2) repeated elbow flexion for averaging, 3) 5 sec rest between elbow movements.
Results
We observed consistent (~90%) beta decrease in one patient and intermittent (~40%) beta decrease in the other using the real-time ECoG spectrogram. Based on the degree of beta changes, we were able to estimate which of the six electrode contacts was closest to the arm area of M1. However, the gamma increase was hard to detect in real-time (single spectrogram, <10%, noisy) even when we used EMG to align spectrograms based on arm motion onsets and average the spectrograms. In offline analysis, we observed prominent gamma changes when spectrograms were aligned and averaged using the accelerometer signals, suggesting that ECoG arrays were above the arm area of M1.
Conclusion
We were able to observe movement-related beta decreases in near real time from rapid spectral analysis of ECoG potentials. Reliable gamma increase detection requires both averaging (over multiple elbow flexions) and accurate movement onset detection for spectrogram alignment. Our real-time system used EMG, which is less accurate than an accelerometer for detecting movement onsets, so future work will incorporate an accelerometer for spectrogram alignment.

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Nanosymposium

286. Structural Changes, Connectivity, and Deep Brain Stimulation Treatment in Parkinson's Disease

Location: SDCC 30B

Time: Monday, November 14, 2016, 8:00 AM - 10:30 AM

Presentation Number: 286.08

Topic: C.03. Parkinson's Disease

Support: National Science Foundation Career Award 58501963

Title: DBS of the STN creates impulse control disorders and fails to restore parkinsonian apathy and action selection deficits.

Authors: *C. ANDERSON;
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Abstract: As many as 70% of Parkinson's Disease (PD) patients experience symptoms of apathy, frequently unresolved or worsened by deep brain stimulation (DBS) of the subthalamic nucleus (STN). Additionally, as many as 56% of patients receiving DBS for PD may experience new-onset impulse control disorders of varying severity following therapy initiation. Some animal-based studies have been performed to examine these symptoms and side effects, but more work is needed to understand their origin and how to prevent them. We created a behavioral configuration and tested the behavioral effects of DBS in the context of healthy-control and hemiparkinsonian (hPD) rodents. Rats were trained on either a go / stop or a go / no-go task and their behavior was quantified under each of control, control + DBS, hPD, and hPD + DBS conditions. We found that DBS of the STN in healthy rodents lead to more impulsive behavior in the form of stop and no-go task failure, impulsive reward seeking, and non-instructed task attempts. Additionally, based on the timing of stop and no-go cue failures, we found evidence to support that STN-DBS interrupts signals responsible for action cancellation. Finally, we demonstrated that hemiparkinsonism leads to slowed cue response times and greatly reduced response rates, unrestored by DBS. Since DBS functions by overriding pathological electrophysiological activity, rather than restoring the activity seen under healthy conditions, it may be that certain neurological signals responsible for healthy action selection are interrupted by PD and not restored by DBS, leading to apathy and mis-weighting of various actions in the context of reward-based action selection. This work will enable future electrophysiological studies into the mechanisms of DBS-induced action suppression deficits and PD-induced apathy and action selection deficits.

Disclosures: C. Anderson: None.

Nanosymposium

286. Structural Changes, Connectivity, and Deep Brain Stimulation Treatment in Parkinson's Disease

Location: SDCC 30B

Time: Monday, November 14, 2016, 8:00 AM - 10:30 AM

Presentation Number: 286.09

Topic: C.03. Parkinson’s Disease

Support: NIH Grant R01 NS 70872

The Grainger Foundation

Title: Dopamine release in the nonhuman primate caudate and putamen depends upon site of stimulation in the subthalamic nucleus

Authors: *P. H. MIN, E. K. ROSS, H. JO, S. CHO, M. L. SETTELL, J. JEONG, P. S. DUFFY, S.-Y. CHANG, K. E. BENNET, C. D. BLAHA, K. H. LEE;
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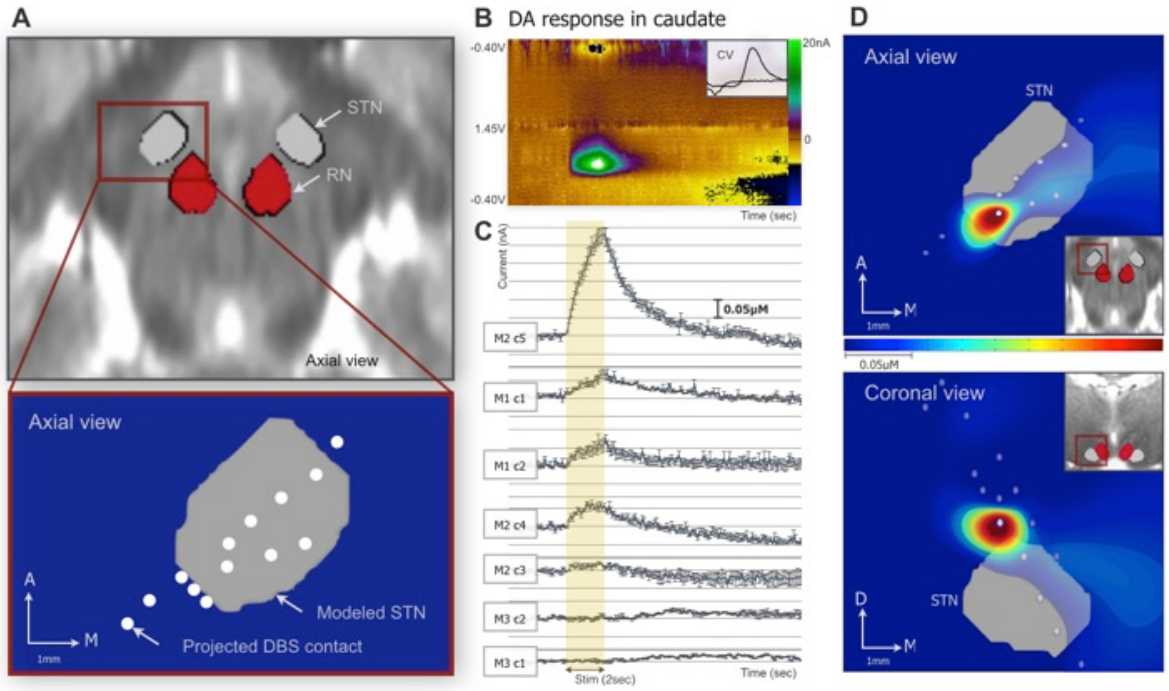
Abstract: Introduction: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is an effective treatment for medically refractory Parkinson's disease. Although it has recognized clinical utility, its biologic mechanisms are not fully understood, and dopamine release as a potential factor in those mechanisms is in dispute. We tested the hypothesis that STN DBS-evoked dopamine release depends on the precise location of the stimulation site in the STN and site of recording in the caudate and putamen. Methods: We conducted DBS with miniature, scaled-to-animal size, multi-contact electrodes and used fMRI to identify the optimal dopamine recording site in the brains of nonhuman primates (n=3, Rhesus macaques), which are highly representative of human brain anatomy and circuitry. Real-time stimulation-evoked dopamine release was monitored using in vivo fast scan cyclic voltammetry (n=51, stimulation sessions). Results: We investigated the relationship between stimulation site in the STN and DBS-evoked dopamine release in the caudate and putamen. The spatial distribution in which the magnitude of DBS-evoked dopamine release increased as the stimulation site moved in a lateral and posterior direction. Specifically, dopamine release maximized in the fMRI targeted sites in both the caudate and putamen when stimulating the dorsolateral posterior border of the STN. Conclusion: We have demonstrated that DBS coincides with changes in dopamine neurotransmitter release in the basal ganglia. Importantly, this study shows that DBS-evoked dopamine release can be minimized or maximized through subtle changes in the stimulation site. Figure: (A) Cross-modality registration for each subject’s MRI-CT fusion image to a NHP brain template; (B)

Representative voltammogram of STN stimulation-evoked change in caudate DA; (C) DA oxidation current vs. time plot of stimulation-evoked dopamine release in the caudate; (D) Overlay of an evoked-dopamine response volume projection color map from the caudate onto the 3-D STN DBS contact location model (n=51, stimulation sessions).



Disclosures: **P.H. Min:** None. **E.K. Ross:** None. **H. Jo:** None. **S. Cho:** None. **M.L. Settell:** None. **J. Jeong:** None. **P.S. Duffy:** None. **S. Chang:** None. **K.E. Bennet:** None. **C.D. Blaha:** None. **K.H. Lee:** None.

Nanosymposium

286. Structural Changes, Connectivity, and Deep Brain Stimulation Treatment in Parkinson's Disease

Location: SDCC 30B

Time: Monday, November 14, 2016, 8:00 AM - 10:30 AM

Presentation Number: 286.10

Topic: C.03. Parkinson’s Disease

Title: Battery longevity of Medtronic Activa PC neurostimulator: nonlinear regression of clinical battery decay curves and supplemental end of service thresholds

Authors: *E. L. HARGREAVES[1], R. P. PATEL[1], S. WONG[2], R. J. DIPAOLA[2], S. F. DANISH[1];
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Abstract: Deep Brain Stimulation (DBS) is an adjunct neurosurgical treatment for Movement Disorders. At the core of the DBS system is the neuromodulation device. Medtronic's most recent Activa Family of neuromodulation devices purports to have a 2-5 year battery life. We have followed 40 of our initial DBS Movement Disorder patients with original Activa PC implants. Presently, almost two-thirds (26/40) have had their neurostimulators exchanged, while another (9/40) still retain their initial functioning neurostimulator. The final (5/40) were lost to follow up (1 passed away, 2 moved away, 2 explanted systems). Thus, we have continuously tracked data from (31/40) implanted individuals, and start and end data from an additional (4/40). A subset of the constructed battery decay curves were fit with nonlinear cubic spline regressions and partial end segments of the curves were fit with hyperbola regressions. Battery values at time of exchange ranged 2.22 (EOS) - 2.60V (ERI) mean: 2.54V (sem .017). Battery life duration of the Activa PCs that crossed the ERI 2.60V value ranged from 2.13years - 5.18years mean: 3.53years (sem .157). Of the neurostimulators that continue to perform, the present durations range from 3.59years - 5.24years mean: 4.52years (sem .178). The cubic spline fit the entire decay curves well, capturing the initial upward concavity of rapid battery decline, the central plateau, and the latent downward concavity towards ERI and EOS thresholds (mean R^2 = .977). However, the cubic spline fits were fairly divergent across the latent downward concavities. The hyperbola regressions of the central plateau and latent downward concavity marginally improved the fits (mean R^2 = .985) but more importantly, converged between curves as battery estimates plummeted towards ERI and EOS values. Days to EOS from the initial ERI crossing were on average 59.72days (sem 2.05), which coincided well with Medtronic's suggested interval of 60 days for the same window. We further generated latencies for the following mean fit battery values of 2.65V, 2.70V and 2.75V revealed averages of 84.72days (sem3.31), 118.05days (sem 5.41) and 174.30days (sem 12.10) to EOS, respectively. Our clinical data coincide well with the 2-5 year battery life purported by Medtronic. Furthermore, our curve fitting analyses provide supplemental battery values, denoting approximately 3, 4 and 6 month warnings until EOS, in addition to the standard ERI threshold, thereby preventing EOS battery failure through more detailed monitoring.

Disclosures: E.L. Hargreaves: None. **R.P. Patel:** None. **S. Wong:** None. **R.J. DiPaola:** None. **S.F. Danish:** None.

Nanosymposium

287. Transplants and Other Treatments of Parkinson's Disease

Location: SDCC 1B

Time: Monday, November 14, 2016, 8:00 AM - 11:00 AM

Presentation Number: 287.01

Topic: C.03. Parkinson's Disease

Title: Transplantation of neural stem cells for parkinson's disease, an update of the first-in-human clinical study

Authors: I. GARITAONANDIA[1], R. GONZALEZ[1], M. POUSTOVOITOV[1], T. ABRAMIHINA[1], A. NOSKOV[1], T. CHRISTIANSEN-WEBER[1], G. SHERMAN[1], A. SEMECHKIN[1], L. LAURENT[2], J. ELSWORTH[3], E. SNYDER[4], D. REDMOND[3], *R. A. KERN[1];
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Abstract: Cell therapy has considerable potential in treating debilitating neurodegenerative disorders such as Parkinson's disease (PD). Grafting fetal neural tissue has shown in significant biochemical and clinical improvements in some PD patients. However, alternative expandable cell sources are needed because fetal tissue is limited and clinically impractical. Here, we used human parthenogenetic stem cells (hpSC), which are pluripotent stem cells that can be expanded and differentiated *in vitro* to generate an unlimited supply of neural tissue. We have manufactured a highly pure population of human parthenogenetic neural stem cells (ISC-hpNSC) with a chemically defined differentiation protocol and created master and working cell banks under cGMP conditions. Clinical grade ISC-hpNSC were extensively tested and used in pharmacology, toxicology, biodistribution, tumorigenicity and efficacy studies. Intranigrostriatal transplantation ISC-hpNSC was shown to be safe, well tolerated and effective in treating rodent and non-human primate models of PD. ISC-hpNSC promoted behavioral recovery without dyskinesias and increased striatal dopamine (DA) concentration, fiber innervation and number DA neurons and induced the expression of genes and pathways down-regulated in PD in comparison to vehicle control animals. Based on the extensive preclinical studies, the Australian Therapeutic Goods Administration and Human Research Ethics Committee have granted us approval to conduct a dose escalating Phase I study to evaluate the safety and tolerability of ISC-hpNSC in PD patients (ClinicalTrials.gov Identifier: NCT02452723). Twelve patients, divided into three cohorts of four, are being recruited in this 12 month study with a 5 year long-term follow-up. The primary endpoint is the assessment of safety and any adverse events related to the administration of ISC-hpNSC. Secondary endpoints evaluate clinical responses compared to baseline using several neurological assessments, ^{18}F-dopa PET and MRI. We expect that ISC-hpNSC will be safe, well tolerated and might even slow down PD progression.

Disclosures: **I. Garitaonandia:** A. Employment/Salary (full or part-time): International Stem Cell Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); International Stem Cell Corporation. **R. Gonzalez:** A. Employment/Salary (full or part-time): International Stem Cell Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); International Stem Cell Corporation. **M. Poustovoitov:** A. Employment/Salary (full or part-time): International Stem Cell Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); International Stem Cell Corporation. **T. Abramihina:** A. Employment/Salary (full or part-time): International Stem Cell Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); International Stem Cell Corporation. **A. Noskov:** A. Employment/Salary (full or part-time): International Stem Cell Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); International Stem Cell Corporation. **T. Christiansen-Weber:** A. Employment/Salary (full or part-time): International Stem Cell Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); International Stem Cell Corporation. **G. Sherman:** A. Employment/Salary (full or part-time): International Stem Cell Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); International Stem Cell Corporation. **A. Semechkin:** A. Employment/Salary (full or part-time): International Stem Cell Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); International Stem Cell Corporation. **L. Laurent:** None. **J. Elsworth:** None. **E. Snyder:** None. **D. Redmond:** F. Consulting Fees (e.g., advisory boards); International Stem Cell Corporation. **R.A. Kern:** A. Employment/Salary (full or part-time): International Stem Cell Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); International Stem Cell Corporation.

Nanosymposium

287. Transplants and Other Treatments of Parkinson's Disease

Location: SDCC 1B

Time: Monday, November 14, 2016, 8:00 AM - 11:00 AM

Presentation Number: 287.02

Topic: C.03. Parkinson’s Disease

Support: Leopold Korn and Michael Korn Professorship (CRF)

Title: Transplants of human fetal dopamine neurons into putamen of Parkinson patients survive for at least 27 years without immunosuppression

Authors: *C. R. FREED[1], R. E. BREEZE[2], B. A. SYMMES[1], S. FAHN[3], D. EIDELBERG[4], W. ZHOU[1];
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Abstract: We have shown that fetal dopamine cell transplants significantly improve objective signs of Parkinson's disease in direct relation to the preoperative response to L-dopa. From our total of 61 patients transplanted between 1988 and 2000, we have had the opportunity to study postmortem brain of 16 subjects who have died 7 months to 27 years after transplant. Dopamine neurons were identified immunohistochemically in transplant tracks using a primary antibody to tyrosine hydroxylase and a secondary antibody labeled with fluorescent FITC. After identification of dopamine neurons by green fluorescence, pigment density was determined by measuring white light transmission through the cytoplasm of each cell. Twenty-five cells in each transplant track were measured. Pigment density increased linearly in transplanted dopamine neurons during the years after transplant. Parallel studies were performed on substantia nigra of children and adults who did not have Parkinson's disease. A similar age-related accumulation of pigment was seen. Despite the absence of immunosuppression at the time of surgery and in subsequent years, every fragment of human embryonic mesencephalon showed surviving dopamine neurons, indicating that the immune system has not destroyed any transplant. While a few isolated Lewy-body like inclusions were occasionally observed, all transplants showed extensive fiber outgrowth into striatum, indicating that the transplanted dopamine neurons were physiologically robust. The number of surviving dopamine neurons was not related to the time since transplant, indicating there is no progressive loss of neurons with time. The largest number of surviving dopamine neurons was found in a patient who died at age 85, ten years after transplant at age 75, indicating that age is not a factor affecting survival of dopamine neurons.. We conclude that transplanted human dopamine neurons undergo morphologic evolution essentially identical to that seen in the normal substantia nigra. This result suggests that the neurotrophic environment of the putamen of patients with idiopathic Parkinson's disease is normal since it can support long term development of transplanted dopamine neurons.

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Nanosymposium

287. Transplants and Other Treatments of Parkinson's Disease

Location: SDCC 1B

Time: Monday, November 14, 2016, 8:00 AM - 11:00 AM

Presentation Number: 287.03

Topic: C.03. Parkinson's Disease

Support: the Swedish Research Council

BAGADILICO-Excellence in Parkinson and Huntington Research

the Swedish Parkinson Foundation

the Torsten Söderberg Foundation

the Strategic Research Area MultiPark

Title: Extensive graft derived dopaminergic innervation in degenerating Parkinsonian brain 24 years after transplantation

Authors: *W. LI[1], E. ENGLUND[2], H. WIDNER[3], B. MATTSSON[4], D. VAN WESTEN[5], J. LÄTT[5], S. REHNCRONA[6], P. BRUNDIN[7], A. BJÖRKLUND[8], O. LINDVALL[3,9], J.-Y. LI[1]; [1]Wallenberg Neurosci. Ctr., Lund, Sweden; [2]Div. of Oncology and Pathology, Lund Univ. Hosp., Lund, Sweden; [3]Div. of Neurology, Lund Univ. Hosp., Lund, Sweden; [4]Neurobio. Unit, Wallenberg Neurosci. Centre, Lund Univ., Lund, Sweden; [5]Ctr. for Med. Imaging and Physiology, Lund Univ. Hosp., Lund, Sweden; [6]ivision of Neurosurgery, Lund Univ. Hosp., Lund, Sweden; [7]Ctr. for Neurodegenerative Science, Van Andel Res. Inst., Grand Rapids, MI; [8]Neurobio. Unit, Wallenberg Neurosci. Center, Lund Univ., Lund, Sweden; [9]Lund Stem Cell Center, Lund Univ. Hospital, Lund, Sweden, Lund, Sweden

Abstract: Parkinson's disease is the most common movement disorder. Here we describe the histopathological analysis of a unique patient with Parkinson's disease who underwent unilateral cell transplantation in the putamen with human embryonic mesencephalic tissue at 24 y before death. The patient enjoyed major clinical benefits for at least a decade after transplantation. After a quarter of a century, complete graft-derived dopaminergic reinnervation was still evident in the transplanted putamen. α-Synuclein-positive inclusions, some with the appearance of typical Lewy bodies, were present in 11-12% of the grafted dopaminergic neurons, reflecting spread of pathology from the host brain to the transplant. The clinical improvements were gradually lost from 14 y posttransplantation, indicating that even extensive graft-derived dopaminergic reinnervation loses its efficacy in a severely degenerating brain.

Disclosures: W. Li: A. Employment/Salary (full or part-time): Neural Plasticity and Repair Unit, Wallenberg Neuroscience Center, Department of Experimental Medical Science, Lund

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Nanosymposium

287. Transplants and Other Treatments of Parkinson's Disease

Location: SDCC 1B

Time: Monday, November 14, 2016, 8:00 AM - 11:00 AM

Presentation Number: 287.04

Topic: C.03. Parkinson’s Disease

Support: Parkinson's disease Foundation

NIH RO1 NS 32842

Title: Robust dopamine graft survival and normalized dopaminergic innervation does not obligate clinical recovery in a patient with Parkinson’s disease

Authors: *J. H. KORDOWER[1,2], C. G. GOETZ[1], Y. CHU[1], G. M. HALLIDAY[3], D. J. MARMION[1], D. A. NICHOLSON[1], T. MUSIAL[1], A. J. STOESSL[4], V. SOSSI[4], T. B. FREEMAN[5], C. W. OLANOW[6];
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Abstract: Dopaminergic cell replacement strategies have been proposed and employed to treat the motor symptoms of Parkinson’s disease (PD). The goal has been robust graft survival and dopaminergic striatal reinnervation coupled with clinical improvement. This report documents a unique case that has come to autopsy sixteen years following fetal nigral grafting. This 55year old female PD patient with tremor predominant PD and a strong response to levodopa received solid pieces of 4 fetal mesencephalon aged 6.5-9 weeks post-conception into the post commissural putamen as part of our double blind NIH sponsored fetal transplant trial (Olanow et al., 2003). Clinically this patient was followed preoperatively, and for 31 months post-

operatively in a blinded fashion as well as for additional 12 years in an open label fashion. Fluorodopa positron emission tomography was also performed preoperatively and at 1 and 2 years post-grafting. Eight-years post-transplantation this patient received bilateral subthalamic nucleus deep brain stimulation to control severe dyskinesias. This patient died 16-years post-transplantation and autolysis time was 6h. This patient never demonstrated any meaningful clinical benefit following grafting as determined by UPDRS in “off” (primary outcome measure) or “on” or on any other secondary but minor “off-medication” diphasic dyskinesia were observed. Robust increases in fluorodopa were seen in the putamen but not caudate bilaterally post grafting. Post-mortem analyses revealed the largest surviving grafts (>300,000 TH positive grafted cells on both sides) and the densest and most extensive putamenal TH-ir fiber innervation reported in patients to date. Reciprocal synaptic contacts between graft and host were seen ultrastructurally. Serine 129-phospho alpha synuclein positive grafted cells were seen in the right (10.7%) and and left (27%) putamen with a subpopulation displaying thioflavin positivity. Normal melanin formation in relative to the age of the cells was observed. Extensive dopaminergic graft viability and robust host innervation did not induce a short- or long-lasting positive clinical response in this patient and suggests that the goal of robust graft survival and striatal reinnervation is not always sufficient to translate into clinical benefit in PD.

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Nanosymposium

287. Transplants and Other Treatments of Parkinson's Disease

Location: SDCC 1B

Time: Monday, November 14, 2016, 8:00 AM - 11:00 AM

Presentation Number: 287.05

Topic: C.03. Parkinson’s Disease

Support: National Center for Advancing Translational Sciences, through grant UL1TR000117

Tom Dupree for Parkinson's Disease Research

Pro's Players Fore Parkinson's

Gifts to the Brain Restoration Center

Title: Combining deep brain stimulation surgery with autologous peripheral nerve graft to the nucleus basalis of Meynert to treat non-motor symptoms in Parkinson's disease

Authors: J. E. QUINTERO[1,2], J. T. SLEVIN[3,2], A. J. ANDERSON-MOONEY[3,2], J. A. GURWELL[3,2], W. KIMMERER[2], *G. A. GERHARDT[1,2], C. G. VAN HORNE[1,2,4];
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Abstract: We recently published a study describing the implantation of autologous peripheral nerve grafts (APNG) to the substantia nigra at the time of deep brain stimulation (DBS) surgery. However, addressing non-motor symptoms of Parkinson's disease remains a challenge. Peripheral nerve is rich in Schwann cells, and after injury, Schwann cells can produce neurotrophic factors. In a 24-month, open-label, single-center, Phase I trial to determine safety and feasibility, we implanted APNG to the nucleus basalis of Meynert (NBM) in 6 individuals with idiopathic Parkinson's disease and declining cognitive performance. DBS electrodes were placed in the globus pallidus internus and a unilateral implantation of sural nerve to the NBM. Unified Parkinson's disease rating scale (UPDRS), neurocognitive performance, speech, and gait were measured at baseline and every 6 months after surgery. Adverse event profiles for all 6 participants were consistent with standard DBS surgery. The first individual who completed the 6-month time point had UPDRS Part III (motor) score OFF medication/stimulation of 37 compared to baseline (40) and an ON score of 24 compared to 30 at baseline. Speech showed an increase in the harmonic to noise ratio parameters. Gait velocity increase by 20%. Neurocognitive performance improved in the executive functioning and language domains. Early findings show that APNG delivery to the NBM was safe and feasible. Monitoring the ongoing outcome of the other participants will be critical to fully assess this potential new therapy.

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Nanosymposium

287. Transplants and Other Treatments of Parkinson's Disease

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Title: Sargramostim improves regulatory T cell and motor functions in a Phase 1 randomized clinical trial for Parkinson's disease

Authors: H. E. GENDELMAN[1], Y. ZHANG[1], P. SANTAMARIA[6], K. E. OLSON[1], C. R. SCHUTT[1], D. BHATTI[2], B. L. DYAVAR SHETTY[1], Y. LU[1], K. A. ESTES[1], E. HEINRICHS-GRAHAM[1], L. LARSON[3], J. L. MEZA[4], M. FOLLETT[1], E. FORSBERG[7], G. SIUZDAK[8], T. W. WILSON[5], C. PETERSON[3], *R. MOSLEY[1];
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Abstract: Inflammatory responses herald the onset and progression of Parkinson's disease (PD). Recent studies support the idea that both are controlled by innate microglial and the functional balance of effector T cells (Teffs) and regulatory T cells (Tregs). The mechanism(s) by which T cells modulate and affect clinical outcomes in PD remains enigmatic. In a randomized, placebo-controlled, double-blinded phase 1 trial, sargramostim (human granulocyte-macrophage colony stimulating factor, GM-CSF, Leukine) was tested as a potential PD therapy (ClinicalTrials.gov: NCT01882010). Herein, 20 PD patients and 17 age-matched non-parkinsonian control subjects were enrolled and monitored for 2 months prior to treatment initiation. PD patients exhibited reduced levels of CD39+ Tregs and Treg function, but increased Teff subset frequencies compared to non-parkinsonian controls. The 20 PD patients were randomized into 2 groups of 10 to self-administer placebo (saline) or drug at 6 μg/kg/day s.c. for 56 days. Primary study outcome was safety as measured by adverse event (AE) monitoring, physical examination, blood counts, blood chemistry, anti-GM-CSF antibody, and motor deficit severity by Unified PD Rating Scale (UPDRS) part III scores. Secondary outcomes included T cell phenotypes, Treg function, and cortical motor activities. Sargramostim treatment was generally well-tolerated in PD populations with expectedly higher AE rates of increased WBC, injection site reactions, and upper extremity/bone pain. AE frequencies in other categories were not significant, and only a hypersensitivity reaction was deemed drug-related. The overall severity index was greater in sargramostim group. UPDRS scores did not worsen during treatment; in fact, after 6 and 8 weeks on sargramostim, scores improved compared to controls. This paralleled in sargramostim-treated patients, improved cortical motor activities as measured by magnetoencephalography, greater numbers of Tregs and subsets, higher Treg activity, and levels of serum tryptophan metabolites that are conducive to Treg induction; namely higher levels of L-kynurenine and diminished levels of serotonin. Levels of Teffs remained unchanged. Transcriptomic analysis of Treg- and Teff-depleted CD4+ T cells indicated complex profiles of pro- and anti-inflammatory gene expression in patients treated with sargramostim compared to placebo. Sargramostim-mediated

modulation of Teff and Treg populations, Treg activity, and improved motor function are supportive that changes in T cell polarity affect brain-immune axes, and present a novel pathway that corrects aberrant immune responses and neurodegenerative outcomes in PD.

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Nanosymposium

287. Transplants and Other Treatments of Parkinson's Disease

Location: SDCC 1B

Time: Monday, November 14, 2016, 8:00 AM - 11:00 AM

Presentation Number: 287.07

Topic: C.03. Parkinson’s Disease

Title: Parkinson's, oxidative stress and osteopathic manipulation

Authors: N. MIKHAIL[1], S. M. ZAKHARY[1], G. TORRES[1], A. LEDER[2], J. DONOGHUE[2], J. D. MANCINI[2], S. YAO[2], *J. R. LEHESTE[1];
[1]Biomed. Sci., [2]Osteo. Manipulative Med., NYIT Col. of Osteo. Med., Old Westbury, NY

Abstract: Mitochondrial dysfunction and an ensuing surge of oxidative and nitrosative radicals are key pathogenic features of Parkinson’s disease (PD). While prolonged periods of excessive free radical stress are associated with increased somatic dysfunction, cause and effect are not yet fully established for PD. Clinically, increased stiffness, shaking while at rest, slowing of movement and postural instability are cardinal features in the progression of PD and are major contributors to a progressively sedentary lifestyle. There is increasing evidence, that large sedentary skeletal muscles are major contributors to the radical burden observed in PD. Exercise and dietary corrections are commonly recommended lifestyle adjustments staving off free radicals and muscle wasting in senior adults. In PD, however, these recommendations are often not applicable leaving affected individuals in jeopardy. Osteopathic manipulative medicine (OMM) is a form of hands-on treatment that can be applied to resolve musculoskeletal restrictions which burden PD patients. The objective of the present study is to test whether a set OMM protocol (PARK-OMM), designed to treat mobilize the spine and extremities in PD subjects, can sufficiently counter the PD-related radical burden leading to an overall improved state of health. For a clinical pilot 11 PD individuals were accepted into a randomized controlled trial with crossover-design comparing the application of PARK-OMM to counseling. Outcomes were determined as changes in postural stability and balance, motor function as well as serum

levels of oxidative stress biomarkers to protein, lipids and nucleic acids. Results indicate PARK-OMM as an effective complementary treatment to improving structural and functional aspects of PD as well as the associated systemic oxidative stress. While these findings require further research in a larger cohort of subjects, underlying cellular and molecular mechanisms are being further investigated in an animal model of progressive PD (aphakia mice).

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Nanosymposium

287. Transplants and Other Treatments of Parkinson's Disease

Location: SDCC 1B

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Presentation Number: 287.08

Topic: C.03. Parkinson’s Disease

Support: RiMED Foundation

Blechman Family Foundation

DSF Charitable Foundation

American Parkinson Disease Association

NIH grant NS095387

Title: A central role for LRRK2 in idiopathic Parkinson disease

Authors: ***R. DI MAIO**, E. K. HOFFMAN, E. ROCHA, J. MCCOY, E. A. BURTON, T. G. HASTINGS, J. T. GREENAMYRE;
Neurology, Pittsburgh Inst. for Neurodegenerative Dis., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Mutations in LRRK2 cause familial Parkinson disease (PD) and, in some populations, may account for up to 40% of all cases. The LRRK2 gene locus also contains a risk factor for ‘idiopathic’ PD (iPD); however, the role of LRRK2 in typical iPD is not clear. While the mechanism(s) by which mutant LRRK2 causes neurodegeneration are not clear, it is believed that disease-causing mutations may be associated with increased kinase activity. Assessment of the kinase activity state of LRRK2 under various conditions has also been problematic and somewhat cumbersome, although there appears to be a growing consensus that autophosphorylation at Ser1292 correlates with activity. Phosphoserine1292 (pS1292) has generally been detected by western blotting rather than immunocytochemistry, which limits

anatomical or cellular resolution. The activity of LRRK2 is also regulated by its interaction with 14-3-3 proteins, whose binding to LRRK2 is associated with reduced activity. The interaction between LRRK2 and 14-3-3 has generally been assessed by co-immunoprecipitation. We have developed a pair of novel proximity ligation assays with excellent anatomical resolution that can rapidly provide information regarding activation state, cellular localization and physiological regulators of LRRK2. Assays have been validated using CRISPR/Cas9 engineered $LRRK2^{-/-}$ and $LRRK2^{G2019S/G2019S}$ HEK and SH-SY5Y cells. The assay is based on (i) S1292 phosphorylation and (ii) dissociation of 14-3-3 from LRRK2. Using this and other assays, we have compelling evidence that (i) LRRK2 is activated in nigrostriatal neurons in iPD; (ii) sublethal concentrations of rotenone activate LRRK2; (iii) α-synuclein overexpression activates LRRK2; (iv) rotenone-induced S129 phosphorylation of α-synuclein is LRRK2-dependent; (v) rotenone-induced inhibition of glucocerebrosidase is LRRK2-dependent. Together, our results suggest that LRRK2 plays a central role in idiopathic PD. Supported by the RiMED Foundation, Blechman Family Foundation, the DSF Charitable Foundation, the American Parkinson Disease Association and NIH grant NS095387.

Disclosures: **R. Di Maio:** A. Employment/Salary (full or part-time): University of Pittsburgh. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ri.MED Foundation. **E.K. Hoffman:** None. **E. Rocha:** None. **J. McCoy:** None. **E.A. Burton:** None. **T.G. Hastings:** None. **J.T. Greenamyre:** None.

Nanosymposium

287. Transplants and Other Treatments of Parkinson's Disease

Location: SDCC 1B

Time: Monday, November 14, 2016, 8:00 AM - 11:00 AM

Presentation Number: 287.09

Topic: I.05. Biomarker and Drug Discovery

Title: Interrogative Biology identifies p53-inducible gene 3 (PIG3) as a potential contributor to LRRK2-mediated neuronal cell death in Parkinson's Disease

Authors: J. CHAUFTY[1], S. PHAT[1], J. RANJAN[1], K. HA[1], S. KIM[2], S. AKELLA[1], R. DEGAONKAR[1], C. BARLOW[2], *K. THAPA[1], M. KIEBISH[1], S. GESTA[1], B. SCHUELE[2], V. K. VISHNUDAS[1], N. R. NARAIN[1], R. SARANGARAJAN[1], P. NARAIN[1], J. LANGSTON[2]; [1]Berg, LLC, Framingham, MA; [2]Parkinson's Inst., Sunnyvale, CA

Abstract: Mutations in the *LRRK2* gene represent a major genetic risk factor for both non-familial and familial Parkinson’s disease (PD). However, the mechanistic link between LRRK2 variants and PD-related neurodegeneration remains unclear. To determine the biological drivers of LRRK2 pathology in PD, primary skin fibroblasts from PD patients harboring the

LRRK2^{G2019S} mutation, idiopathic PD patients, and their matched mutation-negative controls were compared using Berg's Interrogative Biology® consisting of a functional proteomics platform (activity dependent protein enrichment, phospho-proteomics and global proteomics) paired with Bayesian Network Inference analytics. This proprietary data-agnostic discovery platform identified PIG3, an oxidoreductase that is linked to ROS production in p53-mediated cell death, as a key differential protein between LRRK2-mediated PD and healthy patients. Proteomics analysis of fibroblasts from LRRK2-PD patients confirmed an increase in basal PIG3 expression that correlated with upregulation of MKK3/6 activity, p38 MAPK phosphorylation, and accumulation of p53. Given the role of LRRK2 in MAPK signaling, oxidative stress and p53 in PD, PIG3 represents a promising mechanistic link between LRRK2 and PD risk. Thus, the role of PIG3 in a neuronal model of PD was examined. In human dopaminergic SH-SY5Y cells, rotenone and 6-hydroxydopamine (6-OHDA) induced an increase in PIG3 expression that correlated with apoptosis. Notably, siRNA directed against PIG3 significantly reduced rotenone and 6-OHDA cell death in SH-SY5Y cells. Furthermore, inhibition of p38 MAPK by SB203580 similarly reduced apoptosis in these models and importantly was associated with attenuated induction of PIG3 expression. Taken together, our findings demonstrate that PIG3 contributes to apoptosis in a neuronal model of PD and may explain the mechanism behind LRRK2^{G2019S} and putative hypersensitivity to environmental neurotoxins. This study also showcases the predictive power of Interrogative Biology® as a discovery tool for mining fundamental biology from peripheral cells to better understand inherent molecular drivers in neurodegenerative diseases.

Disclosures: The Disclosure Block has exceeded its maximum limit. Please call Tech support at (217) 398-1792 for more information.

Nanosymposium

287. Transplants and Other Treatments of Parkinson's Disease

Location: SDCC 1B

Time: Monday, November 14, 2016, 8:00 AM - 11:00 AM

Presentation Number: 287.10

Topic: C.03. Parkinson's Disease

Support: UGC

Title: Combination of curcumin and ellagic acid mitigates rotenone induced oxidative and mitochondrial deficits in parkinson's disease (pd) in mice

Authors: *A. JUVEKAR, D. KHATRI;
Dept. of Pharmaceut. Sci. and Technol., Inst. of Chem. Technol., Mumbai, India

Abstract: Introduction: Curcumin and Ellagic acid (EA), are natural polyphenolic and bioactive compounds and exhibit pharmacological activities including potent antioxidant, anticancer, anti-inflammatory effects. They are used in cardiovascular and neurodegenerative disorders.
Aim and Objective: The aim was to explore the neuroprotective role of Combination of curcumin and EA against rotenone induced oxidative and mitochondrial dysfunction.
Methods: Chronic administration of rotenone (1mg/kg i.p) for a period of three weeks significantly impaired oxidative defense (Decreased activity of superoxide dismutase, catalase and reduced glutathione level) and mitochondrial Complex-II-Succinate Dehydrogenase (SDH), ComplexIII-MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-H-tetrazoliumbromide) mitochondrial enzymes activities as compared to normal control group in the brain of mice.
Results: Three weeks of treatment (50, 100 and 200 mg/kg, p.o) significantly improved oxidative damage and mitochondrial enzyme complex activities as compared to negative control group. Also, EA restored motor deficits and enhanced the activities of antioxidant enzymes suggesting its antioxidant potential *in vivo*.
Conclusion : The findings suggest neuroprotective role of EA against rotenone induced PD and offers justification for the therapeutic prospective in the management of PD.
Key words: -Curcumin; Ellagic acid; rotenone; neuroprotective; parkinson's disease; mitochondrial dysfunction; oxidative stress

Disclosures: A. Juvekar: None. **D. Khatri:** None.

Nanosymposium

287. Transplants and Other Treatments of Parkinson's Disease

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Presentation Number: 287.11

Topic: C.03. Parkinson’s Disease

Support: SSHRC

Rozsa Foundation

Parkinson Alberta

Title: Dance and Parkinson's disease: a community-based dance program improves performance in functional daily activities in people with Parkinson's disease.

Authors: *A. FOROUD[1,2], A. P. FLYNN[2];
[1]Dept. of Psychology, The Univ. of Lethbridge, Lethbridge, AB, Canada; [2]Dept. of Creative and Performing Arts: Div. of Dance, The Univ. of Calgary, Calgary, AB, Canada

Abstract: Studies of dance and Parkinson's disease (PD), an emerging topic in neuroscience, are demonstrating that dance may be an effective rehabilitative tool for PD. After participation in a dance program, people with PD, a neurodegenerative disease that leads to the loss of movement initiation and motor dysfunction, have shown improvement in standardized tests of balance, gait, mobility, and quality of life (QOL) (Hackney et al., 2007, 2009, 2010; Heiberger et al., 2011). Studies have also shown low attrition rates, and that outcomes on qualitative measures from interviews are positive even in groups with marginal improvement (Houston 2011; Houston & McGill, 2012; Westheimer et al., 2015). Thus experiences gained from a dance program likely have a greater impact on PD than what is captured with standardized tests. Indeed, it is not yet known how the effects of dance translate into daily function. The purpose of this study is to evaluate how participation in a community-based dance program influences daily activities essential for maintaining independent living. Thirty participants (18 PD, 12 healthy controls) were video-recorded while performing a series of tasks that reflect functional behaviour in a natural setting, such as pouring a drink, getting dressed, and moving about in a room. Five months later, participants repeated the tasks having completed the weekly Dancing Parkinson's YYC program offered at Decidedly Jazz Danceworks. Detailed frame-by-frame analysis was made using Laban Movement Analysis and Eshkol Wachman Movement Notation. Movement patterns derived from the notated scores where then quantified for each participant. Preliminary results (first 9 PD and 9 controls) using repeated measures ANOVA show significant differences between the PD and control groups and that both groups improved after completing the dance program. For e.g., in a task involving buttons, both groups improved in grasping the buttons and orienting their limbs in relation to the fabric, with performance in the PD group matching that of the control group prior to the dance program ($p<0,0001$). The PD group also improved in articulation of the digits, wrists, elbows and shoulders ($p<0.005$) suggesting a gain in distal movement initiation. These findings show participation in a weekly dance program facilitates fine motor skills and indicate how dance may be beneficial for daily functional activities. This study is part of a larger project in progress on emotional expression and social communication, QOL, and performance in the dance class thus bridging quantitative and qualitative methodology in order to increase our understanding on how dance effects people with PD.

Disclosures: A. Foroud: None. **A.P. Flynn:** None.

Nanosymposium

287. Transplants and Other Treatments of Parkinson's Disease

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Presentation Number: 287.12

Topic: C.03. Parkinson's Disease

Support: NIH grant AG023630

South Carolina Spinal Cord Injury Research Fund

Title: A method to describe relative 3D motion between the rear and front body segments of rodents: Application om neurodegenerative diseases

Authors: *T. KARAKOSTAS[1], L. MIDDAUGH[2], A.-C. GRANHOLM[2];
[1]Motion Analysis Ctr., Rehabil. Inst. of Chicago, Chicago, IL; [2]Neurosciences and Psychiatry, and The Ctr. on Aging, Med. Univ. of South Carolina, Charleston, SC

Abstract: Parkinson disease (PD) is a neurodegenerative disorder where only recently 3D motion analysis has been used to track progression and the effects of surgical or pharmaceutical treatment paradigms. Because most drug trials for PD involve pre-assessment in models of the disease, translatable and accurate motion capture methods need to be developed for mouse/rat models. Our group was the first to report, on the feasibility of using an optical motion capture system to study aging related changes on a mouse while walking, and on a three body segment rodent model comprised of the head, the anterior and hind body segments with application to PD. The model quantified the distinct 3D motion of each segment. However, like human motion, it may be of greater interest if the motions of the rodent's body segments are described relative to each other. Consequently, the purpose of this study is to propose a model to describe the 3D motion of the rodent's anterior body segment relative to its rear body segment.
Briefly, for the purposes of this report, one $GDNF^{+/-}$ mouse and a wildtype littermate (WT) were anesthetized and 2mm diameter retro reflective markers were fixed to their hair via hypoallergenic double-sided tape. The markers were placed on the anterior rim of the pelvis bilaterally, the greater tubercle bilaterally, and the middle of the back at L4 to define the posterior and anterior body segments. A 6 camera optical capture system captured at 240Hz the 3D position of each marker in time. Rodents walked freely in a 4 feet constrained walkway. Our model was implemented in a custom computer program. For this report, the model ultimately determined the relative angles between the rear and anterior body segments assessing flexion/extension, tilting and spin. The overall forward velocity of forward progression of the rodent's body was estimated from the marker positioned at L4.
Table 1 shows the results of the model.

Table 1

Parameter	**Sagittal/Pitch** (Deg) Max Fl(+)/Ext(-)	**Frontal/Yaw** (Deg) Max Spin Rt (+)/Lf(-)	**Transverse/Roll** (Deg) Max Tilt Right (-)/Left (+)	**Velocity (max): m/s**
GDNF$^{+/-}$	50/58	10/-4	-18/-2	0.13
Wildtype	54/76	8/-22	-13/21	0.24

The WT walked almost twice as fast as the GDNF$^{+/-}$. In the sagittal and transverse planes the motion of the WT rodent was also greater than that of the GDNF$^{+/-}$. Our results correlate very well with the current knowledge about the motor deficits of individuals with PD. This is the first model to treat distinctly the rear from the front body-segments. Our results appear to be clinically valid.

Disclosures: T. Karakostas: None. **L. Middaugh:** None. **A. Granholm:** None.

Nanosymposium

288. Neural Coding in the Somatosensory System

Location: SDCC 2

Time: Monday, November 14, 2016, 8:00 AM - 11:00 AM

Presentation Number: 288.01

Topic: D.03. Somatosensation: Touch

Support: DARPA contract N66001- 10- C- 4056

Title: The dynamics of neural signals about contact pressure - implications for bionic hands

Authors: *S. J. BENSMAIA, T. CALLIER, H. P. SAAL, B. DELHAYE;
Dept. of Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: Tactile signals from the hand are critical to our ability to dexterously manipulate objects. Information about contact pressure is encoded in the responses of mechanoreceptive afferents and of neurons in primary somatosensory cortex (S1). This information allows us to exert enough pressure on objects so that we do not drop them but not much more than that. In the present study, we sought to quantitatively examine how information about pressure is encoded in

the responses of populations of primary afferents and of S1 neurons. To this end, we measured neuronal responses evoked when ramp-and-hold indentations were delivered to the glabrous skin of the hand at different pressure levels. We found that the phasic responses evoked during stimulus onset and offset are much stronger than is the sustained response to the hold phase of the indentation. This phenomenon was observed in the nerve, which is not surprising given that two of the three afferent types only respond to the ramp phases and the third responds more strongly to the on-ramp than it does to the hold. However, this phenomenon was even more pronounced in S1, where most neurons did not respond to the hold phase and those that did, did so very weakly. These results suggest that information about contact pressure is primarily conveyed during the contact transients and that these phasic signals likely play an important role in guiding object manipulation.
The default approach to restoring somatosensation in upper-limb neuroprostheses is to implement a linear mapping between the output of sensors on the prosthesis and strength of electrical stimulation delivered to the nerve or to the brain. Our findings suggest that the mappings should not only include the time varying pressure but also its derivative, and that the latter will more strongly drive the electrical stimulus.

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Nanosymposium

288. Neural Coding in the Somatosensory System

Location: SDCC 2

Time: Monday, November 14, 2016, 8:00 AM - 11:00 AM

Presentation Number: 288.02

Topic: D.03. Somatosensation: Touch

Support: The Gretel and Gordon Bootes Medical Research Foundation

Title: Activity hotspots evoked from peripheral nerves are asymmetrically organised across the dorsal column nuclei surface

Authors: ***A. J. LOUTIT**, T. MADDESS, J. R. POTAS;
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Abstract: A key requirement for developing sensory prostheses is adequate knowledge of the sensory representation in the target region. The brainstem dorsal column nuclei (DCN) is one such potential target. While somatotopography of afferents has been reported in the cross-sectional plane of the DCN, it remains unclear if such organisation translates to activity on the DCNs surface. To investigate the topography of DCN surface activity, we mapped somatosensory potentials evoked from electrical stimulation (0.01 ms; 0.53-1.1 mA) of left and

right sural (LSN, RSN) and peroneal nerves (LPN, RPN) of 8 week old urethane anaesthetised male Wistar rats. We recorded 11 repeated trials with a platinum wire electrode at 25 locations across the DCN surface. Signals were filtered offline to extract and quantify low-frequency (LF, 200 Hz low-pass) and high-frequency (HF, 550-3300 Hz) components. A surface was interpolated between recording positions: LF signals quantified as the peak-to-peak amplitude; HF signals quantified as the integral of the rectified signal between 5-15 ms post-stimulus. Mean 'hotspot' locations were found on the surface of the ipsilateral gracile nuclei. Contralateral nerve activity was significantly different in location. RPN and RSN, but not LPN and LSN, LF and HF hotspot regions were significantly different ($p<0.05$), indicating asymmetrical neural activity organisation. In addition to primary hotspots (HS1), the majority of animals displayed a secondary hotspot (HS2), separated by a saddle region (SR) in LF and/or HF signals for all 4 nerve evoked responses. Of those animals expressing dual hotspot responses, compared to HS1, HS2 was significantly reduced by 20% (LF: 80.3 ± 4.5 % of that of HS1[100.8 ± 0.6], $p = 0.0004$; HF: 81.3 ± 3.4 % of that of HS1[100.4 ± 0.3], $p < 0.0001$), and SRs were significantly reduced by 35% (LF: 64.1 ± 5.6 %, $p = 0.0001$; HF: 64.4 ± 3.9, $p < 0.0001$). While activity was reduced at the SR, the level of activity at this point remained significantly greater than regions of minimal activity within the recording field (LF: 0.6 ± 0.7%; HF: 1.5 ± 0.8%). There was no obvious pattern for HS1, HS2 or SR rostrocaudal/mediolateral locations; in some animals HS1 was located more rostral or more caudal with respect to HS2. For HF signals, there was a significant rostrocaudal separation between LSN HS1 and HS2 responses ($p = 0.004$), and for LF signals, RPN HS1 was significantly more lateral compared to both RSN HS1 and HS2 ($p = 0.038$). These findings suggest that peripheral nerves have defined response hotspot locations over the ipsilateral DCN surface, most nerves evoked activity with two peaks, and these responses are not symmetrically arranged.

Disclosures: **A.J. Loutit:** None. **T. Maddess:** None. **J.R. Potas:** None.

Nanosymposium

288. Neural Coding in the Somatosensory System

Location: SDCC 2

Time: Monday, November 14, 2016, 8:00 AM - 11:00 AM

Presentation Number: 288.03

Topic: D.03. Somatosensation: Touch

Support: The Gretel and Gordon Bootes Medical Research Foundation

Title: Peripheral nerves evoke reproducible signals with machine learnable features in the dorsal column nuclei

Authors: *J. R. POTAS[1], A. J. LOUTIT[1], S. J. REDMOND[2], G. STUART[1], J. W. MORLEY[3,2], T. MADDESS[1];
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Abstract: The brainstem dorsal column nuclei (DCN) may be an ideal neuroprosthetic target for restoring tactile somatosensation. Surface recordings are a practical approach for long term brain-machine interfaces, but little is known about DCN surface potentials. We therefore aimed to characterise these potentials. We mapped and characterised gracile nuclei somatosensory potentials evoked from electrical stimulation (0.01 ms; 0.53-1.1 mA) of all fibres in left and right sural (LSN, RSN) and peroneal nerves (LPN, RPN) of 8 week old urethane anaesthetised male Wistar rats. 11 repeated trials were recorded from each nerve. Responses from activity hotspot regions on the DCN surface contained prominent positive (P1), followed by negative (N1) waves. P1 was significantly shorter, N1 longer, and the P1N1 slope reduced in peroneal compared to sural nerves, but there were no differences between the same contralateral nerves. Surface potential waveform similarity was measured by a bootstrapping mean correlation coefficient method. Response similarity was high among signals derived from the same nerve of the same animal (smallest similarity, LSN, 0.73 ± 0.07; largest similarly, LPN, 0.93 ± 0.02), from the same nerves across different animals (smallest similarity, RSN, 0.58 ± 0.03; largest similarly, LPN, 0.88 ± 0.02), and the same contralateral nerves from different animals (sural, 0.61 ± 0.03; peroneal, LPN, 0.80 ± 0.02). Signals were filtered offline to extract and examine low-frequency (LF, 200 Hz low-pass) and high-frequency (HF, 550-3300Hz) features. Most significant were that peroneal nerves had a longer LF signal duration ($p<0.01$) and more HF events ($p<0.001$) with greater signal energy ($p<0.001$) between 15-65 ms post-stimulus compared to sural nerve responses. We then took 5 key features to train and test a supervised back-propagation neural net algorithm to generalise a feature set derived from surfaced potentials recorded from a 7-electrode array on the surface of the brainstem. The neural network was able to predict, with 96.3 ± 0.3% accuracy, the correct nerve when training (70%), validation (15%) and testing (15%) data sets were randomly allocated from 7 animals, or 80.0 ± 2.0% accuracy when training (70%) and validation (30%) was restricted to 4 animals and testing (100%) performed on the remaining 3 animals (n=10 repeated training/validation and testing cycles for both approaches). These findings demonstrate that machine learning can accurately resolve the location of peripheral sensory input by learning a set of DCN signal features derived from an array of surface potentials. This indicates that the DCN may be a practical target for a somatosensory prosthesis.

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Nanosymposium

288. Neural Coding in the Somatosensory System

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Topic: D.03. Somatosensation: Touch

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Title: Perceptual and neural effects of cuneate nucleus microstimulation in primates

Authors: T. H. LUCAS, S. Y. SRITHARAN, I. M. PLANELL-MENDEZ, *A. G. RICHARDSON;
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Abstract: The cuneate nucleus (CN) is the first supraspinal site of hand and arm somatosensory processing. We recently developed the first chronic neural interface to the CN in monkeys to test whether the CN is a suitable target for encoding somatosensory information after spinal cord injury. Our initial studies have focused on the detectability and discriminability of CN microstimuli in healthy animals and on evoked downstream activity in the primary somatosensory cortex (S1). Four macaques were chronically implanted with 32-channel microelectrode arrays targeting the CN at the level of the obex. Two of these animals were also implanted with chronic arrays in S1. Single CN stimulus pulses consistently evoked a sequence of fast excitation, inhibition, and rebound excitation in single unit and multiunit activity of topographically-aligned S1 sites. Under some conditions, the CN stimuli evoked rhythmic S1 unit and field potential activity at alpha band (8-14 Hz) frequencies, specifically in the S1 granular layer. To explore perceptual effects of CN microstimuli, the monkeys were trained to detect and discriminate vibrotactile stimuli. When the vibrotactile stimuli were replaced by CN microstimuli, detection performance gradually improved over the course of about 10 sessions. Detection probabilities were on average 75% higher than chance after this transition. Detection thresholds (<40 μA) were comparable to those found by others with S1 microstimulation. These results provide the first assessment of artificial CN activation in behaving primates. Ongoing experiments are exploring the discriminability of CN microstimuli, with a specific focus on whether the spread of electrical current at threshold intensities is limited enough to allow independent percepts in the closely-packed representations of the CN.

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Nanosymposium

288. Neural Coding in the Somatosensory System

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Presentation Number: 288.05

Topic: D.03. Somatosensation: Touch

Support: AHFMR Interdisciplinary Team Grant

Title: Restoring somatic sensation with thalamic microstimulation

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Abstract: A useful tactile somatosensory prosthesis must (i) evoke somatosensory percepts in limited body regions, (ii) provide graded sensation, (iii) have reproducibility and persistence, (iv) provide the perception of slip and pressure, and (v) provide proprioception. With thalamic stimulation we have been able to produce 3 of these 5.

Microelectrode recording and stimulation in non-sedated humans prior to deep brain stimulator (DBS) implantation allows investigation of the percepts induced by electrical stimulation of somatosensory pathways. In thalamus we target the kinesthetic nucleus to treat tremor, and the immediately adjacent ventrocaudal (Vc) tactile nucleus to treat pain. Electrical stimulation (333 Hz, 0.2 ms pulses, 1-25 uA, 2-10 s trains) is applied through 25-40 um microelectrodes and patients describe what they feel.

Application of various patterns of microstimulation, including ‘natural’ spike trains digitized from single units in Vc thalamus, rarely produced totally natural percepts; most subjects described stimulation as unnatural and ‘tingling’. High frequency stimulation was just as good as digitized spike trains at evoking ‘natural’ percepts. Lower currents were more likely to induce natural percepts. Different patterns could evoke different percepts in the same brain location and these were generally reproducible. Persistence of percepts was related to the time that stimulation was applied: higher duty cycles (i.e. continuous stimulation) reduced the duration of percepts evoked.

Recently we tried to improve the naturalness of percepts by applying interleaved patterns of stimulation through 2 microelectrodes 200-300 um apart in 6 subjects at 12 sites in Vc thalamus. While this did not change the ‘naturalness’ (in 80%), different interleaved patterns did alter 68% of the percepts described. Therefore, applying concurrent stimulations and manipulating interactions between two closely spaced sites may evoke a larger range of percepts within similar projected fields.

In thalamus we have been unable to evoke sensations of slip, however we could evoke pressure in rare cases. We were also unable to elicit body movement / limb position percepts. This remains the most challenging aspect in the design of a somatosensory neural prosthesis and

suggests that thalamus may provide some percepts, but other sites may be required for the full range of somatic sensation.

Disclosures: **Z.H. Kiss:** None. **L.H. Kim:** None.

Nanosymposium

288. Neural Coding in the Somatosensory System

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Title: Intracortical microstimulation in human somatosensory cortex

Authors: *R. A. GAUNT[1], S. N. FLESHER[2], J. L. COLLINGER[1], S. T. FOLDES[1], J. E. DOWNEY[2], E. C. TYLER-KABARA[3], S. J. BENSMAIA[5], A. B. SCHWARTZ[4], M. L. BONINGER[1];
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Abstract: Intracortical microstimulation (ICMS) in the primary somatosensory cortex (S1) offers a potential method to restore somatosensory perception in people that have lost this capacity through injury or disease. Animal work has shown that somatosensory percepts can be elicited through ICMS in S1. However questions about the quality of the evoked sensations and how much learning may be required to interpret them are difficult or impossible to address in animal experiments.
Under an Investigation Device Exemption, a twenty-eight year old participant with a long-term cervical spinal cord injury was implanted with two microelectrode arrays in primary motor cortex and two microelectrode arrays in area 1 of S1. The S1 arrays were implanted in locations that were responsive during observed cutaneous stimulation of the right hand and fingers during presurgical imaging. We tracked the quality of the evoked artificial sensations over time, the projected locations of these sensations, and the participant's sensitivity to ICMS, measured using classical psychophysical methods.
ICMS-evoked sensations with projected fields in digits 2-5 and at the base of each of these fingers. Fifty nine of the sixty four microelectrodes elicited percepts and no pain was ever reported. The majority of the percepts were described as pressure-like, while some electrodes

evoked tingling sensations. Importantly, all ICMS-evoked sensations were distinctly different than sensations associated with surface electrical stimulation of the skin, which were described as being parenthetic and/or painful. Detection thresholds had a median value of 34.9 μA, with upper and lower quartiles at 60.0 and 24.8 μA, respectively. Thresholds were generally stable over the 11 months of testing. Of the 32 electrodes with three or more measured thresholds, four electrodes had thresholds that decreased significantly while another three increased, suggesting the thresholds were not globally increasing. Just noticeable differences were 15.4 ± 3.9 μA and did not depend on the amplitude of the reference stimulus. Furthermore, increasing the stimulation amplitude resulted in a linear increase ($R^2 = 0.98$) in the perceived intensity of tested electrodes.

Overall, we found that percepts were evoked at somatotopically relevant locations and that the perceived intensity of stimuli scaled linearly over a wide range. Restoring these two streams of somatosensory information could have a major impact on neuroprosthetic hand dexterity and embodiment. Together, these results suggest that ICMS is a promising approach to establish artificial somatosensation.

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Nanosymposium

288. Neural Coding in the Somatosensory System

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Title: Direct cortical stimulation for sensory feedback

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Abstract: Stimulation of human somatosensory cortex has been a tool to guide neurosurgical resections for over 100 years. Direct stimulation of sensory cortex results in somatotopically organized perceptions thought the quality of the perceptions is typically vague or unnatural. It is an emerging question in the implementation of bidirectional neuroprosthetics as to whether direct cortical stimulation would be an effective modality for sensory feedback.
Cortical stimulation of somatosensory cortex was carried out in patients with temporarily implanted electrodes on the cortical surface. Subjects were enrolled in stimulation studies following the completion of clinical stimulation mapping. We examined the information that could be carried in bipolar cortical stimulation by altering parameters such as location, polarity, intensity, pulse width and frequency of stimulation. Specifically, perception from stimulation was applied as a feedback channel during a motor task and incorporated into multimodal perceptions, specifically as part of the rubber hand illusion.
Results: Graded sensory responses were perceived during different amplitudes and frequency of stimulation. Changes in pulse width of a stimulus altered the intensity threshold. Polarity of stimulation did not change perception in this approach. During a motor task, feedback as to the desired position of the hand contralateral to the electrodes was encoded only through cortical stimulation. Subjects were able to keep the hand in the intended window using graded sensory stimulation as the feedback. Reaction times and interpretation of the task were confounds, but the reliability of the perception was robust. For the rubber hand illusion, cortical stimulation was sufficient to create a strong illusion in two patients. As with the use of natural touch, stimulation had to be timed in synchrony with the visual illusion and spatial congruence of the perceived sensation and the visual illusion was mandatory.
Direct cortical stimulation for sensory feedback may have sufficient information to drive prosthetic function. Future work will include steering current to deeper parts of sensory cortex through different electrode configurations. Understanding the temporal dynamics of sensory stimulation perception will be critical to implementation of feedback.

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Nanosymposium

288. Neural Coding in the Somatosensory System

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PVA 2978

Title: Towards artificial proprioception for brain-machine interfaces

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Abstract: Tremendous progress has been made toward brain-machine interfaces (BMIs) for the control of prosthetic devices. Still, BMI-controlled movement remains slow, effortful and imprecise, especially compared to natural movement. We hypothesize that natural fluidity and ease will not be attained without an artificial replacement for proprioception. We are taking a principled approach to developing and optimizing artificial proprioception through intracortical mircrostimulation (ICMS) of somatosensory cortex (S1).

The cortical coding for proprioception remains poorly understood. Moreover, it's not clear that current stimulation technology permits precise enough spatial, temporal and cell-specific neural activation to recapitulate naturalistic proprioception. However, theoretical (Makin, Fellows & Sabes, 2013) and experimental (Dadarlat, O'Doherty & Sabes, 2015) evidence suggests that useful non-biomimetic signals can be readily provided to the brain. Therefore we are taking a learning-based approach to providing artificial proprioception that does not demand biomimetic stimulation.

We trained a rhesus macaque to make reaches to visual targets using closed-loop BMI, decoding motor cortical activity (M1) and stimulating S1. Importantly, the monkey was required to hold its physical arm motionless during BMI-control to remove natural proprioceptive feedback. We then delivered time-varying multichannel ICMS feedback that encoded, along with vision, the moment-by-moment position and velocity of the actuator.

This experiment raised two key technical challenges. First, we must measure the efficacy of the artificial feedback. Performance with bimodal feedback (vision and ICMS) was compared to the unimodal conditions and with no feedback. We varied the reliability of visual feedback both to quantify the reliability of the ICMS signal with respect to the visual standard and to determine if ICMS is efficiently combined with vision. Given the importance of proprioception for online feedback control, it is crucial to assess performance in a dynamic setting: we used a critical stability task (Quick, Card, Whaite, Mischel, Loughlin & Batista, 2014) where feedback is used to control an unstable dynamical system.

Closed loop control with ICMS feedback suffers from stimulation artifacts (SA) in the neural control signal. The challenge of SA contamination increases with the number of stimulation channels. We used an adaptive-filtering scheme that exploited the statistical correlations between multiple recording channels to remove SA and allow truly concurrent stimulation and recording.

Disclosures: J.E. O'Doherty: None. **P.N. Sabes:** None.

Nanosymposium

288. Neural Coding in the Somatosensory System

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Title: Microstimulation of residual nerve fibers with Utah Slanted Electrode Arrays can restore biologically realistic cutaneous and proprioceptive percepts after hand amputation

Authors: *G. A. CLARK, D. M. PAGE, D. T. KLUGER, S. M. WENDLEKEN, T. S. DAVIS, C. DUNCAN, D. T. HUTCHINSON;
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Abstract: Multi-electrode arrays implanted in severed but still functional peripheral arm nerves after long-term amputation of the hand provide a potential means to restore rich, biofidelic sensory and motor function with advanced neuroprosthetic limbs. Five human subjects (S1-S5) received chronic (up to 3 months) implants of one to two 100-electrode Utah Slanted Electrode Arrays (USEAs) in residual median and/or ulnar nerves. S5 also received a 32-electrode electromyogram (EMG) assembly implanted in residual extrinsic hand muscles, for additional motor control. Evoked percepts were mapped by passing increasing current levels through individual USEA electrodes (biphasic, 200-μs pulses; 100-200 Hz, 200-500 ms trains) until the subject reported a percept, or until stimulation maximum was reached and testing of that electrode was discontinued. Subjects reported the perceived location, type, and intensity of the percept. S4 and S5, each with two above-elbow USEA implants, reported over 100 different cutaneous percepts (e.g., pressure, vibration) or proprioceptive percepts (e.g., joint movement, muscle force) evoked by microstimulation with individual USEA electrodes. The percepts covered most of the phantom hand, usually corresponding to normal afferent fiber distributions, and were typically enjoyed by subjects. Median stimulation thresholds were low and biologically

safe (e.g., 3-30 μA). Percept stability varied across electrodes, but most percepts showed within-session stability, and a few percepts remained stable for ≥ 1 month. Subjects were able to discriminate functionally among percepts having different phantom spatial locations or qualities, evoked by individual electrodes or combinations of electrodes. For example, S5 discriminated among 4 different pressure intensities at 3 receptive field locations (index and middle finger and palm; $p < .0005$), and among 4 joint angles for two different fingers (index and middle; $p < .0001$). Recent subjects also used sensory feedback evoked by biofidelic afferent fiber stimulation to guide motor control in a virtual reality environment (VRE) that allowed interactions with virtual objects and simulated activities of daily living (e.g., opening door with handle, playing a virtual guitar). Reciprocally, active engagement with the VRE also influenced subjects' perceptions. Because real-world cutaneous and proprioceptive stimuli rarely engage only a single afferent or type of afferent, the emerging ability to provide a relatively complex repertoire of somatosensory inputs may enhance sensory processing, sensorimotor control, and even a sense of embodiment for advanced neuroprosthetic limbs.

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Nanosymposium

288. Neural Coding in the Somatosensory System

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Title: On the use of intraneural transversal thin-film electrodes to develop bidirectional bionic limbs

Authors: *S. MICERA[1,2];
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Abstract: Replacing a missing upper limb with a functional one is an ancient need and desire. Historically, humans have replaced a missing limb with a prosthesis for many reasons, be it cosmetic, vocational, or for personal autonomy. The hand is a powerful tool and its loss causes severe physical and often mental debilitation. The need for a versatile prosthetic limb with intuitive motor control and realistic sensory feedback is huge and its development is absolutely necessary for the near future.
Among the possible solutions to achieve this goal, interfaces with the peripheral nervous system, and in particular intraneural electrodes, are a very promising choice. In this presentation, the results achieved so far by using thin-film transversal intraneural electrodes (TIMEs) for sensory feedback are summarized.
First, we are going to show the results achieved during a short-term implant of TIMEs in a trans-radial amputee to restore sensory feedback. With the first subject, it was possible to restore several component of the sense of touch such as contact events, grasping force, object shape and stiffness. We also showed that texture discrimination can be restored by implementing a neuromorphic algorithm reproducing the firing dynamics of nerve fibers connected to mechanoreceptors. Recent results achieved of the first long-term implant in another amputee confirm and extend previous results.
Finally, the next steps to achieve a fully implantable devices will be briefly summarized.
These findings show that these interfaces are a valuable solution for delivering sensory feedback to subjects with transradial amputation. Further experiments are necessary to better understand the potentials of this approach during chronic experiments.

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Nanosymposium

288. Neural Coding in the Somatosensory System

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Title: Evolution of human-in-the-loop neuroprosthesis - toward an artificial hand

Authors: ***D. J. TYLER**[1,2], E. GRACZYK[1], M. SCHIEFER[3], I. CUBEROVIC[1], K. MALONE[4], M. KEITH[5], J. ANDERSON[4];
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Abstract: We have implanted peripheral nerve interfaces in upper extremity limb loss subjects for more than four years for restoration of sensory feedback in the missing hand. We have discovered neural coding paradigms to restore naturalistic sensory perceptions ranging from paresthesia to vibration to motion to natural touch; defined paradigms to encode perception intensity; demonstrated improved functional performance; nearly eliminated phantom pain; and created a sense of embodiment of a prosthesis. In a home use trial, the subject reported that he perceives the prosthesis as his hand and that he uses the hand to perform bimanual tasks previously not possible. The non-penetrating, flat interface nerve electrodes (FINEs) have remained on the median, radial, and ulnar nerves of two subjects for over three years. Each FINE has 8 individual points of stimulation evenly distributed around the circumference of the nerve. In these first subjects, greater than 95% of the individual contacts on the FINEs result in tactile, proprioceptive, or rarely, nociceptive sensation. Perceptions are distributed over the hand, and the perception location and stimulation thresholds have remained stable for the life of the implants. In April 2016, we implanted a second generation peripheral nerve stimulation system that provides more stimulation channels on the peripheral nerve for sensation and implanted EMG recording for motor control to create an implanted, bidirectional, closed-loop, artificial hand for individuals with limb loss. Two Composite Flat Interface Nerve Electrodes (CFINEs) with 16 channels each were placed on the median and ulnar nerves of the residual limb, and eight bipolar intramuscular electromyography (EMG) electrodes were placed into four flexor and four extensor muscles. Sensation is critical for fine control of complex function of the hand. Loss of sensation, even in intact hands with normal motor abilities, results in significant functional deficits. Sensation and visual-tactile integration are critical to device embodiment. Even the most complex and anthropomorphic robotic devices to replace the hand will only be perceived as a tool attached to the residual limb. Only natural somatosensory feedback and motor control will enable an artificial hand to be incorporated into the user's body image. As our interfaces mature and demonstrate long-term stability, we are approaching realization of an artificial hand.

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Nanosymposium

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Title: Engineering an optimal afferent interface based on the brain's representation of limb state

Authors: *L. E. MILLER[1], R. CHOWDHURY[2], T. TOMLINSON[1], C. VERSTEEG[2];
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Abstract: The past 15 years have seen a host of technical developments in Brain Computer Interfaces (BCIs) that have moved the field from two- or three-dimensional control of a cursor to multi-dimensional control of a robotic limb, and even activation of multiple, paralyzed muscles. However, in nearly all BCIs, the feedback guiding movement is exclusively visual. As loss of proprioception causes devastating motor control deficits, researchers are increasingly recognizing that supplying somatosensory input directly *to* the user's brain is as important as extracting motor information *from* the brain.

The discharge of most proprioceptive neurons in the primary somatosensory cortex (S1) appears to be tuned to the direction of hand movement and can be summarized reasonably accurately by single "preferred direction". We trained a monkey to make a judgment about the direction of a force perturbation delivered to its hand, while we concurrently stimulated groups of intracortical electrodes with similar PDs. Stimulation biased the monkey's reports of the mechanical perturbations, suggesting that it induced perception of limb motion similar to that caused by actual movement. By stimulating different groups of electrodes with different PDs, we induced biases in different directions, the magnitude of which was dependent of the stimulus current. However, we have preliminary evidence that S1 neurons may be more closely tuned to the muscle-based coordinates of the afferent receptors than they are to egocentric hand coordinates. For small movements around a fixed limb posture these two coordinate systems are roughly linearly related. However, we would expect stimulation based on endpoint PDs to cause a directionally distorted perceptual effect when the limb is placed in a posture other than that used to compute PDs because of the nonlinear mapping between muscle lengths and hand position. We developed a visual motion tracking system using the Kinect depth camera. We estimated muscle lengths during reaching by feeding these signals into an OpenSim musculoskeletal model. We had the monkey reach to targets in two workspaces that required significantly different limb postures. If S1 neurons actually reflect hand coordinates, their PDs should remain invariant to limb posture. Instead, the PDs computed in the two workspaces rotated

systematically. To explain these results, we simulated neurons as linear combinations of muscle stretch velocity, a stimulus to which muscle spindles are highly sensitive. The simulated neural discharge approximately recreated the statistics of the PD rotations of the actual data. We hope to use this model to improve the accuracy of our afferent interface.

Disclosures: L.E. Miller: None. **R. Chowdhury:** None. **T. Tomlinson:** None. **C. Versteeg:** None.

Nanosymposium

289. Voluntary Movements: Oral Motor and Speech

Location: SDCC 24A

Time: Monday, November 14, 2016, 8:00 AM - 10:00 AM

Presentation Number: 289.01

Topic: E.04. Voluntary Movements

Support: NIHR01DC010145

NIHR01DC013979

NSF grant BCS 1262297

Title: Sensorimotor adaptation to real-time formant shifts is influenced by the direction and magnitude of shift.

Authors: *H. KOTHARE[1], V. RAMANARAYANAN[2], B. PARRELL[3], J. F. HOUDE[1], S. S. NAGARAJAN[1];
[1]Univ. of California, San Francisco, San Francisco, CA; [2]Educational Testing Service R&D, San Francisco, CA; [3]Univ. of Delaware, Newark, DE

Abstract: Auditory feedback plays an important role in speech production. Any alteration in auditory feedback usually engenders a change in speech production. The speech motor control system learns to anticipate and compensate for consistent feedback alterations. This counteractive response or sensorimotor adaptation persists temporarily even after feedback returns to normal. Does the accuracy and consistency of sensorimotor adaptation depend on the size and direction of the feedback alteration? To investigate this, we employed real-time auditory feedback alteration to shift the frequency values of the first and second formants (F1 and F2) of participants' speech. The experiment comprised six cases; the shift was different in each case (from /ɛ/ to /I/, /i/, /e/, /æ/, /a/ and /u/). In each case, participants produced 90 repetitions of the nonsense word 'bep' (vowel /ɛ/). A case started with a non-altered block of 10 trials, followed by a block of 50 trials with a constant alteration and then by a non-altered washout block of 30 trials. Shifts were designed on a subject-by-subject basis using pre-collected baseline formant

frequencies of vowels. We find that adaptive control of vowel formant frequency depends on the magnitude and direction of the applied shift in the two-dimensional F1-F2 vowel space. We also observe that all shifts, except the one from /ɛ/ to /u/, elicit a response of a compensatory nature. In general, smaller shifts lead to a relatively larger adaptation. A two-dimensional vector resolution analysis of the response vectors reveals that both the component orthogonal to the axis of the shift and the component parallel to the shift axis influence the magnitude of adaptation. These results are consistent with related auditory feedback studies. Taken together, these findings suggest that the adaptive feedback response in speech is complex and specifically more sensitive to errors in a local neighbourhood around speech motor targets. The response could depend on the magnitude and direction of the auditory error rather than giving equal weight to any possible error.

Disclosures: H. Kothare: None. **V. Ramanarayanan:** None. **B. Parrell:** None. **J.F. Houde:** None. **S.S. Nagarajan:** None.

Nanosymposium

289. Voluntary Movements: Oral Motor and Speech

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Topic: E.04. Voluntary Movements

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NSF grant BCS 1262297

Title: Speech production without vocal tract sensory feedback

Authors: *M. THOMPSON[1], J. HOUDE[2], S. NAGARAJAN[2];
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Abstract: Sensory feedback plays a crucial role in speech production in both healthy individuals and in individuals with production-limited speech disorders such as aphasia, stuttering, and Parkinson's-induced hypophonia. However, the vast majority of research on the sensory consequences of speech production has focussed on auditory feedback while relatively little is known about the role of vocal tract somatosensory feedback. This study investigates speech production in the absence of vocal tract somatosensory feedback by training subjects to use a touchscreen-based speech production platform. Contact with the touchscreen results in instant playback of a vowel dependant on the location selected. Because the axes of the touchscreen are

associated with continuous F2 and F1 frequencies, every possible vowel within a wide formant range can be produced. Over the course of 480 trials, subjects with no initial knowledge of the mapping of screen areas to playback sounds were asked to reproduce auditory vowel targets. Their responses were evaluated for accuracy and precision. Accuracy was defined as the distance between each target and response, precision as the average distance between each response and all other responses sharing a target. As subjects performed the experiment, both accuracy and precision rapidly increased. The average distance between the target and response fell from 115.5 mm ± 21 mm in the first 30 trials to 84.72 mm ± 28 mm in the subsequent 30 trials, finally plateauing around 45mm (final 30 trials 43.9 mm ± 19 mm). Similarly, the mean distance between responses fell from 111.3 mm ± 25 mm in the first 30 trials to 69.7 mm ± 21 mm in the next 30 trials, plateauing between 35 and 48 mm for the final 300 trials. Stable, rapid increases in both precision and accuracy imply the development of an internal sensorimotor map, allowing subjects to predict the auditory consequences of each touch and to develop the stereotyped responses necessary to achieve the desired feedback. Results suggest the development of individualized production analogous to the personalized vowel production seen in vocal speech. Findings provide evidence that healthy adults are capable of rapidly learning a new platform of speech production without vocal tract feedback that bears similarities to vocal speech. This study has implications not only for sensory feedback research, but also for diagnosis and quality of life in individuals with production-limited disorders.

Disclosures: **M. Thompson:** None. **J. Houde:** None. **S. Nagarajan:** None.

Nanosymposium

289. Voluntary Movements: Oral Motor and Speech

Location: SDCC 24A

Time: Monday, November 14, 2016, 8:00 AM - 10:00 AM

Presentation Number: 289.03

Topic: E.04. Voluntary Movements

Title: Investigating the role of auditory feedback in the production of speech and non speech vocal behaviours

Authors: *Z. K. AGNEW[1], H. KOTHARE[2], S. NAGARAJAN[3], J. F. HOUDE[2];
[1]UCSF Med. Sch., San Francisco, CA; [2]OHNS, [3]Radiology, UCSF, San Francisco, CA

Abstract: Whilst the neural basis of speech production has been the target of numerous investigations over the last few decades, the neural control of emotional vocalisations has gone relatively under studied. A number of lines of evidence suggest that the neural control of speech production and the production of emotional vocalisations may be distinct, and further, that emotional vocalizations may be more akin to vocalizations made by non-human primates than to

human speech. Many emotional vocalizations, including those employed here, are recognizable across cultures. Conversely, speech sounds are highly over-learnt articulations that are not common across different cultures. Given these differences, it has been suggested that emotional vocalisations may rely on evolutionarily older, or different neural systems. Here we investigate the hypothesis that feedback control of emotional vocalisations is distinct from that employed during highly learnt vocal behaviours such as speech.
Subjects were trained to either produce emotional vocalisations for the categories of laughter, disgust, or speech sounds, in response to a written cue. In separate blocks, subjects heard these same sounds and their task was simply to listen. This allowed us to specifically investigate motor induced suppression (MIS), which is a neural marker for feedback processing, defined by a reduced response to production compared to listening in auditory regions of temporal cortices. We have previously demonstrated that different cortical regions are active during overt production of the two articulations and that sensory cortices show significantly different responses during the production of speech and emotional vocalisations using functional magnetic resonance imaging. Here we use magnetoencephalography imaging (MEGI) to investigate time resolved neural responses in auditory cortex to self produced speech and non-speech vocalisations, and compare time resolved MEGI findings to our previous findings in fMRI.
We report activity in sensorimotor cortices associated with speech production during the production of both speech sounds and emotional vocalisations. In addition we report distinct responses in auditory regions during production compared to listening, for both speech and emotional vocalisations. These data suggest that motor control of speech may be distinct from that of other vocal behaviours and serve to elucidate distinct neural networks for the production of non-speech vocalisations. These data are discussed with respect to current feedback control models of speech production and primate functional neuroanatomy.

Disclosures: **Z.K. Agnew:** None. **H. Kothare:** None. **S. Nagarajan:** None. **J.F. Houde:** None.

Nanosymposium

289. Voluntary Movements: Oral Motor and Speech

Location: SDCC 24A

Time: Monday, November 14, 2016, 8:00 AM - 10:00 AM

Presentation Number: 289.04

Topic: E.04. Voluntary Movements

Title: A supralaryngeal neuromuscular apparatus for sonar beam-forming in echolocating bats

Authors: ***S. TRENT**[1,2], M. SMOTHERMAN[1,2];
[1]Biol., Texas A&M Univ., College Station, TX; [2]Texas A&M Inst. for Neurosci., College Station, TX

Abstract: There is behavioral evidence that echolocating bats can manipulate the acoustic projection pattern of their sonar pulse emissions, but the mechanism(s) for this are unknown. We hypothesized that the Mexican free-tailed bat (*Tadarida brasiliensis*) achieves this by finely adjusting the shape of its mouth (beam-forming) in a behavior akin to supralaryngeal speech motor control by humans. This hypothesis arose from our discovery that *Tadarida brasiliensis* raise their nose and lips preceding each echolocation pulse and that they possess a hypertrophied set of specialized facial muscles possibly analogous to the *levator labii aleque nasi* . We investigated whether this muscle complex 1) is active during sonar performance, 2) displays anatomical and histological specializations consistent with the high-speed demands of echolocation, and 3) can effectively perform beam-forming through the fine manipulations of mouth-gape. Firstly, EMG recordings from awake echolocating bats confirmed that these muscles were activated in a temporally precise coordination with pulse emissions. Secondly, we described the anatomical organization of the muscle complex, its origin and insertions, and its innervation patterns. Histochemical analyses confirmed that these were highly aerobic, fast-twitch muscles, as expected for muscles supporting rapid pulse emissions for extended periods. Lastly, we directly measured how changes in face shape affected the sonar beam-width. This muscle complex allows bats to lift the nose tip to create a small aperture producing a wide-angle beam, or to lift both the nose and the upper lips simultaneously creating a wider aperture but narrower beam. We confirmed that for a typical pulse (downward FM sweep, 50-20 kHz), raising and pulling back the lips narrowed the projection beam relative to just raising the nose tip with lips held down. These results confirm that *Tadarida* possesses a specialized supralaryngeal neuromuscular apparatus for sonar beam-forming.

Disclosures: S. Trent: None. **M. Smotherman:** None.

Nanosymposium

289. Voluntary Movements: Oral Motor and Speech

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Presentation Number: 289.05

Topic: E.04. Voluntary Movements

Title: Individuals with cerebellar degeneration correct for within-category variation of vowels even in the absence of auditory feedback

Authors: *B. PARRELL[1], Z. AGNEW[2], J. HOUDE[2], S. NAGARAJAN[2], R. IVRY[3]; [1]Linguistics and Cognitive Sci., Univ. of Delaware, Newark, DE; [2]Univ. of California, San Francisco, San Francisco, CA; [3]Univ. of California, Berkeley, Berkeley, CA

Abstract: Our understanding of the sensorimotor control of speech has greatly benefitted from studies in which external perturbations are applied while participants are speaking, and their behavioral change in response to the perturbation is measured. This work has shown that sensory feedback plays a critical role in speech motor control, as healthy speakers correct for perturbations to both auditory and somatosensory feedback. Interestingly, similar corrections are observed in the absence of externally applied perturbations: Productions of vowels near a category boundary appear to be treated as small errors, with on-line adjustments of these productions towards the vowel target. This "vowel centering" effect is partially reduced, but not eliminated, by masking auditory feedback, suggesting a role for auditory feedback in online error correction. However, the retention of centering behavior in the absence of auditory feedback suggest that this process may, at least in part, be driven by a (cerebellar-generated) predicted sensory error, similar to models of in-flight saccade corrections.
We examined vowel centering behavior in patients with cerebellar degeneration (CD, n=13), comparing conditions with and without auditory feedback. In previous work with this population, we have made two observations. First, the CD group exhibits increased sensitivity to externally introduced auditory errors, suggesting an over-reliance on feedback control. Second, the group is impaired in adapting their speech in response to a consistent perturbation, suggesting a problem with correctly predicting the consequences of the speech movements. Thus, we hypothesized that patients would show a larger-than-normal centering effect in clear speech (no masking noise) due to their over-reliance on sensory feedback but a substantially reduced effect when auditory feedback was unavailable due to their inability to generate accurate sensory predictions. In line with the first prediction, the CD group showed a slight increase in the centering effect, although this effect was not reliable. However, contrary to the second prediction, the patients showed no difference in centering magnitude between clear speech and masking noise conditions. This suggests that, while the CD group correct for online errors, auditory feedback is not critical for these corrections, at least when the variability is endogenous. This suggests that the CD group may rely on somatosensory feedback to a greater extent than normal speakers, and that this increased use of somatosensory information may occur even before the onset of vocalization.

Disclosures: **B. Parrell:** None. **Z. Agnew:** None. **J. Houde:** None. **S. Nagarajan:** None. **R. Ivry:** None.

Nanosymposium

289. Voluntary Movements: Oral Motor and Speech

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Presentation Number: 289.06

Topic: E.04. Voluntary Movements

Support: NIH Grant P50 DC013027

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Title: Neural correlates of language phenotypes in Autism Spectrum Disorder

Authors: *J. A. SEGAWA[1], J. A. TOURVILLE[1], Q. T. H. NGUYEN[2], F. I. KARAHANOGLU[2], P. WIGHTON[2], A. VAN DER KOUWE[2], M. D. TISDALL[2], R. A. FOWLER[2], J. SMALL[3], D. S. MANOACH[2], F. H. GUENTHER[1];
[1]Boston Univ., Boston, MA; [2]Massachusetts Gen. Hosp., Boston, MA; [3]Salem State Univ., Salem, MA

Abstract: Communication and language deficits are amongst the most striking symptoms of autism spectrum disorder (ASD), and up to 25% of people with the disorder remain minimally verbal into adulthood. Despite its large size, the minimally verbal ASD population is extremely underrepresented in the neuroimaging literature, in large part because communication and other deficits make it difficult for these participants to comply with neuroimaging requirements such as remaining still. As a result, little progress has been made in characterizing the neural bases of speech deficits in ASD.

To address this dearth, we collected diffusion weighted images of the brains of adolescents (aged 14-21 years) with ASD who were classified based on speech output as minimally verbal, verbal but language impaired, or verbal with normal language, as well as typically developing control participants. We conducted a probabilistic tractography analysis between anatomically defined regions of interest implicated in speech motor processes.

In the left hemisphere, white matter pathways between the supplementary motor area (SMA) and ventral premotor cortex (vPMC) and between the SMA and ventral motor cortex (vMC) showed significant differences in strength across groups. Not only were these pathways weaker in ASD participants compared to typically developing controls, but the SMA-vPMC/vMC pathways were weaker for minimally verbal/language impaired ASD participants compared to ASD participants with normal language. No other pathways within the speech network, including the right hemisphere SMA-vPMC/vMC pathways, were significantly different across groups.

The left vPMC, vMC, and SMA are believed to comprise a network responsible for initiating and executing speech movements. The current results confirm our prior finding of impaired left SMA-vPMC connectivity in high-functioning adults with ASD and further reveal that reduced speech output in adolescents with ASD is associated with reduced connectivity within the speech initiation network. In addition to identifying neural correlates of speech deficits in ASD, our findings suggest potential targets for therapy to improve the verbal output – and the quality of life – of adolescents with ASD.

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Nanosymposium

289. Voluntary Movements: Oral Motor and Speech

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Topic: E.04. Voluntary Movements

Support: NIH RO1DE023816

Title: Neural population dynamics in the primary motor, primary somatosensory, and cortical masticatory areas of the orofacial cortex during bite force generation at varying gapes

Authors: ***C. F. ROSS**[1], F. ARCE-MCSHANE[1], N. HATSOPOULOS[1], B. SESSLE[2], Y. LANKA[1];
[1]Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL; [2]Univ. of Toronto, Toronto, ON, Canada

Abstract: Human mastication relies on the precise control of the generation of bite force at varying degrees of mouth opening, i.e. gapes. The role of orofacial sensorimotor cortex in the coordination of gape and bite force is unknown. Here we examined the spiking activity recorded with microelectrode arrays implanted chronically in the primary motor (MIo), primary somatosensory (SIo), and cortical masticatory (CMA) areas of the orofacial sensorimotor cortex in monkeys (Macaca mulatta) trained to generate one of three bite force levels at one of three gapes per trial. Over 60% of neurons in MIo, SIo, and CMA showed significant modulation of their preparatory- (0.5 s before force onset to force onset) and/or movement-related firing rate relative to the hold period (Paired t-Test, $p<0.001$). In all three areas, neurons whose spiking activity varied significantly with gape were predominant (36-64%) while neurons whose spiking activity varied significantly with instructed force level were fewer, ranging between 5-8% (Two-way ANOVA, $p<0.05$). Using an analysis based on nearest-neighbor classifier revealed that spiking activity of populations of simultaneously recorded neurons in the three areas was able to predict the trial condition (gape and force) above chance level. Lastly, projection of the population response onto a two-dimensional state space (jPCA, Churchland et al, 2012) revealed a prominent rotational feature of the neural state-space for MIo but not for SIo or CMA. This rotational structure was consistent with that found in arm motor cortex during reaching. Overall, the results suggest that while single-unit and population responses in all three areas play a role in

the coordination of bite force and gape, MIo may perform computations that are different from those performed by SIo and CMA.

Disclosures: **C.F. Ross:** A. Employment/Salary (full or part-time): University of Chicago. **F. Arce-McShane:** A. Employment/Salary (full or part-time): University of Chicago. **N. Hatsopoulos:** A. Employment/Salary (full or part-time): University of Chicago. **B. Sessle:** A. Employment/Salary (full or part-time): University of Toronto. **Y. Lanka:** A. Employment/Salary (full or part-time): University of Chicago.

Nanosymposium

289. Voluntary Movements: Oral Motor and Speech

Location: SDCC 24A

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Topic: E.04. Voluntary Movements

Support: NIH RO1DE023816.

Title: Intracortical microstimulation of primary orofacial motor cortex and it's effect on jaw and tongue muscle recruitment

Authors: ***Y. V. RAM**, C. F. ROSS, N. HATSOPOULOS;
Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: Intracortical micro stimulation (ICMS) of the orofacial area of the primary motor cortex (MIo) reveals overlapping and redundant representations of jaw, tongue, and face movements. Direct connections have been identified between MIo and the motor nuclei of various cranial nerves including the hypoglossal, trigeminal, and facial nuclei. However, the functional consequences of these connections have yet to be fully characterized. In the current study, we determine how ICMS of MIo influences the strength and timing of muscle recruitment in muscles innervated by the hypoglossal and trigeminal nerves. Individual electrodes from a 96 channel multi-electrode array implanted in MIo were stimulated with single biphasic pulses at 15 Hz and 28 μA for 2.5 minutes each. Stimulus-triggered averaging was used to calculate the mean EMG responses of 14 jaw and tongue muscles to the stimulation of each electrode. The jaw and tongue muscles we consider were left and right superficial masters, geniohyoids, sternohyoids, mylohyoids, anterior digastrics, styloglossus, genioglossus. Preliminary findings showed that ICMS generated a positive correlation between activations of jaw opening muscles: geniohyoid, anterior digastric, and mylohyoid ($P<0.04$). These muscles are recruited together to depress the mandible and elevate the floor of the mouth. Jaw depressors were represented laterally on the array while the jaw elevators were represented medially. There was a negative correlation

between jaw depressor and elevator activity ($P<10^{-9}$). These findings indicate that cortical contributions to jaw movements may include simultaneous jaw depressor inhibition and jaw elevator excitement.

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Nanosymposium

290. Microbiome Gut Brain Axis

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Presentation Number: 290.01

Topic: G.05. Anxiety Disorders

Support: Ontario Mental Health Foundation (OMHF)

Title: Changes in behavior and gut microbiome induced by chronic treatment with the dopamine agonist quinpirole

Authors: *H. SZECHTMAN, T. JUNG, P. JUNG, L. RAVEENDRAN, Y. FARBOD, A. DVORKIN-GHEVA, B. SAKIC, M. SURETTE;
McMaster Univ., Hamilton, ON, Canada

Abstract: Chronic treatment of rats with the D2/D3 dopamine agonist quinpirole (QNP) yields several concurrent transformations in the behavior of the animal; e.g., it induces compulsive checking (proposed as animal model of OCD[1]) and increases locomotor activity[2]. The latter effect is termed 'locomotor sensitization'. The mechanism by which chronic QNP produces those behavioral transformations is not known. One suggestion has been that underlying the transformations in behavior is a QNP-induced heightened activity in a central dopaminergic system controlling energy expenditure[2], consistent with subjective reports of "feelings of energy" with psychostimulant drugs. Reminiscent of a heightened sense of energy are empirical findings that in QNP sensitized rats, energy metabolism is shifted in favor of free fatty acid utilization, as measured by VCO2/VO2 respiratory quotient[3]. Here we report a plausible peripheral mechanism contributing to the shift in energy metabolism, namely, a QNP-induced modification in the composition of gut microbial populations. **Methods**: Two groups of rats received 9 injections of saline (n=16) or QNP (n=15; 0.25 mg/kg), at weekly intervals for the first 5 weeks and then 2 injections per week until end of treatment. After each injection, rats were placed on a large open field for 55 min and their behavior was video recorded for subsequent analysis. Fecal matter was collected after each trial and frozen for bacterial community profiling of the 16S rRNA gene using paired end reads of the V3 region. **Results**: As expected, QNP induced compulsive checking and locomotor sensitization. A number of significant shifts in gut bacterial composition

were observed in both groups, as well as differential changes due to QNP. Of note, QNP raised the relative abundance of Lachnospiraceae. Members of this family have been implicated in energy metabolism and immunomodulation in the gut. **Conclusion**: It is possible that altered gut microbiota may contribute to the mechanism mediating the transformation in behavior induced by chronic treatment with QNP.

REFERENCES:[1]Szechtman H, Sulis W, Eilam D. *Quinpirole induces compulsive checking behavior in rats: a potential animal model of obsessive-compulsive disorder (OCD).* Behav Neurosc, 1998, **112**:1475-1485.
[2]Szechtman H, Talangbayan H, Canaran G, Dai H, Eilam D. *Dynamics of behavioral sensitization induced by the dopamine agonist quinpirole and a proposed central energy control mechanism*. Psychopharmacol, 1994, **115**:95-104. [3]Coscina D, Currie P, Szechtman H. *Association of altered whole-body metabolism with locomotor sensitization induced by quinpirole*. Physiol Behav, 1998, **63**:755-761.

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Nanosymposium

290. Microbiome Gut Brain Axis

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Topic: G.05. Anxiety Disorders

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Boris Family Grant

Title: Mice colonized with GAD microbiota display anxiety and depressive-like behaviour and changes in brain BDNF expression.

Authors: *E. PEREZ GUZMAN[1,3], R. ANGLIN[2], G. DE PALMA[3], R. POTTS[3], J. LU[3], M. AMER[2], M. BAILEY[4], S. M. COLLINS[3], M. SURETTE[3], P. BERCIK[3];
[1]Med., [2]Psychiatry, McMaster Univ., Hamilton, ON, Canada; [3]Med., Farncombe Family Digestive Hlth. Inst., Hamilton, ON, Canada; [4]Ctr. for Microbial Pathogenesis, Columbus, OH

Abstract: Background: The pathophysiology of anxiety and depression is multifactorial and remains largely unknown. Recent studies have demonstrated that gut microbiota, apart from instructing and shaping the host immune system and metabolism, also has ability to modulate behavior and brain chemistry.

Study Objectives: To investigate whether gut microbiota from patients with Generalized Anxiety Disorder (GAD) can induce anxiety-like behaviour in gnotobiotic mice and to study the underlying mechanisms.
Methods: Germ-free NIH Swiss mice (n=18) were colonized with microbiota from either a patient with GAD and comorbid depression (n=10) or from an age and sex-matched healthy control (HC) (n=8). Both the GAD patient (female, 19 years) and healthy control (female, 20 years) were well characterized and selected based on their Depression, Anxiety, and Stress Scale (DASS) scores from a pool of participants of an ongoing clinical study. Three weeks post-colonization, the mice underwent behavioural assessment using standard psychometric tests including the open field, marble-burying, digging and tail suspension tests. Stool β-defensin levels were measured by ELISA. Microbiota profiles were assessed by 16S rRNA based Illumina analysis. Lastly, brain BDNF expression was measured by immunofluorescence staining.
Results: GAD-colonized mice had a distinct microbiota profile compared to HC-colonized mice and each group clustered around their respective human donor. Fecal β-defensin levels were six fold higher in the GAD donor than the HC. Similarly, mouse fecal β-defensin levels were higher in GAD-colonized mice than in HC-colonized mice ($p=0.03$). Mice colonized with GAD microbiota exhibited significantly greater anxiety and depressive-like behaviour than HC-colonized mice, as they spent less time in the center of the open field arena ($p=0.01$), buried a greater number of marbles ($p=0.0002$), spent a greater time digging ($p=0.005$) and remained more time immobile ($p=0.04$). BDNF levels were higher in the amygdala and lower in the hippocampus of GAD-colonized mice compared to HC mice.
Conclusions: Our results demonstrate that GAD microbiota can induce anxiety and depressive-like behavior in the murine host, which is accompanied by elevated immune markers and altered expression of BDNF in brain regions involved in emotional processing. Altogether, our data suggest that gut microbiota may contribute to the pathophysiology of anxiety and depression, likely through immune mediated mechanisms.

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Nanosymposium

290. Microbiome Gut Brain Axis

Location: SDCC 4

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Presentation Number: 290.03

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Support: NARSAD YI Award

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KL2

Title: Prenatal stress alters intrauterine environment and contributes to adult female microbiome and behavioral changes

Authors: T. L. GUR[1], L. A. SHAY[1], A. VADODKAR[1], S. L. FISHER[1], *M. T. BAILEY[2,3]; [1]Psychiatry, [2]Dept. of Pediatrics, The Ohio State Univ., Columbus, OH; [3]Ctr. for Microbial Pathogenesis, The Res. Inst. at Nationwide Children's Hosp., Columbus, OH

Abstract: Background: Exposure to stressful stimuli changes the composition of the intestinal microbiota, which is associated with development of stress-induced behavioral changes. Psychiatric disorders have been associated with *in utero* and early neonatal exposure to maternal stress, though the underlying mechanisms are not fully understood. Commensal microbes from the maternal gastrointestinal and reproductive tracts are the first to colonize the developing fetus and newborn, thus raising the possibility that commensal microbes are involved with the transmission of the maternal experience during pregnancy. **Study Objectives:** To determine the contribution of maternal stressor exposure and changes in commensal microbes on the development of adult psychopathology. **Methods:** Pregnant C57BL/6 female mice were exposed to an acute restraint stressor using well ventilated restraining tubes between gestational/embryonic day (E) 10-E16, for 2 hrs per day. Pregnant females in the non-stress control group were left undisturbed throughout pregnancy. Placental tissue and amniotic fluid were collected from pregnant females and fetuses at E17.5 in one cohort of mice. Microbial diversity was assessed using the Illumina MiSeq platform, for targeted 16S ribosomal RNA gene sequencing. RT-PCR and Bioplex assays were used to examine placental cytokines and growth factors. In a second cohort of mice, behavior was assessed with the elevated plus maze (EPM), the tail suspension test (TST) and the novel object recognition test when the pups reached adulthood. **Results:** Prenatal stress led to alterations in intestinal microbial populations in both the pregnant females and their female offspring. Differences in the intestinal microbiome may actually begin in utero, since the microbial communities of female (but not male) placentas were significantly changed by stressor exposure. These changes in microbial composition were associated with altered placental gene expression, including Interleukin-6 (IL-6), monoamine

oxidase A, and O-GlcNAc transferase (OGT). In addition, alterations in cognition and anxiety, as well neural gene expression, were evident in the female offspring of stressor-exposed dams, even when the offspring reached adulthood. **Conclusions:** These data indicate that gestation is a critical window in contributing to the development of adult psychopathology, and that the microbiome may be a key link between early life environment and later life behavioral changes. **Funding**: This study was funded by a NARSAD YI Award, KL2, and a March of Dimes Transdisciplinary Scholar award to TLG.

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Nanosymposium

290. Microbiome Gut Brain Axis

Location: SDCC 4

Time: Monday, November 14, 2016, 8:00 AM - 9:45 AM

Presentation Number: 290.04

Topic: G.05. Anxiety Disorders

Support: Office of Naval Research #N00014-14-1-0787

Canadian Institute of Health Research GSM-136180

Title: Gut-brain signalling modulates behavioural deficits in chronic stress independent of the microbiota

Authors: *A. BHARWANI[1,2,4], F. M. MIAN[1,4], M. G. SURETTE[3,5], J. BIENENSTOCK[2,4], P. FORSYTHE[3,4];
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Abstract: The gut microbiota and the brain are engaged in persistent bidirectional interplay—a phenomenon that influences host neural function and behaviour. Exposure to psychosocial stress induces complex structural changes in the profile of the microbiota, suggesting a potential role for gut bacteria in stress-induced deficits. However, whether the microbiota influence the behavioural and neural changes induced by stress, and the associated pathways of communication underlying this influence remain unclear. Using a neuroactive bacteria strain,

Lactobacillus rhamnosus JB-1^{TM}, we investigated whether gut-brain signalling modulates neural and behavioural alterations stemming from chronic stress exposure.
Male C57BL/6 mice were orally administered 10^9 CFU of JB-1 over 28 days and subjected to chronic social defeat during the final ten days of treatment. Mice were assessed for alterations in social, exploratory, and anxiety-like behaviours using multiple behavioural assays. Spleens were examined using fluorescence-associated cell sorting for systemic changes in the immunoregulatory phenotype. 16S rRNA sequencing and mass spectrometry were used to analyze the microbiota community and functional changes in metabolite production.
Chronic stress induced various behavioural deficits—reduced sociability and exploration, and elevated anxiety-like behaviour—that were partially corrected with JB-1-treatment, with the exception of avoidance behaviour towards aggressors, which remained evident for up to 3 weeks following stressor cessation. Defeated mice exhibited markers of immunoregulatory alterations, such as dendritic cell activation and transiently elevated levels of IL-10+ T regulatory cells (Treg) that were suppressed over time; these shifts were prevented by JB-1 while further increasing IL-10+ Treg. Stress exposure induced complex alterations in the microbiota profile and reduced species richness and diversity, none of which were prevented by treatment. Both stress exposure and treatment altered specific metabolite levels, suggesting candidate pathways for bottom-up gut-brain signalling.
These findings demonstrate the ability of neuroactive bacteria to modulate gut-brain signalling independently of structural changes in the microbiota, and correct certain deficits induced by chronic stress. Collectively, these results reveal the potential for microbe-based adjuncts or therapies as a novel strategy in the treatment of psychiatric conditions.

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Nanosymposium

290. Microbiome Gut Brain Axis

Location: SDCC 4

Time: Monday, November 14, 2016, 8:00 AM - 9:45 AM

Presentation Number: 290.05

Topic: G.05. Anxiety Disorders

Title: Neonatal colonization of the gastrointestinal tract with Bifidobacterium species alters CNS expression of synaptic plasticity-related genes

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[1]Baylor Col. of Med., Houston, TX; [2]Texas Children's Hosp., Houston, TX

Abstract: Background: *Bifidobacterium* species have been shown in recent studies to alter CNS gene expression, neurotransmitter function, and behavior in adult rodents. In humans, bifidobacteria are detectable within the first week after birth and are a predominant genus of the infant intestinal microbiota. We have previously shown that colonization of adult germ-free mice with *Bifidobacterium dentium* results in altered CNS expression of genes important to postnatal neurodevelopment. Given that *Bifidobacterium* spp. colonize the human system during a critical period of neural circuit development and organization, *Bifidobacterium*-specific CNS modulation early in life may have pervasive and lasting effects on brain function and behavior. We hypothesized that neonatal colonization with a consortium of "infant-type" *Bifidobacterium* species would alter CNS expression of genes important to synaptic plasticity and neural circuit development. **Method and Results:** In order to examine the effects of early *Bifidobacterium* colonization, pregnant female mice were orally gavaged with a mixture of "infant-type" *Bifidobacterium* species, including *B. bifidum*, *B. longum* subsp. *infantis*, *B. breve*, and *B. dentium*. Control mice received sterile saline gavages. Co-housing of dam and pups during the pre-weaning period resulted in colonization of the pups during the neonatal stage. The cerebellum was used as a model to study how the microbiota alters gene expression and circuit development. Cerebellar tissue was collected from mouse pups at postnatal days 4, 10, and 20. Gene expression was analyzed via qRT-PCR and circuit development was assessed via immunohistochemistry. One proposed method of microbiota-host crosstalk is via the production of microbial metabolites such as short-chain fatty acids (SCFAs), which are known histone deacetylase inhibitors that can epigenetically modulate neuronal gene expression. SCFA concentrations were analyzed via LC-MRM-MS, and preliminary data suggest that *Bifidobacterium* increases systemic concentrations of several SCFAs. **Conclusion**: These data demonstrate that colonization with *Bifidobacterium* species results in altered expression of genes important to neurodevelopment. *Bifidobacterium*-mediated increases in certain SCFAs may modulate expression of these genes in the cerebellum and other brain regions.

Disclosures: B. Luk: None. **M. Engevik:** None. **J. Versalovic:** None.

Nanosymposium

290. Microbiome Gut Brain Axis

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Time: Monday, November 14, 2016, 8:00 AM - 9:45 AM

Presentation Number: 290.06

Topic: G.05. Anxiety Disorders

Support: NARSAD Brain and Behaviour Research Foundation Grant Number 20771

Science Foundation Ireland grant number SFI/12/RC/2272

Title: Regulation of microRNAs in the prefrontal cortex by the microbiota: implications for brain and behaviour

Authors: *A. E. HOBAN[1], R. STILLING[2], F. SHANAHAN[3], T. G. DINAN[4], J. F. CRYAN[2], G. CLARKE[4];
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Abstract: Background: The prefrontal cortex (PFC) is a highly emotional key brain region implicated in many neuropsychiatric disorders ranging for schizophrenia, major depressive disorder, anxiety disorders, autism and even obsessive compulsive disorders. Over the past decade the gut microbiota has emerged as a key player in many brain related disorders where disturbance of the bidirectional microbiota-gut-brain axis has been implicated in not only many behavioural-related disorders but also many neurodegrenetive diseases such as Parkinson's and Alzheimer's disease. In relation to altered behaviours related to changes in microbiota, the most consistent findings is in relation to anxiety-like behaviours and the stress response. Germ-free animals which a void of any bacteria display altered HPA axis response to stress and altered baseline anxiety level. The molecular mechanisms underpinning the role of the gut microbiota in relation to anxiety remain poorly understood however, they may well be due alterations in gene expression in the CNS. MicroRNA are an interesting class of gene regulators and it currently remain unknown whether the gut microbiota can influence the expression of brain-specific microRNA. The aim of this experiment was to establish if germ-free animals have altered microRNA expression patterns in the prefrontal cortex. **Methods**: Using the Illumina Next Generation Sequencing platform sourced to Exiqon (Denmark) (NGS), we investigated whether germ-free animals had altered expression of microRNAs in the PFC compared to conventionally raised animals as well as animals that were born germ-free and subsequently colonized at weaning **Results:** Within the PFC of germ-free mice there was 21 microRNA that were significantly differentially expressed that also had a fold change of ±0.5 when compared to conventional mice. Six of these microRNAs appear to be normalized by colonisation. Amongst these 6 microRNAs, mir-219a-2-3p was highly abundant, had a strong fold change (1.6) and was normalized by colonization. *In silico* based target prediction analysis highlight that this microRNA may target mRNA that could be relevant to anxiety such as the serotonin receptor 2a (*Htra2*). **Conclusion**: This results demonstrates that the microbiota can regulate the expression of microRNAs in the PFC. Moreover, our results demonstrated that targeting the gut microbiota later in life, post weaning has a normalizing effect on the expression of several microRNAs. Future studies will aim to verify the exact role these altered microRNAs play in relation to anxiety-related disorders.

Disclosures: **A.E. Hoban:** 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; Science Foundation Ireland. **R. Stilling:** 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; Science Foundation Ireland. **F. Shanahan:** 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; Science Foundation Ireland. **T.G. Dinan:** 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; Science Foundation Ireland. **J.F. Cryan:** 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; Science Foundation Ireland. **G. Clarke:** 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; Science Foundation Ireland.

Nanosymposium

290. Microbiome Gut Brain Axis

Location: SDCC 4

Time: Monday, November 14, 2016, 8:00 AM - 9:45 AM

Presentation Number: 290.07

Topic: G.05. Anxiety Disorders

Support: Ontario Brain Institute

Title: The influence of microbiota on brain structure

Authors: *J. A. FOSTER[1], S. L. THOMPSON[1], J. ELLEGOOD[2], J. LERCH[2];
[1]Psychiatry & Behav Neurosci, McMaster Univ., Hamilton, ON, Canada; [2]The Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Researchers in psychiatry and neuroscience are increasingly recognizing the importance of microbiota to brain communication in mental health. Scientists have established the link between gut bacteria and anxiety-like behaviours in animal models and with emotional brain regions in healthy people. During postnatal development, colonization and maturation of the commensal bacteria occurs in parallel with brain development. This study used MRI to investigate how microbiota influence the neuroanatomical phenotype in male and female Balb/C, C57Bl/6, and FVB mice. Bacterial community profiling of 16SrRNA gene was carried out using a modified bar coded Illumina sequencing method in the McMaster Genome Center. Imaging was performed using a 7T MRI with a T2 weighted, 3D fast spin echo sequence at the Mouse Imaging Centre (SickKids). Strain-specific differences in microbiota diversity were observed

with reduced alpha diversity in Balb/C mice compared to C57Bl/6 and FVB. Beta diversity analysis revealed strain-specific differences in microbiota composition; principal coordinates analysis (PCoA) showed 3 distinct clusters separated by strain. The taxonomic profile of the microbiota showed significant strain differences in relative abundance of clinically relevant commensals such as *Bifidobacterium, Lactobaccilus, Alistipes,* and *Prevotella.* Initial analysis of several significant strain differences in normalized brain volume in several key brain regions implicated in stress-related behaviour. For example, Balb/C mice showed increase amygdala volume compared to C57Bl/6 and FVB mice. No differences were observed in hippocampal volume by strain, however a significant sex difference was observed in C57Bl/6 mice. Interestingly, significant strain and sex effects between brain regions (cortical, amygdala, corpus callosum) and anxiety-like behaviour are observed. Additional integrated analysis linking specific microbiota to brain structure and behaviour is ongoing. Overall, this approach will help us understand how microbiota and host genetics influence differences in brain structure.

Disclosures: J.A. Foster: A. Employment/Salary (full or part-time): University Health Network, McMaster University. 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; Ontario Brain Institute. **S.L. Thompson:** A. Employment/Salary (full or part-time): Ontario Graduate Scholarship. **J. Ellegood:** None. **J. Lerch:** None.

Nanosymposium

291. Marmoset Neurobiology

Location: SDCC 7B

Time: Monday, November 14, 2016, 8:00 AM - 10:30 AM

Presentation Number: 291.01

Topic: H.01. Animal Cognition and Behavior

Support: A-MED Grant Brain/MINDS

Title: MRI-based structural and functional mapping of marmoset brains

Authors: *H. OKANO[1,2], J. HATA[2], T. KANEKO[2];
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Abstract: Investigation of brains of non-human primate brain provides us an essential cue to understand the human brain and to develop therapeutic strategies for human psychiatric/neurological disorders. Thus, mapping the brain of a small New World monkey, the common marmoset (*Callithrix jacchus*), is one of the most important characteristics of Brain/MINDS (the brain mapping project in Japan). Here, we wish to update the progress of

structural and functional mapping the marmoset brain.
Our team focus on macro level understanding of the marmoset brain using a high field MRI (9.4T). We have been obtaining population data of various types of MR contrast such as common structural map, diffusion tractgraphy and cortical myelination map. We have obtained functional connectivity map by resting-state fMRI. We integrated these MRI data with a digital 3D atlas of anatomical parcellation based on cytoarchitectonic definition and developed a macro-level connection matrix of marmoset whole brain. These dataset has provided unique opportunity to assess variety of physiological alternation in disease model marmosets with minimally-invasive procedures. This is especially important for longitudinal study such as therapeutic study or marmosets with genetic modification.
We are also working on functional mapping of the marmoset brain by task-based fMRI with a fully awake animal. Recently, we developed an 8-channel phased array coil for awake study, and obtained robust bold response by visual stimulation in our system. We also observed that visual motion stimulation activated at MT complex, and the activity extended to rostral on ventral part of lateral surface of temporal cortex. This suggest that functional segregation for visual motion processing is similar to some extent with those of other primate species such as macaques and humans, and thus shed light on one of utility of marmosets as a non-human primate model.

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Nanosymposium

291. Marmoset Neurobiology

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Presentation Number: 291.02

Topic: H.01. Animal Cognition and Behavior

Support: the Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) from Japan Agency for Medical Research and development, AMED

Title: Axonal projection map of areas around the superior temporal sulcus in the common marmoset

Authors: *H. ABE[1], T. TANI[1], H. MASHIKO[1], N. KITAMURA[1], K. SAKAI[2], H. MIZUKAMI[3], A. WATAKABE[1], T. YAMAMORI[1], N. ICHINOHE[1,2];
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Abstract: The common marmoset (Callithrix jacchus) is becoming a popular non-human primate model in neuroscience research due to several advantages including well-developed prefrontal cortex, the availability of transgenic technologies and their social behavior including rich vocal communication and monogamy family form. For social behavior, brain areas located around the superior temporal sulcus have been considered important because those areas are activated during understanding other's actions. In order to study the underlying neural mechanisms in more detail, their anatomical connections must be revealed, but the axonal projection is poorly known because anatomical tracing studies in the marmoset have been mainly done using retrograde tracers. To reveal the axonal projection from areas around the superior temporal sulcus, we injected an AAV, which works as an anterograde tracer by expressing a green fluorescent protein in infected neurons, to a posterior part of the FSTv, which is located ventral to the posterior part of the superior temporal sulcus, to an adult marmoset. After a three week waiting period, the animal was perfused and the post-mortem brain was sectioned in a thickness of 50 um. The sections were served to obtain fluorescent images to examine fluorescently labeled axonal projections and processed with myelin and Nissl substance staining to identify brain areas. The posterior part of FSTv had axonal projections to frontal areas 10, 46, 8, 6Va, 6Vb, 12O, and ProM. In the temporal cortex, the axons terminated in V4, MTc, MT, TEO, TPO, TF, TE1, TE2 and TE3. Especially for TE1 to TE3, the axons were almost exclusively labeled in the layer 4 suggestive of feed-forward connection, whereas for area MT in the layers 1 and 6 suggestive of feedback connection. For the parietal and occipital cortices, the axons terminated in PF, PFG, LIP, V3A and insular GI, DI, and IPro. Thus the posterior part of FSTv had projections to a large area of the marmoset brain including frontal cortex implicated in cognitive control, parietal occipital temporal cortices in visual processing, insular cortex in feeling of sympathy, and interestingly the ventral premotor cortex, parietal PF and PFG where mirror neurons have been found.

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Nanosymposium

291. Marmoset Neurobiology

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Presentation Number: 291.03

Topic: H.01. Animal Cognition and Behavior

Support: AMED Brain/MINDS

MEXT Kakenhi 16K07036

Title: Mapping connectivity of marmoset prefrontal cortex by serial two-photon tomography

Authors: *A. WATAKABE[1], J. WANG[1], M. TAKAJI[1], H. MIZUKAMI[2], A. WOODWARD[1], T. KAWASE[3], H. SKIBBE[3], Y. YAMAGUCHI[1], S. ISHII[3], T. YAMAMORI[1];
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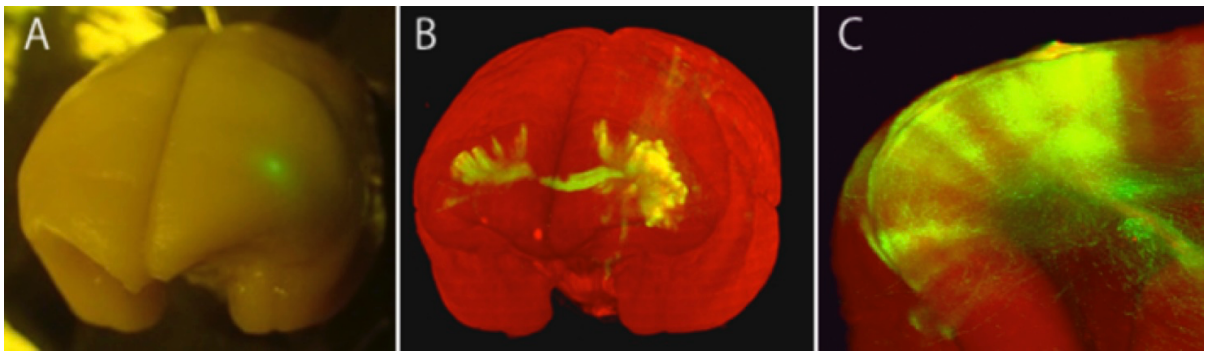
Abstract: The advancement in imaging techniques has opened an era of big data science for neuroanatomy. One of the best examples of such approach is the mapping of mouse brains by Allen Institute for Brain Science. In their study, high throughput imaging by serial two-photon tomography (STPT) generated massive data from hundreds of mouse brains. In an attempt to elucidate the structural basis for high cognitive function of primates, we employed a similar strategy to map marmoset prefrontal cortex (PFC). Despite small size and lissencephalic brains, marmosets possess differentiated PFC with comparable areas to those in macaques. By systematic injection of AAV-based tracers followed by STPT imaging, we aim to obtain detailed knowledge about how PFC areas interact with each other and with distant brain regions. Here we present the results of our pilot experiments, in which we tested our new AAV tracer system that exhibits much brighter labeling than normal synapsin-EGFP tracer. Marmoset brains are comparably bigger, less translucent and difficult to process. Nevertheless, STPT imaging went successful and we obtained complex 3D representation of PFC projection patterns. In our injections, AAV tracer transduced neurons within 1mm radius along the cortical column. These neurons sent intracortical projections in columnar fashion, consistent with the macaque studies. They also projected to distant cortices, claustrum, striatum, thalamus etc. The computational pipeline to analyze these image data is being developed, including new global tracking method that can detect connectivity even to the cellular resolution. Figure legend; A: Fluorescent image of tracer-injected marmoset brain. B: 3D-reconstruction after STPT imaging. C: Example of PFC connections viewed at high magnification.



Disclosures: **A. Watakabe:** None. **J. Wang:** None. **M. Takaji:** None. **H. Mizukami:** None. **A. Woodward:** None. **T. Kawase:** None. **H. Skibbe:** None. **Y. Yamaguchi:** None. **S. Ishii:** None. **T. Yamamori:** None.

Nanosymposium

291. Marmoset Neurobiology

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Title: A high-throughput neurohistological pipeline for whole-brain mesoscale circuit mapping for Marmoset

Authors: *P. P. MITRA[1], Y. S. TAKAHASHI[4], K. WEBER[4], M. K. LIN[4], K. HOSSAIN[4], B. HUO[4], A. S. TOLPYGO[2], D. D. FERRANTE[3], J. HATA[4], J. CHAN[5], H. MIZUKAMI[6], A. WATAKABE[4], T. YAMAMORI[4], N. KISHI[4], A. IRIKI[4], M. G. P. ROSA[5], E. SASAKI[4], H. OKANO[4];
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Abstract: Our knowledge of primate brain connectivity remains partial after decades of neuroanatomical research, with some species and brain regions well covered (cf. the Macaque visual system) but with gaps elsewhere. The Marmoset Brain Architecture Project aims to close this knowledge gap through systematic mesoscale connectivity mapping of the brain of the common Marmoset (Callithrix Jacchus). To this end, a neurohistological pipeline has been established at the RIKEN Brain Science Institute as part of the Japan Brain/MINDs project. This pipeline replicates the corresponding setup for the Mouse Brain Architecture Project at CSHL, along with capacity enhancements required for the 8x larger size of the Marmoset brain. The project utilizes female marmosets (~8 years of age) and divides the left hemisphere into ~400 injection targets, which will be covered during the project period. Both anterograde tracers (AAV x 2 colors) and retrograde tracers (Fast Blue (FB), Diamidino Yellow (DY) and Cholera Toxin B Subunit (CTB)) are being utilized for a total of 4-5 injections per individual brain. A full coverage of the brain is expected over the duration of the project. A suite of supplementary MRI image volumes (pre and post-mortem) as well as DTI volumes are being gathered, and the brains processed to include tracer series as well as alternating histological series for purposes of atlas

mapping. The brains are frozen in a custom mold to preserve alignment along the cardinal axes and sectioned using tape-transfer assisted cryosectioning to preserve the geometrical integrity between successive sections. Fluorescently labeled sections (AAV red, green; FB, DY) are automatically coverslipped after sectioning. Alternate series of sections are histologically processed (Nissl, Myelin, CTB immunohistochemistry) before coverslipping. Both types of sections are imaged in high throughput mode using a whole-slide scanner and the images transferred to storage following appropriate quality control steps. The resulting image series are reconstructed into 3D volumes containing alternating series with tracer and histological information and co-registered with the MRI volumes to produce multi-modal whole-brain data sets. The pipeline has now successfully processed multiple brains, and examples of the resulting 3D multimodal data volumes will be presented along with the custom protocols developed for processing marmoset brains.

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Nanosymposium

291. Marmoset Neurobiology

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Title: The marmoset brain architecture project: sharing data on primate corticocortical connectivity through an open access web platform

Authors: *M. G. ROSA[1,3], P. MAJKA[2,4,3], J. M. CHAN[2], S. BAI[2], D. FERRANTE[5], P. P. MITRA[5,3];
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Abstract: The marmoset (*Callithrix jacchus*) is an emerging animal model for large-scale attempts to understand primate brain connectivity. Here we describe an open access, web-based platform for sharing data from neuroanatomical experiments on this species. The initial release (accessible at marmoset.braincircuits.org) is focused on corticocortical connectivity, and includes 37 retrograde tracer injections in 18 animals. We have adopted a continuous publication approach, with new data being uploaded as they become available. Cases representing new experiments, as well as archival data covering the last 20 years are being digitized.
For each case, the site gives access to images of Nissl-stained sections with overlays showing the locations of injection sites and labeled neurons, providing a co-registered atlas. Gigapixel images of individual sections were stored in JPEG2000 format. Our approach is to store a single multi-resolution data stream, which is unpacked in real time at a desired resolution, using a customized Java-based Djatoka image server (*https://sourceforge.net/projects/djatoka/*) for dynamic dissemination and rendering. Selectively decoding only tiles and layers needed by the viewer, saves bandwidth and time while preserving zooming functionality. We developed a web-based viewer (based on customized OpenLayers), allowing the user to freely overlay multiple layers of information, without downloading and installing software.
Identification of likely areas is based on registration of a subject's data to a marmoset brain template, generated from a freely available stereotaxic atlas (Paxinos et al., 2012; *The Marmoset Brain in Stereotaxic Coordinates*; ftp://ftp.space.intersect.org.au/neura/). This allows fast data throughput in comparison with expert-based manual determination of histological boundaries. Comparison between the results of the automated and human-based processing reveals that the centers of injection sites can be reconstructed, on average, to within 0.6 mm (range 0-1.4 mm). The initial release also gives access to data summaries, in the form of flat map reconstructions, and metadata (animal age, sex, weight, tracer type, amount, post-injection survival time). Planned future development includes a connectivity matrix that is updated as new injections are added.
By laying the foundations of an open access resource, we intend to enable comparison and visualization of large data sets, allowing in turn integration and analysis of results from many cases. The applicability to archival materials may reduce the number of additional experiments required to produce the first detailed cortical connectome of a primate brain.

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Nanosymposium

291. Marmoset Neurobiology

Location: SDCC 7B

Time: Monday, November 14, 2016, 8:00 AM - 10:30 AM

Presentation Number: 291.06

Topic: H.01. Animal Cognition and Behavior

Support: AMED Brain/MINDS

MEXT Kakenhi 16K18371

Title: Two-photon calcium imaging using genetically-encoded calcium indicator in awake marmoset

Authors: *O. SADAKANE[1], M. UEDA[2], A. WATAKABE[1], H. MIZUKAMI[3], T. YAMAMORI[1];
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Abstract: The combination of two-photon microscopy with genetically-encoded calcium indicators has made it possible to monitor neuronal population activity chronically in behaving animals. In mice studies, this technique has been used extensively to investigate the organization of neural circuits and its plasticity. However, there were limitations in the application of this technique to primate brains, even though the investigation of primate brains is crucial for the understanding of the human brain. Recently, we developed a novel system to chronically image the activity of cortical neurons of the adult marmoset (*Callithrix jacchus*), a small New World monkey. We used a tetracycline-inducible system to robustly amplify the expression of GCaMP6f. In the previous report, we conducted the experiments using animals under anesthetized condition. Here, we applied our system to the early visual cortex of awake marmosets to test the feasibility of the method in behaving marmosets. In our system, the head of a subject was fixed under a two-photon microscope. Visual stimuli, including natural scene images and artificial bar stimuli, were presented onto the display in front of the animal. The animal was allowed to view the visual stimuli freely, and the movement of their gaze was continuously monitored using a video-based eye tracking system. A chronically implanted optical window provides access to the cortical surface. Using this system, we succeeded in monitoring visual responses of cortical neurons at the frame rate of 30 Hz. We were able to follow a population of same neurons over a month. In conclusion, we succeeded in extending our system to monitor neuronal population activities from primate neocortex in awake condition. This method will be applicable to functional analysis of neuronal population activity in behaving marmosets.

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291. Marmoset Neurobiology

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Support: NIH grant DC-03180

Title: Subthreshold response properties of the primary auditory cortex in awake marmosets studied by intracellular recordings

Authors: ***L. GAO**, X. WANG;
BME, Johns Hopkins Univ., Baltimore, MD

Abstract: Extracellular recording studies have shown that neural spiking activities in primary auditory cortex (A1) of awake animals often differ from those observed in anesthetized animals, which suggest that their underlying subthreshold mechanisms may be distinct from those of anesthetized animals. In the present study, we systematically studied the basic properties of subthreshold responses of A1 neurons using a novel intracellular recording technique developed for awake marmosets. The main observations are as follows: (1) In contrast to transient responses observed in A1 of anesthetized animals, sustained depolarization during the stimulus and long-lasting subthreshold responses (depolarization or hyperpolarization) after the stimulus offset were commonly observed in A1 of awake marmosets; (2) The bandwidth of frequency tuning measured by subthreshold response was generally broader than that of firing rate, and both appear narrower in comparison with those measured in A1 of anesthetized animals; (3) For neurons with monotonic rate level functions, the threshold of subthreshold responses was typically lower than that of firing rate; (4) For neurons with non-monotonic rate level functions, the subthreshold response was less non-monotonic than spiking response, suggesting an enhancement of non-monotonicity from subthreshold to spiking responses in A1 of awake animals; (5) A1 neurons can be classified into regular-spiking and fast-spiking types based on spike waveform; (6) A greater proportion of fast-spiking neurons exhibited monotonic rate-level function whereas more regular-spiking neurons showed non-monotonic rate-level function. These findings provide important insights into cortical processing of acoustic information at cellular and circuit levels.

Disclosures: **L. Gao:** None. **X. Wang:** None.

Nanosymposium

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant DC003180

Title: Pitch perception in marmosets

Authors: *X. SONG[1], M. S. OSMANSKI[2], Y. GUO[2], X. WANG[3];
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Abstract: The mechanisms of pitch perception have been one of auditory neuroscience's central questions due to the importance of pitch in music and speech perception. Yet the evolutionary origins of pitch perception, and whether its underlying mechanisms are unique to humans, is unknown. It has been shown that humans perceive pitch of harmonic sounds through spectral or temporal features. Previous studies have described three primary features of human pitch perception: (1) Lower resolved harmonics have a stronger pitch strength compared to a pure tone at the fundamental frequency (F0) and also to higher unresolved harmonics; (2) pitch of resolved harmonics is sensitive to the quality of spectral harmonicity; (3) pitch of unresolved harmonics is sensitive to the salience of temporal envelope cues. Among these features, the first two have never been demonstrated in any non-human species. We have conducted a series of psychophysical experiments to explore pitch perception behaviors in the marmoset (*Callithrix jacchus*), a highly vocal New World monkey species separated from humans by about 30 to 40 million years. Our results showed that, for a standard musical tuning fundamental frequency of 440Hz (ISO 16), the marmoset exhibits all three primary features of pitch perception mechanisms as found in humans. Combined with previous findings of a specialized pitch processing region in both marmoset and human auditory cortex, this finding suggests that the mechanisms for pitch perception, which have long been thought unique to humans, may have originated early in primate evolution, before the separation of New World and Old World primates. Additionally, one remarkable property of human pitch perception is that it is largely intact in the absence of the fundamental frequency component, the so-called "missing fundamental" phenomenon. Our recent experiments have provided new evidence that the marmoset perceives missing fundamental in a pitch discrimination task, even when the pitch change is as small as one semitone (the smallest pitch step in Western music). Together, our findings demonstrate that the marmoset has the capability to process pitch information similar to humans and suggest that these animals may be able to discriminate notes in a human musical scale, which is crucial for any music melody perception.

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Topic: H.01. Animal Cognition and Behavior

Support: NIH R01 DC012087

Title: The role of frontal cortex in marmoset conversations

Authors: *C. T. MILLER;
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Abstract: There is a growth of marmoset neuroscience worldwide. Mapping of brains of common marmoset provides us a essential clues to understand the human brain and to develop therapeutic strategies for human psychiatric/neurological disorders. Conversations represent a unique facet of communication, whereby individuals engage in cooperative, reciprocal exchanges of vocal signals. Like many primate species, including humans, marmoset conversations occur naturally and function to mitigate various social interactions. Here we present data from a series of experiments in which frontal cortex neurons were recorded from freely-moving marmosets while they engaged in their naturally occurring antiphonal conversations. Throughout these experiments, we utilized a novel, interactive playback method in which marmoset subjects conversed with 'Virtual Marmosets'. This method affords the opportunity to manipulate various aspects of the vocal signal and vocal behavior in order to better experimentally test functional characteristics of specific aspects of the conversational exchange. We will discuss the three following recent findings from this line of research. (1) In our initial neurophysiology experiment, we observed that marmoset frontal cortex neurons exhibited robust vocal-motor related changes in neural activity during natural vocal production, but little evidence that neurons were responsive when hearing vocalizations during this vocal behavior. (2) Despite the dearth of sensory responses in this frontal cortex population, we were able to reliably predict (92% accuracy) whether a particular vocalization would elicit a response from subjects. In fact, we observed that even neural activity preceding the initial vocalization stimulus was sufficient to predict the behavioral outcome. This suggested that the state of frontal cortex at the time marmosets hear a vocalization determines whether a conversation will occur. (3) In order to further test the role of marmoset frontal cortex, we implemented a version of the VM paradigm in which unexpected 'Probe' vocalization stimuli are broadcast during conversations. The goal is to determine whether this stimulus is perceptually detectable and elicit

more robust responses from frontal cortex neurons. When presenting subjects with Probe stimuli that represent a change in social category - the individual identity of the VM - preliminary evidence showed that some frontal cortex neurons exhibit strong responses for only these stimuli. Overall, this line of work is beginning to unravel the role of frontal cortex in naturally occurring marmoset conversations.

Disclosures: C.T. Miller: None.

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291. Marmoset Neurobiology

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Brain/MINDS

Title: Sustained motivation and its brain mechanisms in object manipulation by marmosets

Authors: *Y. YAMAZAKI[1,4], S. WATANABE[2], K. HIKISHIMA[3,6], E. SASAKI[6,3], C. J. PRICE[7], R. LEMON[8], H. OKANO[3,5], A. IRIKI[4];
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Abstract: There is a growing interest in the common marmoset as a model for neuroscientific research because of the cognitive and social abilities they share with humans. Among such abilities, self-control, or sustained motivation for reward is observed both in wild and captive marmosets. In the wild, they depend on gum trees for food and must patiently await for the gum to be exuded. In our laboratory, they will wait for up to two minutes to get a small amount of reward in a positional delayed matching task. Does this disposition make a difference to their cognitive abilities and brain mechanisms? We examined object manipulation tasks, a forced choice task, and tool use training. In the choice task, when presented with two strings of cloth with different lengths, one of which supported a food item and the other which did not, they spontaneously chose to pull the one which supported the reward. They could even learn to reject a more tempting, but unobtainable, reward in favor of a smaller one. This result indicated the

marmosets' ability to abstract the rules behind the task facing then, even if it involved inhibiting more direct but ultimately unrewarded behaviors. In the tool use task, when marmosets were trained to in a step-wise manner to manipulate a rake-shaped tool to retrieve a food item, they took about a year to complete the full training protocol, using both hands depending on the task conditions. This was a remarkable difference from Japanese macaques, who need only two weeks to master the task, using either hand. When we identified the marmoset brain structures involved in tool learning, using voxel based morphometry, there were several regions which increased their volume during the learning. Among then, we found that the nucleus accumbens increased its volume especially in the later phase of the training, during which marmosets made many more errors than in the earlier phase, suggesting an elevated and sustained motivation to use the tool even when the task became more demanding and the goal more difficult to achieve. Our results demonstrate unique learning characteristics in tool use by marmosets, which could be supported by brain mechanisms similar to those which enable humans to keep learning in novel conditions.

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Nanosymposium

292. Computational Models of Decision Making and Confidence

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Presentation Number: 292.01

Topic: H.02. Human Cognition and Behavior

Support: The John Templeton Foundation, Grant # 57876

Title: Multitasking capability versus efficiency of representation in neural network architectures

Authors: ***S. MUSSLICK**[1], K. ÖZCIMDER[2], B. DEY[2], M. A. PATWARY[4], P. KRIEGER[1], T. L. WILLKE[4], J. D. COHEN[3];
[2]Mechanical and Aerospace Engin., [3]Princeton Neurosci. Inst., [1]Princeton Univ., Princeton, NJ; [4]Parallel Computing Lab., Intel Corp., Santa Clara, CA

Abstract: Our limited ability to conduct multiple control-demanding tasks at once is one of the most salient and well-recognized features of human goal-directed behavior. In this work we investigate the source of this constraint from the perspective of a tradeoff between the efficiency of task representations and multitasking (parallel processing) capability in neural systems. We introduce a graph-theoretic analysis that is based on the assumption that cross-talk between tasks

arises due to shared task representations, and use this to analyze the parallel processing capability of two-layer neural networks as a function of task pathway overlap and network size. We describe how this analysis can be applied to task representations encoded in neural networks or neuroimaging data, and use this to compare predictions about multitasking performance with actual performance of trained three-layer neural networks. We further assess how shared task representations and associated multitasking limitations may arise as a function of the task environment that is defined over the degree of feature-overlap across tasks. Finally, we analyze how priors on weight similarity between tasks may affect learning speed, generalization performance, and multitasking performance in trained neural networks. Our results yield that the parallel processing capability of two-layer networks drops precipitously as a function of process overlap, and scales highly sublinearly with network size. Our simulations show that the introduced graph-analytic tools allow predicting how well the network performs a given set of tasks simultaneously. Under the assumption of task-set inertia, parallel processing limitations extend to networks in which multitasking is implemented sequentially, by switching between tasks as rapidly as possible: tasks predicted to be interfering were subject to greater switch costs that constrained serial multitasking performance. In accordance with observations that shared task representations are likely to develop in environments with high correlations between task features, we observe that the multitasking capability of trained networks drops in such environments. We also observe that weight priors on task similarity improve learning speed and generalization but lead to strong constraints on parallel processing capability. Altogether our analytical and numerical results identify a tradeoff between efficiency of representation and multitasking capability. We seek to contrast these findings with an ongoing behavioral study by assessing learning and multitasking performance of human subjects across tasks with varying degrees of feature-overlap.

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Nanosymposium

292. Computational Models of Decision Making and Confidence

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Topic: H.02. Human Cognition and Behavior

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Title: Optimal policy for multi-alternative decision-making

Authors: *S. TAJIMA[1], J. DRUGOWITSCH[2], A. POUGET[1];
[1]Univ. of Geneva, Geneva, Switzerland; [2]Harvard Med. Sch., Boston, MA

Abstract: While studies with binary choice paradigms have demonstrated the canonical mechanism of decision-making in such simple situations, much less is known about the computational principles underlying complex decisions with more than two options. A classical implementation of multi-alternative decisions is the "race model (RM)", in which momentary choice preference is signaled by a competition among ramping-up neural activities, that terminates once one of them reaches a constant decision threshold. On the other hand, recent physiological recordings suggest puzzling properties of neural dynamics that require extending standard RMs; in perceptual and value-based decision tasks, neurons often show activity normalization over the neural units (e.g., Louie et al., *J. Neurosci.*, 2011; 2014) and a time-dependent bias input to the network (urgency signal) that promotes rapid decisions (e.g., Churchland et al., *Nat. Neurosci.*, 2008). Although some ad-hoc models have been proposed to fit neural behavior, why the nervous system ought to have such properties and how they relate to each other remain poorly understood. To address these problems, we theoretically derive the normative strategies for general N-alternative decisions in perceptual and value-based domains. This reveals nonlinear and time-dependent decision-boundaries in a high-dimensional belief space, which differ from those in previous approximate models. Although such complex decision strategies may appear intractable for nervous systems *in situ*, we find that a geometric symmetry in those decision boundaries allow the optimal strategies to be well-approximated by a remarkably simple neural mechanism. The resulting circuit is interpreted as a novel extension of RM in which activity-normalization and the urgency signal cooperate to implement the optimal decision boundaries, thus providing a consistent explanation for these neural mechanisms from a normative perspective. Moreover, the model reveals how the optimal decision policy relates to reported physiological and behavioral phenomena in multi-alternative decisions (e.g., Hick's law about how reaction times depend on the number of options), and predicts an unreported time-dependent normalization that constrains neural population activity during decision-making.

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Nanosymposium

292. Computational Models of Decision Making and Confidence

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Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust (S.H.C.: 099741/Z/12/Z)

Title: Human noise blindness and decision suboptimality

Authors: *S. HERCE CASTAÑÓN, D. BANG, J. DING, C. SUMMERFIELD;
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Abstract: Human judgments about sensory stimuli are well described by optimal inference models in which the different sources of decision-relevant information are weighted according to their reliability. For example, during sensorimotor judgments, humans rely more on information about stimulus base rates (prior probability of occurrence) when the stimulus quality is experimentally degraded, e.g. when it is harder to perceive. Conversely, in cognitive tasks, humans show systematic deviations from optimality. For example, when asked to guess an individual's profession, participants neglect the base rate of occurrence of different professions. Understanding this discrepancy in optimality between perceptual and cognitive tasks is a major challenge for the cognitive sciences.

Here, we tested and confirmed one explanation: that decision optimality is driven by differences in human metacognitive sensitivity to (i) noise that arises "early", e.g. during the encoding of information, and (ii) noise that arises "late", e.g. during the integration of different pieces of information. We asked human observers to categorize the average orientation of set of gratings as clockwise or counterclockwise of the horizontal axis, providing fully informative feedback after each decision. We independently manipulated the impact of early and late noise on choices. Higher early noise was achieved by lowering the contrast of each grating (poor encoding); higher late noise was achieved by increasing the variability of orientations within a set of gratings (harder to integrate). Both manipulations lead to a significant drop in decision accuracy by about 10%. We used 3 methods for testing observers' metacognitive sensitivity to early and late noise: (i) recording whether observers adjusted (according to their performance) their use of a cue about the base rate of each stimulus category, (ii) eliciting second-order confidence judgments, and (iii) offering observers the option to "opt out" out of making a decision for a fixed probability of receiving reward. In all three cases, humans adjusted their decision strategy optimally in response to increased early noise but failed to do so in response to increased late noise. We conclude that humans are relatively blind to noise arising from their imperfect integration of different sources of information. These findings offer an explanation for human decision suboptimality on cognitive and economic tasks.

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Nanosymposium

292. Computational Models of Decision Making and Confidence

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DARPA SUBNETS

UC Irvine School of Medicine Bridge Fund

Title: Fast encoding of current and past value computations in human Orbitofrontal Cortex

Authors: *I. SAEZ[1], J. LIN[2], E. CHANG[3], J. PARVIZI[4], G. SCHALK[5], R. T. KNIGHT[1], M. HSU[1];
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Abstract: Damage to orbitofrontal cortex (OFC) results in severe impairments in the ability to make everyday decisions, while leaving many other cognitive faculties intact. Recent lesion evidence suggests that the core deficit caused by OFC damage is impaired value updating according to current internal states, an observation consistent with the existing representation of multiple aspects of valuation related to both external cues and internally represented information in OFC. Important insights into the exact nature of this multi-dimensional representation have come from studies of decision-making that use computational frameworks in which agents try to learn the expected value (utility) of potential actions, and use said value distributions to generate adaptive behavior. Abundant human fMRI and animal single unit studies link neural activation in OFC to a variety of computational aspects of reward, but direct neural evidence about their representation in human prefrontal cortex remains scarce.
Here we sought to overcome existing methodological limitations by directly recording electrophysiological activity in the human orbitofrontal cortex. We conducted multi-electrode intracranial recordings during a neuroeconomic task using electrocorticography (ECoG) in

patients with intractable epilepsy. We focused on power in the high frequency range (high gamma, HG; 70-200 Hz), which is the key marker of cortical activation, correlates with neuronal spiking, has fast dynamics and an excellent signal to noise ratio, and is therefore uniquely suited to reflect cortical computations.
We show that HG encodes a rich variety of computations relevant to decision-making, including aspects of current choice (gamble risk, decision to gamble or not, win probability and chosen value) and outcome (gamble win, gamble loss, RPE and regret). Interestingly, HG activations also reflected past choices and outcomes, which we hypothesize could be used to guide present choices and to update values.
This study demonstrates the feasibility of combining computational models of valuation with intracranial multi-array recordings in neurosurgical patients. Future experiments should provide additional insight into how information related to decision-making is encoded in and transferred between cortical brain areas engaged in action selection and memory, and the potential role of low frequency oscillations in these processes.

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Title: Computational and neural underpinnings of individual differences in human confidence

Authors: *J. NAVAJAS, C. HINDOCHA, P. E. LATHAM, B. BAHRAMI;
Univ. Col. London, London, United Kingdom

Abstract: Confidence, the “feeling of knowing” that accompanies our decisions, plays an important role in guiding processes such as learning, error monitoring, and social interactions. Previous research has sought to understand the computations underlying this subjective belief by combining model-based approaches with neuroimaging data. In this approach, inter-individual variability is usually neglected or assumed to be a random effect. In contrast to this perspective, recent studies have shown that individual differences in confidence are consistent, and suggest that different individuals may perform different computations to estimate confidence from uncertain evidence. Here, we investigate the computational and neural underpinnings of these

individual differences. To this end, we developed a task where 30 oriented Gabor patches were serially flashed in the fovea at 4 Hz. Participants were instructed to decide whether the mean orientation of the sequence was tilted clockwise or counter-clockwise relative to the vertical meridian. We manipulated the uncertainty in these decisions by drawing the orientation of the patches from distributions with different variance. We found that different individuals consistently reported, to different extents, two different probabilistic quantities: their perceived probability of being correct and the precision of their probability representation. This was stable across several weeks and consistent across tasks involving uncertainty in different domains. We recorded electroencephalography (EEG) signals and show that the power and phase-locking of occipital oscillations entrained to the visual stimuli encodes uncertainty in this task. Overall, these results provide novel insights into the neural and computational mechanisms underlying the computation of confidence.

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Topic: H.02. Human Cognition and Behavior

Support: NIH R01 NS088628-01

Title: Intracranial electrocorticography supports dissociable representations for perceptual decisions and confidence judgments

Authors: ***M. A. PETERS**[1], T. THESEN[3], Y. D. KO[4], B. MANISCALCO[5], C. CARLSON[3], M. DAVIDSON[3], W. DOYLE[3], R. KUZNIECKY[3], O. DEVINSKY[3], E. HALGREN[6], H. LAU[2]; [2]Psychology, [1]UCLA, Los Angeles, CA; [3]New York Univ., New York, NY; [4]Columbia Univ., New York, NY; [5]NIH/NINDS, Bethesda, MD; [6]UCSD, San Diego, CA

Abstract: When we perceive a stimulus, we also have a sense of confidence in our perceptual decisions. But how does the brain compute such confidence? Popular theories posit that the perceptual system optimally judges confidence according to the balance of evidence favoring and against a perceptual choice: we should be less confident about more ambiguous stimuli. However, recent behavioral and computational evidence suggests that while perceptual *decisions* depend on this Balance of Evidence (BE) among stimulus alternatives, *confidence judgments* disproportionately rely on the amount of evidence favoring the decision (Response-Congruent Evidence, RCE), giving less weight to evidence favoring other possible choices (Response-

Incongruent Evidence, RIE). In other words, we are more confident when judging more salient stimuli, even if they are just as ambiguous.
Here, we investigated whether neural evidence would support RCE-based confidence. We recorded electrocorticography from 1060 electrodes implanted in six patients during a perceptual discrimination (face/house) and confidence judgment task. Using cross-validated linear support vector machine (SVM), we investigated whether four trial-by-trial behavioral factors could be classified as a function of time: Stimulus, Response, Evidence Rate (slope of Single-Trial Linear Ballistic Accumulator) in the brain, and Confidence. We found that all four can be predicted above chance by gamma (30-80Hz) and high-gamma (80-160Hz) power. To evaluate the representational similarity among the four, we trained SVMs on each behavioral factor and cross-predicted to the others. Classifiers trained on Stimulus or Response generalized their predictive capacity to each other, suggesting similar representations. However, rectified classifiers (according to signal detection theory) trained on Stimulus or Response could predict Evidence Rate but not Confidence, suggesting that Confidence depends on a separable representation from the other three. We confirmed this dissociation by defining RCE and RIE as a function of the SVM feature weights trained on Stimulus and the gamma/high-gamma power at each time point, and used Receiver Operating Characteristic (ROC) analysis to predict Response, Evidence Rate, and Confidence based on the mean BE (BE = RCE - RCI), RCE, and RIE for each trial. Results confirmed the above findings: Response and Evidence Rate were best predicted by BE, but Confidence was best predicted by RCE. Our findings provide the first neural evidence in support of RCE-based confidence, and argue against popular hypotheses that espouse humans' optimality in confidence judgments.

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Presentation Number: 292.07

Topic: H.02. Human Cognition and Behavior

Support: DFG GRK1589/2

Title: The P300 in sequential perceptual decision making: from subjective evidence to confidence

Authors: *J. HERDING[1], S. LUDWIG[1], B. SPITZER[2], F. BLANKENBURG[1];
[1]Freie Univ. Berlin, Berlin, Germany; [2]Dept. of Exptl. Psychology, Universtity of Oxford, Oxford, United Kingdom

Abstract: The classic P300 event-related potential is currently undergoing a comeback in perceptual decision making (cf. O'Connell et al., 2012, Nat Neurosci). Recent studies suggest that the central-parietal positivity in the human EEG signal tracks evidence accumulation in two-alternative forced choice (2-AFC) tasks in which a single noisy stimulus is presented at a time (e.g., random dot motion: Kelly et al., 2013, J Neurosci; house vs. car images: Philiastides et al., 2014, J Neurosci). Here, we investigate whether the P300 also reflects the evidence driving decisions in 2-AFC tasks wherein two sequentially presented stimuli have to be compared in order to make a choice. To this end, we recorded EEG data (64 channels) while participants had to decide whether the latter of two sequentially presented vibrotactile stimuli had a higher or a lower frequency than the former. Both stimuli were presented for 250 ms separated by a 1000 ms retention interval. We collected 73 datasets using four different variants of this sequential frequency comparison (SFC) task varying both the response modality (button press/saccade) and response timing (immediate/postponed). In the SFC task, the objective sensory evidence for either choice is given by the signed difference between stimulus frequencies, whereas the absolute difference determines task difficulty. Based on individual behavioral performances, we refined these objective measures and estimated subjectively perceived frequency differences (SPFDs) for each participant in a Bayesian model. We used the signed and absolute values of the SPFDs from each trial as covariates in a general linear model of the individual EEG data. On the group level, we found, for each variant of the task, the P300 was initially modulated by the signed SPFDs (~300 - 600 ms) and then by the absolute values of the SPFDs (~350 - 800 ms). The early modulation of the P300 by the signed values of the SPFDs is in line with the current view that the P300 reflects sensory evidence in perceptual decision making. The latter result agrees with the classic finding that associates increases in P300 amplitude with increases in absolute difference between standard and deviant stimuli in oddball paradigms. In the given context of perceptual decision making, we suggest that this modulation can be related to task difficulty and may index the confidence in a given choice. Taken together, the SFC paradigm allowed us to manipulate sensory evidence independently from task difficulty, and thus to show a separate impact of both measures on the P300.

Disclosures: J. Herding: None. **S. Ludwig:** None. **B. Spitzer:** None. **F. Blankenburg:** None.

Nanosymposium

379. Axon and Dendrite Development and Regeneration

Location: SDCC 30B

Time: Monday, November 14, 2016, 1:00 PM - 3:45 PM

Presentation Number: 379.01

Topic: A.05. Axon and Dendrite Development

Support: RGC GRF HKUST 16103315

RGC GRF HKUST 16101414

Title: Enhancing neuronal activity by melanopsin/GPCR signaling promotes axon regeneration in the adult CNS

Authors: *K. LIU, C. YANG, X. WANG;
HKUST, Kowloon, Hong Kong

Abstract: During development, neuronal activity is essential for axon guidance and wiring. However, its function in axonal regeneration in the mature CNS remains elusive. We found that overexpression of the light-sensitive GPCR melanopsin in the retina enhanced neuronal activities of retinal ganglion cells in adult mice. Interestingly, melanopsin overexpression stimulated axonal regeneration after optic nerve crush by upregulating mTOR complex 1 (mTORC1). Dark adaptation and Kir2.1 expression suppressed the mTOR signaling and the axon regeneration, indicating that neuronal activity was required. Furthermore, inhibiting Gq/11 signaling, downstream of heterologous expression of melanopsin, blocked the growth effect. Specifically activating Gq in retinal ganglion cells through a chemogenetic approach that has been widely used in boosting neuronal firing also elevated mTOR activation, and promoted axonal regeneration. We also extended similar strategies onto the corticospinal tract and examined the sprouting after unilateral pyramidotomy. Thus, our results provide evidence that enhancing neuronal activity promotes axon regrowth after CNS injury.

Disclosures: K. Liu: None. **C. Yang:** None. **X. Wang:** None.

Nanosymposium

379. Axon and Dendrite Development and Regeneration

Location: SDCC 30B

Time: Monday, November 14, 2016, 1:00 PM - 3:45 PM

Presentation Number: 379.02

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant R01DC013066

Title: Vangl2 directs stereotyped turning of peripheral neuronal processes during development of the mouse cochlea

Authors: *M. R. DEANS[1], S. R. GHIMIRE[2];
[1]Surgery, [2]Neurobio. & Anat., Univ. of Utah, Salt Lake City, UT

Abstract: The cochlea is innervated by the sensory neurons of the spiral ganglia that relay sound information from the sensory receptor hair cells to central auditory targets. A subset of neurons in the spiral ganglion is dedicated to a fundamentally important feedback circuit that provides neuroprotection in extreme noise and facilitates hearing and speech discrimination in noisy environments. This circuit is dependent on the Type2 spiral ganglion neurons (SGN2) that innervate the outer hair cells. The morphological development of SGN2s is unique because their peripheral process projects beyond the inner hair cells before making a distinct 90° turn towards the base of the cochlea in order to synapse with 8 to 10 outer hair cells. While many aspects of SGN2 development and outer hair cell innervation are not known, our laboratory has found evidence that the planar cell polarity (PCP) protein Vangl2 contributes to at least one step in this process; the turning event that directs the SGN2 peripheral process to the base of the cochlea. In Pax2-Cre;Vangl2 CKO mutant mice in which gene deletion is restricted to tissues that include the inner ear, approximately 30% of SGN2 processes turn incorrectly and project towards the apex of the cochlea. Similar errors occur in other PCP mutants including CELSR1 KO mice suggesting a role for the non-canonical Wnt-signaling pathway in SGN2 turning. Using a series of additional Cre lines to further restrict Vangl2 gene deletion to either the neurons or the cochlear duct, we demonstrate that Vangl2 functions in a non-cell autonomous manner and is not required in the growth cone for turning. While these experiments are focused on developmental processes guiding process outgrowth and target cell innervation we anticipate that these events must be recapitulated during hair cell re-innervation and repair, and therefore are significant for restoring function to the deafened cochlea.

Disclosures: **M.R. Deans:** None. **S.R. Ghimire:** None.

Nanosymposium

379. Axon and Dendrite Development and Regeneration

Location: SDCC 30B

Time: Monday, November 14, 2016, 1:00 PM - 3:45 PM

Presentation Number: 379.03

Topic: A.05. Axon and Dendrite Development

Support: NINDS NS047484

NINDS NS046357

NINDS NS095615

JSPS Postdoctoral Fellowship for Research Abroad

Title: Sonic Hedgehog is a midline switch for Wnt/planar cell polarity signaling in commissural axons

Authors: *K. ONISHI, Y. ZOU;
Div. of Biol. Sci., Univ. of California San Diego, La Jolla, CA

Abstract: A widely used strategy in axon wiring is to break down complex trajectory into segments separated by intermediate targets. Pathfinding axons change responsiveness to guidance cues at these intermediate targets and take on new directions. We report here a novel gene-expression-based switch mechanism. Sonic Hedgehog (Shh)-Smoothened signaling suppresses the expression of mShisa2 in commissural neurons, which inhibits glycosylation and cell surface presentation of Frizzled3, essential for their proper anterior-posterior (A-P) guidance after midline crossing. mShisa2 expression in commissural neurons using a heterologous promoter causes randomized growth of post-crossing commissural axons along the A-P axis. This novel regulatory loop between Shh and PCP signaling may also be essential in many other developmental processes.

Disclosures: K. Onishi: None. **Y. Zou:** None.

Nanosymposium

379. Axon and Dendrite Development and Regeneration

Location: SDCC 30B

Time: Monday, November 14, 2016, 1:00 PM - 3:45 PM

Presentation Number: 379.04

Topic: A.05. Axon and Dendrite Development

Support: March of Dimes 6-FY10-296

NIH NS 063999, NS 085097

Title: Netrin1 establishes short-range axon guidance boundaries in the developing spinal cord

Authors: *S. G. VARADARAJAN[1], J. H. KONG[1], K. D. PHAN[1], C. S. PANAITOF[3], J. CARDIN[4], A. KANIA[4], B. G. NOVITCH[2], S. J. BUTLER[2];
[1]Neurobio., [2]Dept. of Neurobio., UCLA, Los Angeles, CA; [3]Dept. of Biol., Univ. of Nebraska, Kearney, NE; [4]Inst. de Recherches Cliniques de Montreal, Montreal, QC, Canada

Abstract: Localized diffusible chemotropic signals are canonical sources of guidance information for axons extending towards their synaptic targets. Equally important, but less well

understood, are contact-dependent regional boundaries that provide either permissive or non-permissive substrates for axon growth. Studies using the developing vertebrate spinal cord as a model system have identified the key secreted cues that act from the dorsal and ventral midlines to orient dorsal commissural axons. However, all spinal axons grow precisely around the ventricular zone (VZ) suggesting that the edge of the VZ represents a “hederal” axon growth boundary: it orients axon extension, promotes fasciculation and prevents local innervation. This boundary appears to be mediated by netrin1: netrin1 is present in the VZ where it extends into the dorsal spinal cord and axons project aberrantly into the VZ in absence of netrin1. Our studies suggest that different populations of spinal neurons use specific combinations of Unc5 and/or Dcc to avoid the VZ and that Dcc is the key receptor mediating netrin1 signal. Classic work has demonstrated the importance of netrin1 as a floor plate chemoattractant; our studies now suggest that netrin1 also defines a “hederal” growth boundary in the VZ that promotes axon fasciculation while preventing inappropriate innervation. Understanding the developmental mechanisms by which extrinsic guidance signals direct the formation of neural circuits is key to developing therapeutic strategies to promote regeneration after injury.

Disclosures: **S.G. Varadarajan:** None. **J.H. Kong:** None. **K.D. Phan:** None. **C.S. Panaitof:** None. **J. Cardin:** None. **A. Kania:** None. **B.G. Novitch:** None. **S.J. Butler:** None.

Nanosymposium

379. Axon and Dendrite Development and Regeneration

Location: SDCC 30B

Time: Monday, November 14, 2016, 1:00 PM - 3:45 PM

Presentation Number: 379.05

Topic: A.05. Axon and Dendrite Development

Support: ERC StG#311159 - ZebraTectum

Title: A gradient of Reelin regulates axonal lamination in the vertebrate visual system

Authors: *F. DEL BENE[1], V. DI DONATO[1], T. AUER[1], K. DUROURE[1], J.-P. CONCORDET[2];
[1]Inst. Curie - Ctr. de Recherche, Paris, France; [2]Muséum Natl. d'Histoire Naturelle, Paris, France

Abstract: Neural circuits require the formation of precise connections between neurons to be functional. Axons and dendrites of functionally related neuronal populations form synapses, which are often spatially organized in discrete layers. In the vertebrate visual system, the clustering in synaptic laminae of projections from retinal afferents to retino-recipient areas promotes a rapid establishment of the network. One way to achieve a fast and precise axonal targeting is hypothesized to be the pre-patterning of neuropil layers by extracellular factors, as

the repulsive Slit1. However, other guidance molecules involved in this process are unknown. Here we show that one major guidance cue, the extracellular matrix (ECM) protein reelin, imparts, through a concentration gradient, lamina-specific positional information on retinal axons. Taking advantage of the zebrafish retinotectal system as a model, we demonstrate that the reelin secreted by a class of superficial inhibitory neurons (SINs) in the optic tectum exerts an attractive role on Retinal Ganglion Cell (RGC) axons via a signaling cascade mediated by very-low-density lipoprotein (VLDLR) receptor and disabled-1. Furthermore, we found that the spatial distribution of reelin in the tectal neuropil requires heparane sulphate phosfoglycans, unlikely Slit1, guarantying anchoring of the protein at the basement membrane. Finally, by genetically inducing a source of reelin contrasting the endogenous gradient we perturbed axonal lamination, suggesting that single RGCs need a gradient distribution to target a discrete layer. Together, our results prove that the Reelin/VLDLR signaling pathway functions synergistically with Slit1/Robo2 pathway to provide a correct axonal lamination pattern during the development of the vertebrate visual system.

Disclosures: **F. Del Bene:** None. **V. Di Donato:** None. **T. Auer:** None. **K. Duroure:** None. **J. Concordet:** None.

Nanosymposium

379. Axon and Dendrite Development and Regeneration

Location: SDCC 30B

Time: Monday, November 14, 2016, 1:00 PM - 3:45 PM

Presentation Number: 379.06

Topic: A.05. Axon and Dendrite Development

Support: NIH RO1NS062047

Title: MAP7 regulates microtubule bundles during development of axon collateral branches

Authors: ***S. TYMANSKYJ**, B. YANG, L. MA;
Thomas Jefferson Univ., Philadelphia, PA

Abstract: Microtubule bundles are found throughout neurons and can be affected in pathological conditions, but their roles in axonal development and the impact on neural circuit formation is largely unclear. We report a regulatory mechanism mediated by microtubule-associated protein 7 (MAP7 also known as ensconsin) that is critical for collateral branch development of dorsal root ganglion (DRG) sensory neurons. First, MAP7 expression in DRG neurons starts at the inception of collateral branch formation. Second, precocious over-expression of MAP7 results in an increase in branching in cultured DRG neurons, whereas shRNA knockdown leads to a decrease in overall branching. Furthermore, a spontaneous mouse mutant expressing truncated MAP7

(*mshi*) produces increased collateral branches both in culture and *in vivo* identifying the domain responsible for collateral branch formation. Interestingly, both MAP7 and the truncated form promote microtubule bundling both in COS cells and in vitro. Live imaging reveals that these bundles are stable but MAP7 association lags behind EB3-labeled growing plus ends. In axons, MAP7 specifically localizes to branch sitesbut enters into the branch with a delay, revealing a role of MAP7 in branch maturation. Finally, function and localization analysis of a specific phosphorylation site responsible for regulating bundling activity and branch formation. Analysis of phospho-mutants indicates that this site is transiently dephosphorylated during branch initiation and later re-phosphorylated to promote branch stabilization. Together, our study demonstrates the first neuronal function of MAP7 and identifies a mechanism for microtubule bundle regulation during axonal development.

Disclosures: **S. Tymanskyj:** None. **B. Yang:** None. **L. Ma:** None.

Nanosymposium

379. Axon and Dendrite Development and Regeneration

Location: SDCC 30B

Time: Monday, November 14, 2016, 1:00 PM - 3:45 PM

Presentation Number: 379.07

Topic: A.05. Axon and Dendrite Development

Title: Heterogeneity of the axon initial segment in interneuron and pyramidal cells of rat visual cortex

Authors: F. HOEFFLIN[1], A. JACK[4], J. BUCHER[2], C. SCHULTZ[3], P. WAHLE[4], *M. ENGELHARDT[1];
[2]LIMA Core, [3]Inst. of Neuroanatomy, [1]Univ. Heidelberg, Med. Fac. Mannheim, Mannheim, Germany; [4]Developmental Neurobio., Ruhr-University Bochum, Bochum, Germany

Abstract: In polarized neurons, the neuronal domain that orchestrates action potential initiation is the axon initial segment (AIS). In most cortical neurons, the AIS is located at the proximal axon, in relative proximity to the soma. Morphological studies in the recent past have shown that in various cortical regions such as the somatosensory, visual or cingulate cortex, the AIS appears as a more or less homogeneous domain. This has been especially obvious in cortical pyramidal cells, which are predominantly perpendicular in their orientation towards the pial surface. However, different studies that analyze either various brain regions or are based on *in vitro* applications paint a different picture. In the hippocampal CA1 region for example, up to 50% of AIS emerge from distal dendritic branches. In these particular cells, AIS are separated from the soma by a visible gap. Another parameter that seems to vary is overall AIS length, and *in vitro* and *in vivo* studies have shown a direct correlation of AIS length and position with cellular

excitability. We set out to investigate AIS morphology in greater detail, hypothesizing that the initial observation of seemingly homogeneous AIS in a given cortical region is inadequate and depends on which neuronal class is investigated: pyramidal neuron or interneuron. To test this hypothesis, we labeled cortical neurons in organotypic cultures derived from visual cortex with biolistic gene transfer to map the entire neuron and classify the type by established morphological criteria, in combination with immunolabeling of the AIS. Using confocal microscopy and morphometric analysis, we analyzed parameters that have been correlated with functional aspects such as AIS position and length. Cortical interneurons have not been systematically studied so far because their axons may arise from the soma as well as from even third order dendritic branches, and a subpopulation of cells issues more than one axon. Surprisingly, we find a substantial AIS heterogeneity in visual cortex. Pyramidal neurons have significantly longer and more distal AIS than interneurons. Noncanonical AIS phenotypes are observed, ranging from multiple branch points to what we term AIS “extra domains”. The latter show spot-like immunoreaction to AIS markers and appear often at a remarkable distance to the distal end of the actual AIS. Our data contribute to the emerging understanding that AIS morphology and also plasticity can have a significant impact on neuron function and thus, network formation and maintenance. In fact, the observed AIS heterogeneity could be a significant contributor to local cell adaptation and function and thus merits further investigation.

Disclosures: **F. Hoefflin:** None. **A. Jack:** None. **J. Bucher:** None. **C. Schultz:** None. **P. Wahle:** None. **M. Engelhardt:** None.

Nanosymposium

379. Axon and Dendrite Development and Regeneration

Location: SDCC 30B

Time: Monday, November 14, 2016, 1:00 PM - 3:45 PM

Presentation Number: 379.08

Topic: A.05. Axon and Dendrite Development

Support: Packard Center for ALS Research

Travis Roy Foundation

Massachusetts Department of Public Health - SCI cure grant

ALS Association

Title: Hierarchical ordering of molecular controls over corticospinal motor neuron segmental targeting: implications for evolution of motor control

Authors: *V. V. SAHNI, S. SHNIDER, D. JABAUDON, J. SONG, J. MACKLIS;
Stem Cell and Regenerative Biol., Harvard Univ., Cambridge, MA

Abstract: The corticospinal system controls performance of highly skilled and complex movements. Underlying this precise motor control, corticospinal motor neurons (CSMN), extend axons to and innervate distinct target spinal cord segments - from rostral targets in the brainstem and cervical cord (controlling face and arm movements), to caudal targets in the thoracic and lumbar cord (controlling hindlimb movements). The molecular basis for this segmentally specific connectivity is unknown.

Parallel segmental specificity is present in motor neuron diseases (MNDs). CSMN degeneration MNDs such as amyotrophic lateral sclerosis (ALS) causes spasticity and paralysis. However, MNDs do not affect all CSMN equally. In bulbar forms of ALS, for instance, CSMN projecting to the brainstem degenerate, causing craniofacial weakness and spasticity, while in hereditary spastic paraplegia (HSP), lumbar-projecting CSMN degenerate, causing leg weakness and spasticity. The basis for this heterogeneity in different MNDs is unknown.

We isolated functionally distinct CSMN subpopulations during development - $CSMN_C$, projecting to brainstem and cervical cord, and $CSMN_L$, projecting to thoraco-lumbar-cord - and identified differentially expressed genes between them. Using this approach, we identified novel controls that direct CSMN axons to appropriate spinal levels - bulbo-cervical by $CSMN_C$ and thoraco-lumbar by $CSMN_L$. Interestingly, some of these molecular controls associate with subtype-specific forms of CSMN disease in humans.

We identify what appears to be a hierarchical order of controls over CSMN segmental targeting. $CSMN_C$ express specific molecular controls that suppress the expression of $CSMN_L$-specific controls. These $CSMN_C$ genes appear more recently in evolution (orthologs are present only in vertebrates), and are expressed in evolutionarily newer regions of the cortex as compared to $CSMN_L$-specific genes. Further, analysis of the recently available transcriptome of the developing human brain suggests that spatiotemporal expression of these $CSMN_C$ genes is conserved in humans. These results raise the intriguing possibility that $CSMN_C$-specific controls evolved to suppress potentially older "default" $CSMN_L$-specific controls, enabling increased diversity of CSMN projecting to the most dexterous control circuits in the cervical cord, and functionally distinguishing $CSMN_C$ from evolutionarily older $CSMN_L$ subpopulations.

Together, these newly identified controls constitute new mechanisms directing CSMN axonal targeting. This provides foundation for further investigation of mechanisms directing development and evolution of corticospinal circuitry.

Disclosures: V.V. Sahni: None. **S. Shnider:** None. **D. Jabaudon:** None. **J. Song:** None. **J. Macklis:** None.

Nanosymposium

379. Axon and Dendrite Development and Regeneration

Location: SDCC 30B

Time: Monday, November 14, 2016, 1:00 PM - 3:45 PM

Presentation Number: 379.09

Topic: A.05. Axon and Dendrite Development

Support: Intramural research program of the NINDS

Title: Developmental regulation of dendritic dynamics and growth in *Drosophila* larval visual circuit

Authors: *C. SHENG, U. JAVED, J. YIN, B. QIN, C. LONG, Q. YUAN;
NINDS, NIH, Bethesda, MD

Abstract: Highly dynamic dendritic branches are observed in young vertebrate neurons. Their motility and density are determined by the maturity of the neuron, also strongly influenced by the synaptic activity. Previous studies suggested that the exploratory dendrite branches likely serve important functions in regulating dendrite growth and synaptogenesis during early development. However, molecular mechanisms controlling the developmental and activity-dependent regulation of dendrite dynamics are not well understood.
We are using genetic approaches to study activity-dependent plasticity in the developing *Drosophila* larval visual circuit, where dendritic arbors of ventral lateral neurons (LNvs), the postsynaptic targets of larval photoreceptors, exhibit robust structural plasticity when the animal is subjected to different visual experiences. Using high-resolution time-lapse imaging studies on single labeled LNvs in the intact larval brain, we developed a system to study dendrite dynamics in a genetic model. Dynamic branches in LNv dendrites were observed throughout larval development, while being highly prevalent in young larvae and significantly reduced in later stages. In addition, we also observed homeostatic regulation of dendrite dynamics by visual experience, where excessive visual inputs strongly suppressed branch dynamics even at the younger stage. Through a large scale in vivo transgenic RNAi screen followed by genetic studies using mutant flies, we identified candidate cytoskeleton-associated proteins specifically required for the regulation of dendrite dynamics without affecting dendrite growth at early developmental stages. Together, our data revealed the presence of the exploratory dynamic branches on synapse-forming dendrites in the *Drosophila* central nervous system, and identified specific cellular and molecular components involved in the regulation of branch dynamics during dendrite development.

Disclosures: C. Sheng: None. **U. Javed:** None. **J. Yin:** None. **B. Qin:** None. **C. Long:** None. **Q. Yuan:** None.

Nanosymposium

379. Axon and Dendrite Development and Regeneration

Location: SDCC 30B

Time: Monday, November 14, 2016, 1:00 PM - 3:45 PM

Presentation Number: 379.10

Topic: A.05. Axon and Dendrite Development

Support: Howard Hughes Medical Institute

Khan Nanotechnology Development Grant, Duke University

Title: Independent and overlapping ankyrin-B- and β2-spectrin-based mechanisms control axonal organelle transport and development of long axonal tracts

Authors: *D. N. LORENZO[1,3], V. BENNETT[1,3], A. BADEA[2];
[1]Biochem., [2]Radiology, Duke Univ., Durham, NC; [3]Howard Hughes Med. Inst., Durham, NC

Abstract: Long axonal projections require coordinated long-range organelle transport for their formation and maintenance. However, how axonal cargos recruit their motors and how their traffic is regulated is not fully resolved. Ankyrin-B (AnkB) promotes fast axonal transport and axon elongation by coupling dynactin to multiple organelles through binding to phosphatidylinositol 3-phosphate lipids.[1] Additionally, AnkB is required for maintaining the local concentration of its obligatory partner β2-spectrin, which, in turn, is required for the formation of a periodic sub-membranous lattice in axons.[2] Interestingly, β-spectrins also associate either directly or indirectly with kinesin and dynein motors.[3, 4] Here, we report AnkB and β2-spectrin are each key elements in pathways that are both independent and overlapping, which together are responsible for axonal transport of synaptic cargo and other organelles, and are essential for establishing proper brain connectivity. Cultured hippocampal neurons from either AnkB null (*AnkB*$^{-/-}$) or β2-spectrin null (*β2-Sp*$^{f/f}$*; Nestin-Cre/+*) mice each show significant reduction in the transport of multiple organelles and axonal length, where AnkB loss primarily affects retrograde transport and β2-spectrin loss affects bidirectional motility. The axonal transport phenotypes of AnkB null neurons can be rescued by expression of wild-type 220 kDa AnkB, but only partially by AnkB mutants unable to associate with β2-spectrin. Similarly, loss of β2-spectrin linkages to AnkB partially impaired organelle transport. Strikingly, dual loss of AnkB and β2-spectrin by expressing Cre-recombinase in neurons of *AnkB*$^{f/f}$*;β2-Sp*$^{f/f}$ mice causes total abrogation of both anterograde and retrograde axonal transport, and loss of dynactin association with intracellular membranes. Gene dosage-dependent reductions in AnkB and β2-spectrin levels in mice lead to severe loss of motor coordination, tremors, smaller bodies and shortened lifespan, while loss of both AnkB and β2-spectrin is embryonic lethal. Diffusion Tensor Imaging or histological characterization of AnkB- or β2-spectrin-null brains reveals severe defects in the development of long fiber tracts. AnkB and β2-spectrin therefore function both independently and in collaboration as adaptors between motors and their cargos that are

essential for axonal transport and development. Cited Bibliography 1- Lorenzo *et al.* (2014). J Cell Biol. 207(6):735-52. 2- Zhong *et al.* (2014). Elife. eLife.04581. 3- Takeda *et al.* (2000). J Cell Biol. 148(6):1255-65. 4- Holleran *et al.* (1996). J. Cell Biol. 135:1815-1829.

Disclosures: D.N. Lorenzo: None. **V. Bennett:** None. **A. Badea:** None.

Nanosymposium

379. Axon and Dendrite Development and Regeneration

Location: SDCC 30B

Time: Monday, November 14, 2016, 1:00 PM - 3:45 PM

Presentation Number: 379.11

Topic: A.05. Axon and Dendrite Development

Support: Max-Planck Society

Deutsche Forschungsgemeinschaft (Synergy)

Title: EphB2 released via extracellular vesicles, a contact-independent signaling mechanism in axon guidance

Authors: *J. GONG[1], R. KÖRNER[2], R. KLEIN[1];
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Abstract: Eph receptors comprise the largest subfamily of receptor tyrosine kinases and together with their membrane-tethered ligands, the ephrins, they function in many different physiological processes including stem and progenitor cell migration, axon guidance, vascular development, synaptic plasticity, and also pathological processes such as diabetes, cancer and neurodegeneration. Ephrin-Eph signaling requires direct cell contact and is bi-directional: ephrin to Eph signaling is called forward signaling, while Eph to ephrin signaling is called reverse signaling. Biological functions of ephrin-Eph signaling are well understood, whereas our mechanistic understanding is modest. In an effort to learn more about the mechanisms of Eph receptor signaling, we analyzed the interaction proteome of activated Eph complexes in cultured cells and identified members of the ESCRT complex as the strongest EphB2 interactors. Interestingly, we found that endogenous Ephs/ephrins are released to extracellular vesicles (EVs) from gliobastoma U-251MG cells and primary neurons. Cells release membranous vesicles known as EVs or exosomes that represent a novel mode of intercellular communication. We find that biogenesis of Eph- and ephrin-loaded exosomes are modulated by the function of endosomal sorting complex responsible for transport (ESCRT)-machinery. Importantly, Eph-containing exosome preparations are specifically recognized and internalized by ephrin-expressing cells.

This process activates ephrin tyrosine phosphorylation suggestive of reverse signaling in the recipient cell. We further demonstrated that EphB2 containing EVs are able to induce neuronal growth cone collapse, an established assay for repulsive axon guidance. These findings uncover a novel concept of ephrin-Eph signaling at a distance via EVs, in addition to the canonical cell-cell contact dependent bi-directional signalling. It may be a mechanism to establish functional Eph/ephrin gradients for topographic map formation during development and to mediate synaptic plasticity in the adult. It may also modulate Eph/ephrin function in disease.

Disclosures: J. Gong: None. **R. Körner:** None. **R. Klein:** None.

Nanosymposium

380. Mechanisms and Role of Synaptic Pathology in Alzheimer's Disease

Location: SDCC 24A

Time: Monday, November 14, 2016, 1:00 PM - 3:00 PM

Presentation Number: 380.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BrightFocus Foundation Grant A2013328S00

The Cheryl and Haim Saban Family Foundation

The Marciano Family Foundation

Title: Copaxone induces macrophage clearance of amyloid-beta42 oligomers and preserves synapses in AD models

Authors: *M. KORONYO-HAMAOUI[1], S. LI[1,2], Y. KORONYO[1], D. DALEY[1], E. Y. HAYDEN[3], J. SHEYN[1], D.-T. FUCHS[1], T. TORBATI[1,3], A. RENTSENDORJ[1], D. B. TEPLOW[3], K. L. BLACK[1];
[1]Neurosurg., Cedars-Sinai Med. Ctr., Los Angeles, CA; [2]Inst. of Life Sci., Wenzhou Univ., Wenzhou, China; [3]Neurol., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Background. The devastating synaptic loss seen in Alzheimer's disease (AD) is tightly associated with accumulation of amyloidogenic forms of the 42-residue long amyloid β-protein ($A\beta_{42}$). Immunization with Copaxone (also known as Cop1) or blood enrichment with CD115- monocytes lead to reduced cerebral Aβ levels, synaptic preservation and attenuation of cognitive decline in transgenic AD (ADtg) mouse models. These benefits are attributed to increased cerebral recruitment of monocyte-derived macrophages that were shown to engulf and remove Aβ plaques and $A\beta_{42}$ fibrils. However, investigating the cellular effects of the non-fibrillar forms of $A\beta_{42,}$ especially the small-size oligomers, has been very challenging due to their highly metastable and polydisperse nature. Thus, the exact effects of these oligomers on synaptotoxicity

and the ability of innate immune cells to resist them are poorly understood.
Methods. To measure the impact of $A\beta_{42}$ oligomers and fibrils on synapses, we used stable populations of structurally characterized oligomers (cross-linked by PICUP), pre-formed fibrils, and scrambled $A\beta_{42}$ controls. We assessed synaptic and neuritic length integrity in primary postnatal day 1 cortical neurons. Further, synaptic protection was assessed *in vitro* by co-culture with Cop1-stimulated bone marrow-derived macrophages versus untreated macrophages, and *in vivo* in ADtg mice brain following Cop1 immunization or adoptive transfer of CD115 monocytes.
Results. We found that primary cortical neurons exhibit a conformation-dependent susceptibility to $A\beta_{42}$ fibrils, and, moreover, oligomers, which triggered neuritic retraction and massive loss of pre-VGluT1 and post-PSD95 synaptic density. Co-culturing primary neurons with Cop1-treated macrophages substantially protected against these effects by way of increasing members of scavenger receptors and early endosomal-mediated macrophage clearance of $A\beta_{42}$ assemblies. Increased extracellular degradation of $A\beta_{42}$ by Cop1-treated macrophages was also found to be structurally dependent. A detailed analysis of hippocampal and entorhinal cortex substructures in ADtg mice indicated significant pre- and post-synaptic losses, which were reversed by Cop1 and blood grafted CD115-monocyte immunomodulation. Synaptic protection correlated with reduced neuropathology and retained cognition.
Conclusion. Our data suggest that $A\beta_{42}$ exerts synaptotoxicity depending on its conformational structure, and that Cop1 induces a neuroprotective phenotype in macrophages through distinct intra- and extra-cellular mechanisms that enhance clearance of $A\beta_{42}$ fibrils and oligomers.

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Nanosymposium

380. Mechanisms and Role of Synaptic Pathology in Alzheimer's Disease

Location: SDCC 24A

Time: Monday, November 14, 2016, 1:00 PM - 3:00 PM

Presentation Number: 380.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG048077

Alzheimer's Association Grant NIRG-13-283742

Title: ZCCHC17 impairment is a potential disrupter of neuronal activity in Alzheimer’s disease

Authors: *A. F. TEICH[1], Z. TOMLJANOVIC[2], M. PATEL[3];
[1]Pathology and Cell Biology; Taub Inst. for the Aging Brain, [2]Pathology and Cell Biol., Columbia Univ., New York, NY; [3]Mem. Sloan Kettering Cancer Ctr., New York, NY

Abstract: Our laboratory has recently identified ZCCHC17 (or pNO40) as a potential key player in the pathogenesis of late-onset Alzheimer's disease (LOAD). ZCCHC17 was discovered in 2002, and there is limited literature on this protein. It has previously been localized to the nucleus as well as the nucleolus in a variety of tissues, and its structure implies possible roles in RNA processing as well as in ribosomal genesis/function. In addition, it was recently identified as being consistently down-regulated in a meta-analysis of RNA expression data from LOAD brain tissue. Our laboratory is using bioinformatic tools to investigate potential master regulators of gene expression in cortical neurons that are disrupted in Alzheimer's disease (AD). In our analysis, ZCCHC17 is ranked highly in two important ways: 1) As a potential master regulator of gene expression generally, and 2) As a potential master regulator of synaptic genes specifically. Our analysis predicts that ZCCHC17 has impaired activity in AD, and that this impairment may partially explain dysregulation of synaptic gene expression in AD. We have now identified 38 synaptic genes that are predicted to be regulated by ZCCHC17 in human neurons and that are subsequently impaired when ZCCHC17 is knocked-down in rat cortical cultures. Of these 38 synaptic genes, 28 also have decreased mRNA in human AD brain tissue. This both validates ZCCHC17 as a potential master regulator of synaptic genes in AD and also suggests a preserved function of ZCCHC17 in synaptic function across species. To test the effects of ZCCHC17 knock-down on neuronal activity, we performed both patch clamp and calcium imaging in rat cortical cultures after ZCCHC17 knock-down. Our results show that ZCCHC17 knock-down leads to an impairment in excitation (by calcium imaging) as well as an impairment in potassium current (by patch clamp). Taken together, our results support the hypothesis that ZCCHC17 dysfunction contributes to a disruption of neuronal activity in LOAD.

Disclosures: **A.F. Teich:** None. **Z. Tomljanovic:** None. **M. Patel:** None.

Nanosymposium

380. Mechanisms and Role of Synaptic Pathology in Alzheimer's Disease

Location: SDCC 24A

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Presentation Number: 380.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Mitchell Center for Neurodegenerative Diseases

NIH Grant NIA R01 AG042890

Title: Inhibition of mammalian phospholipase D isoform 1 prevents amyloid beta oligomer driven synaptic dysfunction and memory deficits in rodents

Authors: *B. KRISHNAN, W.-R. ZHANG, G. TAGLIALATELA;
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Abstract: Alzheimer's disease (AD), the most common and severe age-associated neurodegenerative dementia, currently affects one in every nine Americans >65 years of age and one in every three >85 years. There is currently no cure and the need to identify innovative targets for prevention and treatment are an urgent need. The accumulation of β-amyloid peptides (Aβ) at the synaptic level is an important mechanism that leads to the progression of cognitive decline, subsequent neuronal degradation and other hallmarks that characterize the loss of long-term memory mechanisms in the progression of AD. Recent studies from our group have demonstrated a role for phospholipase D (PLD) as a key signaling element in the maintenance of long-term memory. Previously, we presented data showing differential expression of PLD isoforms (PLD1 and PLD2) in synaptosomal fractions in AD brains suggesting a role for PLD isoforms in mediating synaptic dysfunction associated with AD progression. In the present study, we demonstrate a role for PLD1 inhibition in blocking Aβ oligomer (Aβo) mediated synaptic dysfunction using electrophysiological and behavioral studies. Using Novel Object Recognition, we observed that the deficit in object discrimination associated with intracereberoventricular injections of Aβo was prevented by intraperitoneal injection of PLD1 inhibitor. We, further, explore the epigenetic regulation of PLD1 and downstream signaling supporting our rationale for exploring the role of PLD1 signaling as a possible biomarker/therapeutic target in preventing the progression of memory deficits in AD.
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Disclosures: B. Krishnan: None. **W. Zhang:** None. **G. Taglialatela:** None.

Nanosymposium

380. Mechanisms and Role of Synaptic Pathology in Alzheimer's Disease

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Presentation Number: 380.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA Grant 1RO3AG04753701A1 (MAM)

NIH/NIA Grant 1R01AG042890 (GT)

Title: NSC-derived exosomes reduce hippocampal synapses vulnerability to the dysfunctional impact of amyloid beta oligomers.

Authors: *M.-A. MICCI[1], B. KRISHNAN[2], W.-R. ZHANG[2], E. BISHOP[1], S. G. KERNIE[3], C. ANACKER[4], R. HEN[4], G. TAGLIALATELA[2];
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Abstract: Alzheimer's disease (AD) is the most common and severe age-associated neurodegenerative dementia for which finding a resolving cure is a pressing national priority. The recent discovery that certain individuals remain cognitively intact despite the presence of neuropathology associated with a fully symptomatic stage of the disease suggests that there is a way for the brain to evade dementia even in the face of AD. It follows that understanding the mechanism(s) involved in such extraordinary resistance would reveal targets for the development of a novel, effective therapeutic concept based on inducing cognitive resilience in anyone challenged with AD neuropathology. We have discovered that brain synapses in these unaffected subjects are resistant to the disruptive binding of the toxic amyloid beta (Aβ) oligomer (an event linked to onset of dementia in AD), and that this resistance is associated with the presence of higher numbers of neural stem cells (NSC) in the hippocampus. While these observations suggest a link between increased neurogenesis and synaptic resistance to Aβ, the involved mechanism (an important possible treatment target) remains to be determined. With this goal in mind, in the present work, we investigated the effect of NSC-derived exosomes (NSC-exo; small secreted vesicles containing cell-specific cargos of proteins, lipids and genetic material, that are transported to other cells and modulate their function and physiology) in affecting synaptic physiology and promoting synaptic resistance to Aβ oligomer binding using both *ex vivo* and *in vivo* models. We found that synaptic excitability was inhibited in NSC-exo-treated hippocampal slices via a GABA-dependent mechanism. We also found that hippocampal synapses in slices or animals treated icv with NSC-exo were significantly less vulnerable to Aβ oligomer binding. Furthermore, transgenic mice in which neurogenesis (and number of NSC) was suppressed showed increased Aβ oligomer binding on hippocampal synapses, a phenomenon which was reversed by treatment with NSC-exo. On the other hand, transgenic mice in which NSC number was conditionally increased showed reduced Aβ binding on hippocampal synapses. These results identify a novel mechanism, mediated by NSC-exo modulation of synaptic susceptibility to the dysfunctional impact of Aβ oligomers, that may underscore the ability of certain individuals with increased neurogenesis to resist the cognitive decline normally associated with AD neuropathology and unmask a previously unappreciated target for the development of a new treatment concept for AD centered on NSC-derived exosome delivery.

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Nanosymposium

380. Mechanisms and Role of Synaptic Pathology in Alzheimer's Disease

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Time: Monday, November 14, 2016, 1:00 PM - 3:00 PM

Presentation Number: 380.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 5R01AG046275

Title: The plasticity disrupting activity of Abeta requires expression of the amyloid precursor protein

Authors: *Z. WANG[1], W. HONG[2], A. SERONO[2], T. WALTER[2], W. LIU[2], T. T. O'MALLEY[2], S. LI[2], T. YOUNG-PEARSE[2], D. M. WALSH[2];
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Abstract: Alzheimer's disease (AD) is a progressive and fatal neurodegenerative disease that mostly affects persons over the age of 65 years. Genetic studies on familial AD indicate that the amyloid precursor protein (APP) and its metabolites, including the amyloid β-protein (Aβ), play a central role in AD. It has been previously proposed that APP could serve as receptors for Aβ and that binding of Aβ to APP may be mediate all or some of the negative effects attributed to Aβ. Studies using synthetic Aβ and brain extracts containing Aβ indicate that oligomeric forms of Aβ can suppress long-term potentiation (LTP) but the mechanisms by which Aβ mediates this effect remain obscure.
Here we used aqueous (Aβ-containing) extracts from brain tissue of individuals who died with very mild forms of AD. Grey matter from Temporal cortex, a region implicated in the early stages of AD, was homogenized in artificial cerebrospinal fluid (ACSF) and portions treated with a pan anti-Aβ polyclonal antibody to remove Aβ. The effect of AD brain extracts (+/- immunodepletion) on LTP and excitatory-inhibitory (E/I) tone were then investigated using hippocampal brain slices from wild type (WT) and APP knock-out (APP KO) mice. Aβ-containing brain extracts, but not extracts immunodepleted of Aβ suppressed LTP in the hippocampus of WT mice, but neither extract effected LTP in hippocampi from APP KO mice. Moreover, whole cell voltage clamp recording to measure E/I balance of individual pyramidal neurons of the CA1 area revealed that AD brain extracts increased excitatory activity and decreased inhibitory input in WT, but not APP KO mice. Taken together our results indicate that the LTP-disruption mediated by brain-derived Aβ requires expression of APP and involves changes in homeostatic synaptic plasticity. This information should enable the development of agents to restore E/I balance and thus reduce synaptic degeneration.

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Nanosymposium

380. Mechanisms and Role of Synaptic Pathology in Alzheimer's Disease

Location: SDCC 24A

Time: Monday, November 14, 2016, 1:00 PM - 3:00 PM

Presentation Number: 380.06

Topic: C.02. Alzheimer's Disease and Other Dementias

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Education and Lifelong Learning, Supporting Postdoctoral Researchers" and "Large Scale Cooperative Project", co-financed by the European Social Fund (ESF) and the Greek General Secretariat for Research and Technology

Title: Microtubule-associated protein Tau is essential for stress-driven hippocampal pathology

Authors: *I. SOTIROPOULOS[1,2], S. LOPES[1,2], J. VAZ-SILVA[1,2], V. PINTO[1,2], C. DALLA[3], N. KOKRAS[3], B. T. BEDENK[4], M. CZISCH[4], O. F. X. ALMEIDA[4], N. SOUSA[1,2];
[1]ICVS, Sch. of Hlth. Sciences, Minho Univ., Braga, Portugal; [2]ICVS/3B's - PT Government Associate Lab., Braga/Guimarães, Portugal; [3]Dept. of Pharmacology, Med. Sch. of Athens, Athens, Greece; [4]Max Planck Inst. of Psychiatry, Munich, Germany

Abstract: Synaptic malfunction is a key pathomechanism in both depressive and Alzheimer´s disease (AD) pathologies with chronic stress and stress hormones, glucocorticoids (GC), being a risk factor for both disorders. Accumulating evidence suggests a continuum between depression, impaired cognition and AD raising stress, a well-known sculptor of brain plasticity, as potential connecting factor. As Tau protein and its hyperphosphorylation have been implicated in neuronal/synaptic malfunction in AD brain, we hereby assessed whether Tau plays a critical role in stress-driven depressive pathology and associated cognitive decline. For that purpose, we exposed Tau knock-out (Tau-KO) mice and their wild-type (WT) littermates in chronic unpredictable stress. Our recent findings demonstrate, for the first time, that stress- and GC-driven neuronal deficits in wild-type mice are accompanied by synaptic missorting of Tau and enhanced Fyn/GluN2B-driven synaptic signaling assessed by both molecular (WB) and ultrastructural (TEM) analysis. In contrast, mice lacking *Tau* (Tau-KO) Tau-KO mice are resilient to chronic stress exhibiting no depressive-like behavior and cognitive impairments while, in contrary to WT, stressed Tau-KO also did not display neuronal/synaptic atrophy,

reduction in synaptic plasticity and MRI-based neuronal activity. These findings identify Tau as an essential mediator in the orchestration of cellular cascades underlying dendritic and synaptic atrophy/loss in stress-evoked depressive pathology and associated cognitive deficits adding to our molecular understanding of how stress precipitates brain pathology.

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Nanosymposium

380. Mechanisms and Role of Synaptic Pathology in Alzheimer's Disease

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Presentation Number: 380.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Research Grant from BrightFocus Foundation

Title: Optogenetic restoration of disrupted slow oscillations halts amyloid deposition and restores calcium homeostasis in an animal model of Alzheimer’s disease

Authors: ***K. KASTANENKA**, S. S. HOU, N. SHAKERDGE, R. LOGAN, D. FENG, S. WEGMANN, J. M. HAWKES, X. CHEN, B. J. BACSKAI;
Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Slow oscillations are important for consolidation of memory during sleep, and Alzheimer’s disease (AD) patients experience memory disturbances. Thus, we sought to examine slow oscillation activity using the voltage-sensitive dye RH1691 in an animal model of AD (APP mice). Slow oscillations at 0.6Hz were disrupted in APP mice starting at 3 months of age. Soluble amyloid-beta was sufficient to disrupt the slow oscillations. Cortical GABA levels were low in APP mice and application of exogenous GABA restored the slow oscillations, indicating that aberrant excitatory activity within the cortical circuit was responsible for slow oscillation dysfunction. In addition, light activation of channelrhodopsin-2 (ChR2) expressed in excitatory cortical neurons restored slow oscillations by synchronizing neuronal activity. Using multiphoton microscopy, we performed longitudinal imaging of senile plaques and monitored intracellular calcium. Cytosolic calcium is a surrogate marker of neuronal activity and is normally tightly regulated. We had previously demonstrated that resting calcium levels measured with the genetically encoded calcium sensor YC3.6 were elevated in a subset of neurons in APP transgenics, and hypothesized that an effective treatment would restore calcium to control levels. Driving slow oscillation activity with optogenetics halted amyloid plaque deposition and

prevented calcium overload associated with this pathology. The treatment also restored the levels of GABA and GABA receptors that were downregulated in APP mice. In summary, these results demonstrate that restoring slow oscillatory activity in an AD animal model interferes with the progression of pathology. Furthermore, treatment of aberrant slow oscillation activity in AD patients might prevent neurodegenerative phenotypes and slow disease progression.

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Nanosymposium

380. Mechanisms and Role of Synaptic Pathology in Alzheimer's Disease

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Presentation Number: 380.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA (AG045571)

Northwestern University Alzheimer’s Disease Center grant from NIA (AG013854)

Title: Heightened synaptic integrity in elderly with exceptional memory capacity (SuperAgers)

Authors: O. MELÉNDEZ-FERNÁNDEZ[1], L. KUKREJA[1], S. WEINTRAUB[1], *C.-K. WU[3], E. BIGIO[2], E. ROGALSKI[1], M.-M. MESULAM[1], C. GEULA[1];
[1]Cognitive Neurol. and Alzheimer's Dis. Ctr., [2]Pathology, Northwestern Univ., Chicago, IL; [3]Neurol., Univ. of California - Irvine Sch. of Medici, Irvine, CA

Abstract: Decline in episodic memory is a common characteristic of aging. However, there is an alternative trajectory that resists the cognitive changes of normal aging. Recently, our center initiated investigations of a subset of individuals over age 80 with episodic memory performance equal to or better than cognitively average individuals 20-30 years younger. We have used the term “SuperAger” to refer to such individuals. SuperAgers show larger cortical volumes on MRI, less ApoE4, more von Economo neurons, and less Alzheimer’s disease (AD) pathology than their cognitively average peers. Synaptic loss is one of the strongest correlates of cognitive decline in the normal elderly and in AD. In fact, alterations in synapses have been shown to correlate with hippocampal-dependent memory impairment in both normal aging and AD. The purpose of this study was to investigate the integrity of synaptic proteins in postmortem brain tissue from cognitive SuperAgers, cognitively average elderly and AD patients. In normal aging and AD, region-specific alterations in synapses correlate with hippocampal-dependent memory

impairment. We examined levels of the post-synaptic proteins spinophilin and PSD95 in the hippocampus using Western blot analysis. Spinophilin is a marker of dendritic spines, which are sites of synaptic contact. PSD95 is a scaffolding protein, which plays a key role in synaptic plasticity through its interactions with NMDA and AMPA glutamate receptors necessary for long-term potentiation, learning, and memory. In accordance with previous reports, levels of post-synaptic proteins were substantially lower in AD compared to age-matched controls, with a certain level of variability. However, in SuperAgers, the trend was the opposite, where levels of spinophilin and PSD-95 were consistently higher than the control group. Our findings suggest that SuperAgers have hippocampal synapses that are unusually resilient to age-related involutional processes. The precise factors that contribute to higher synaptic integrity in SuperAgers remain to be elucidated.

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Nanosymposium

381. Motor Neuron Disease Mechanisms

Location: SDCC 32B

Time: Monday, November 14, 2016, 1:00 PM - 2:45 PM

Presentation Number: 381.01

Topic: C.05. Neuromuscular Diseases

Support: NIH

Title: Overexpression of the Cdk5 inhibitory peptide (CIP) in motor neurons delay disease and extend survival of a mouse model of amyotropic lateral sclerosis.

Authors: *B. BALACHANDRAN KRISHNAMMA[1], S. KESAVAPANY[2], S. SKUNTZ[3], V. SHUKLA[3], N. AMIN[3], M. BHASKAR[1], P. GRANT[1], H. PANT[1];
[1]Building 49, Room 2A35, 49 Convent Drive, MSC 4479, NIH, Bethesda, MD; [2]Singapore University, Singapore, 21 Lower Kent Ridge Rd, Singapore; [3]NINDS, NIH, Bethesda, MD

Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder characterized by the progressive loss of spinal motor neurons. While the aetiological mechanisms underlying the disease remain poorly understood, recent studies suggest that deregulation of Cdk5 activity associated with the hyperphosphorylation of tau and neurofilament (NF) proteins in mice expressing a mutant superoxide dismutase (SOD1(G37R)) linked to ALS. A Cdk5 involvement in motor neuron degeneration is supported by analysis of three SOD1 (G37R) mouse lines exhibiting perikaryal inclusions of NF proteins. Here, we tested the hypothesis that inhibition of Cdk5/p25 hyperactivation *in vivo*, is a neuroprotective factor during

ALS pathogenesis by crossing the new transgenic mouse line that over express CIP (Chat Cre/CIP) in motor neurons with the SOD1(G37R). We report that overexpression of CIP in the motor neuron significantly extends survival, improves motor deficits, and delays pathology in the spinal cord and in brain of SOD1 (G37R) mice. Furthermore, we find that overexpression of CIP in motor neurons significantly delays neuroinflammatory response. Taken together, these data suggest that CIP may serve as a novel therapeutic agent for the treatment of neurodegenerative diseases.

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Nanosymposium

381. Motor Neuron Disease Mechanisms

Location: SDCC 32B

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Presentation Number: 381.02

Topic: C.05. Neuromuscular Diseases

Support: ARISLA 2014. "CONSLA_Setting Of A Conditioning Model To Identify New Therapeutic Molecular Targets In Amyotrophic Lateral Sclerosis"

Title: Preconditioning induced by low doses of L-BMAA in SOD1-G93A mice modulates the ionic transporter NCX3 leading to a state refractory to ALS

Authors: ***G. PIGNATARO**, S. ANZILOTTI, G. SIMEONE, A. VINCIGUERRA, P. BRANCACCIO, P. CEPPARULO, N. GUIDA, L. ANNUNZIATO;
FEDERICO II UNIVERSITY OF NAPLES, NAPLES, Italy

Abstract: The present study characterized for the first time an animal model of preconditioning in ALS. Preconditioning (PC) is a phenomenon wherein a mild insult induces a cellular and tissue resistance to a later severe injury. Here we demonstrated that, PC, induced by low doses of L-BMAA, elicits gene expression changes leading to a state refractory to ALS. First of all we characterized the first preconditioning mouse model of ALS based on sub-threshold treatment with the toxin L-BMAA and then we demonstrated that the plasmamembrane exchanger Na^+/Ca^{2+}, NCX3, represents a target for setting on new strategies in ALS intervention. PC was induced by icv injection of low doses of L-BMAA. The effect of PC was evaluated on disease onset, on motor functions and on motoneurons in terms of functional declines and severity of

histological damage. L-BMAA-induced preconditioning prevented the downregulation of NCX3 in the spinal cord of G93A mice and reduced neuroinflammation. Interestingly, L-BMAA-induced preconditioning determined an increase of survival and a better behavioral motor task performance in G93A mice.These studies allowed us to setting on the first model of preconditioning in ALS and to candidate NCX3 as a new target for setting on new ALS therapies. In particular, the expression of NCX3 protein decreased in the amyotrophic lateral sclerosis affected areas during aging.Interestingly, the preconditioning treatment prevented the downregulation of NCX3. Moreover, we found an increase of NCX3 signaling in neuromuscular junctions of Preconditioned G93A compared to Vehicle-treated G93A mice. Most importantly, the preconditioning stimulus increased the survival rate and improved the motor performance skills. Mice bearing both G93A and NCX3+/- mutation showed a more severe worsening of their motor performances, an earlier onset of disease symptoms and a reduced size of gastrocnemious muscle compared to G93A mice. In conclusion, this study candidates NCX3 as a putative target in ALS intervention. The pharmacological activation or the overexpression of NCX3 could mitigate motor neurons degeneration by handling the deregulation in ionic homeostasis occurring in ALS.

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Nanosymposium

381. Motor Neuron Disease Mechanisms

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Topic: C.05. Neuromuscular Diseases

Support: NIH Grant 1R01NSO77863

TARGET-ALS

THIERRY LATRAN FOUNDATION

ASSOCIATION FRANCAISE CONTRE LES MYOPATHIES

Title: Monosynaptic excitatory inputs to spinal motoneurons are depressed in SOD1-G93A mice, model of Amyotrophic Lateral Sclerosis (ALS)

Authors: M. BACZYK, M. MANUEL, C. MARTINOT, N. DELESTREE, *D. ZYTNICKI; Paris Descartes Univ., Paris, France

Abstract: In ALS, dysfunction of the excitatory amino-acid transporter 2 in astrocytes could lead to a toxic accumulation of glutamate in the synaptic cleft of excitatory synapses (Rothstein et al., *Ann. Neurol.* 1995; Sasaki et al., *Acta Neuropathol.* 2000). Here we investigated whether this alters the size of EPSPs in motoneurons in a mouse model of ALS.
Experiments were carried out on SOD1-G93A mice (mSOD1) and on their controls (wtSOD1 mice) at P45-55, that is before degeneration onset. Mice were deeply anesthetized with pentobarbital sodium, artificially ventilated and curarized. Intracellular recordings of triceps surae (TS) motoneurons allowed recording monosynaptic EPSPs from proprioceptive Ia afferents and from descending mediolateral funiculus (MLF).
Ia monosynaptic EPSPs are significantly reduced in mutant mice compared to controls whether Ia EPSPs were induced by muscle vibration (5.1 ± 3.5 mV in mSOD1, N=33 vs 7.3 ± 4.8 mV in wtSOD1, N=31; p=0.02) or by nerve electrical stimulation (1.5 ± 0.8 mV in mSOD1, N=36 vs 2.2 ± 1.1 mV in wtSOD1, N=35; p=0.003). Similarly, descending monosynaptic EPSPs from MLF (electrically stimulated at 2.2x the threshold for EPSP appearance) were significantly reduced in mSOD1 (0.85 ± 0.2 mV, N=22) compared to wtSOD1 (1.20 ± 0.4mV, N=21; $p<0.001$). The resting membrane potential of motoneurons and their input resistance were unchanged.
Some motoneurons were also intracellularly filled with neurobiotin, and VGlut1 (excitatory proprioceptive inputs) and VGlut2 (other excitatory inputs) boutons were immunostained. The density (VGlut1,VGlut2) and size (VGlut1) of these boutons, on both soma and dendrites, were unchanged in mSOD1 compared to wtSOD1 mice. This indicates that the reduction of the EPSP size is not caused by neuroanatomical alterations, but rather by an impairment of the synaptic function.
We conclude that excitatory synapses to motoneurons are depressed in SOD1-G93A mice before the degeneration onset of the most vulnerable motoneurons. The fact that both proprioceptive and descending inputs are depressed points towards a postsynaptic mechanism.

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Nanosymposium

381. Motor Neuron Disease Mechanisms

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Topic: C.05. Neuromuscular Diseases

Support: NIH NINDS NS091836

Title: Regulation of motoneuron excitability in ALS

Authors: *S. M. ELBASIOUNY[1], T. GARRETT[2], K. QUINLAN[3], C. HECKMAN[3], S. DUKKIPATI[1];
[1]Neuroscience, Cell Biology, and Physiol., [2]Wright State Univ., Dayton, OH; [3]Northwestern Univ., Chicago, IL

Abstract: In ALS, spinal motoneurons experience numerous abnormalities in their cellular properties. These abnormalities include changes in the cell morphology, cell size, cell electrical properties, and ion channels' amplitudes. Despite these profound changes, the cell's net excitability remains unchanged, indicating that the motoneuron experiences several cellular disease and compensatory changes with opposing effects on the cell excitability that offset each other. In this study, we use computational modeling to examine how the motoneuron regulates its excitability in the disease. Specifically, we assess the separate effects of each of these cellular changes on the net excitability and show how they integrate to offset each other. Our simulations were able to reveal additional changes in the cell membrane passive and active properties. Model predictions were verified experimentally using immunohistochemistry methods. **Keyword(s):** ALS, MOTONEURON NEURODEGENERATION. **Support:** NIH NINDS NS091836

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Topic: C.05. Neuromuscular Diseases

Support: Target ALS

Thierry Latran Foundation

Association Française contre les Myopathies

Title: Excitability of adult spinal motor neurons in the FUS-P525L model of ALS

Authors: M. MARTINEZ-SILVA, D. ZYTNICKI, *M. MANUEL;
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Abstract: Alterations in motoneuron excitability have been reported in Amyotrophic lateral sclerosis (ALS), where excitotoxicity, due to a hyperexcitable state, has long been suspected as a disease mechanism. However, recent studies in mutant SOD1-G93A mice (Delestree et al.,

2014), show evidence that spinal motoneurons are not intrinsically hyperexcitable, and that Fast-type motor units (MUs) are affected before their muscle fibers become denervated (Manuel et al., SfN 2014).
In this context, the goal of this study is to analyze the excitability of motoneurons in a novel model of ALS, the FUS-P525L mouse (Sharma et al., 2016), around the time of muscle fiber denervation. For this, we performed intracellular in vivo recordings of motor units from ankle extensor muscles (TS-MUs, including Sol), and ankle flexor muscles (DP-MUs, including TA) in adult FUS-P525L mice. Our results reveal that the excitability of motoneurons from both ankle flexor and extensor muscles is diminished at P180, where a larger proportion of motoneurons (mostly in FF and FR subpopulations) are incapable of firing repetitively in response to stationary inputs in mutants (43%) compared to controls (24%). We analyzed the excitability of the motoneurons that still fire repetitively at this age, and we found that the electrophysiological properties in TS-MUs and TA-MUs in FUS-P525L mice are not statistically different (Mann-Whitney U test) from their control counterparts. For example, recruitment current was 7.3 ± 4.4 nA (N=31) in mutants vs. controls 6.8 ± 4.6 nA (N=31), Gain was 13 ± 9 Hz/nA (N=21) vs. 19 ± 14 Hz/nA (N=22), voltage threshold was -50 ± 9 mV (N=29) vs. -52 ± 7 mV (N=28), and input resistance was 3.0 ± 1.4 MΩ (N=47) vs. 3.1 ± 1.7 MΩ (N=46).
Our results suggest that motoneuron hypoexcitability is a hallmark during the pre-symptomatic phase of ALS. However, a large portion of motoneuron are also incapable of repetitive firing at P180 in WT mice, which implies that this behaviour could be partly due to the normal aging process. For this reason experiments on younger mice (about P30) are under investigation.

Disclosures: **M. Martinez-Silva:** None. **D. Zytnicki:** None. **M. Manuel:** None.

Nanosymposium

381. Motor Neuron Disease Mechanisms

Location: SDCC 32B

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Presentation Number: 381.06

Topic: C.05. Neuromuscular Diseases

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Title: Does it really change? Identifying potential sources of variability in experimental studies of ALS mouse models

Authors: ***S. S. DUKKIPATI**, A. CHIHI, R. E. W. FYFFE, S. M. ELBASIOUNY;
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Abstract: Animal models of neurodegenerative diseases and injury receive a large amount of attention in the greater biomedical scientific community. Impediments to thorough characterization of these models to understand disease pathogenesis exist in many cases, including in amyotrophic lateral sclerosis or ALS. Using a popular transgenic mouse model strain of ALS, the SOD1-G93A high-expressing line, we set out to study cholinergic synaptic inputs on spinal motor neurons. These synapses, also known as C-boutons, remain a contentious and unclear subject within the ALS field. We performed anatomical analysis and meta-analysis using immunohistochemistry and light microscopy to observe possible effects of generalization, bias, and analytical processes on variability in measured cholinergic synaptic properties (bouton size and intensity). These sources of variability identified in this study may lead to inadequate and/or inappropriate analysis in anatomical studies of ALS pathogenesis. We suggest that this may also be present in other ALS studies, and in the study of other animal models of neurodegenerative diseases and injury.

Disclosures: S.S. Dukkipati: None. **A. Chihi:** None. **R.E.W. Fyffe:** None. **S.M. Elbasiouny:** None.

Nanosymposium

381. Motor Neuron Disease Mechanisms

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Topic: C.05. Neuromuscular Diseases

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Title: Loss of tdp-43 contributes to non-coding rna mediated toxicity

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Translational Neuropathology Res. Lab., Perelman Sch. of Med. At Univ. of Pennsylvania, Philadelphia, PA

Abstract: Non-coding RNA species may be toxic via sequestering RNA binding proteins (RNABP) away from their site of action. In fact, an intronic hexanucleotide repeat expansion in *C9orf72*, which was identified as the most common genetic cause of amyotrophic lateral sclerosis and frontotemporal lobar degeneration, forms RNA foci that may sequester RNABPs. Importantly, all *C9orf72* expansion carriers develop TDP-43 pathology, namely the loss of nuclear TDP-43 into cytoplasmic aggregates. TDP-43 is a heterogenous nucleoriboprotein particle (hnRNP) that regulates RNA processing. It is unclear how loss of nuclear TDP-43 contributes to neurodegeneration. To understand the effects of nuclear TDP-43 loss and *C9orf72* mutation, we developed a novel method to fractionate post-mortem human brain to isolate neuronal nuclei with and without TDP-43 for RNA sequencing. Analysis of 1.6 billion uniquely mapped reads from fractionated frontal neocortex of 15 *C9orf72* mutation carriers or controls demonstrated widespread transcriptome differences, including 5576 differentially expressed genes (DEGs) due to loss of TDP-43 compared to 323 DEGs linked to the *C9orf72* mutation. Gene expression changes linked to the *C9orf72* mutation were highly correlated with reductions in *C9orf72* RNA expression. DEG analysis showed that loss of TDP-43 protein led to altered auto-regulation of the gene encoding TDP-43 (*TARDBP*). Further analysis showed that loss of TDP-43 was associated with 5337 differentially used genic elements (DUGEs) with enrichment for non-coding intronic and 3' untranslated RNA segments. Of interest, DUGEs associated with TDP-43 loss were highly enriched for hnRNP binding sites. Similar changes in non-coding RNA elements were recapitulated upon CRISPR-Cas9 knockout of *TARDBP* in 293T cells. Ongoing experiments will test whether experimental manipulation of non-coding RNA elements contribute to toxicity via hnRNP sequestration. We propose a model where loss of TDP-43 leads to aberrant non-coding RNA segment processing, whereby these non-coding RNA segments contribute to cellular toxicity.

Disclosures: **E.Y. Liu:** None. **J. Russ:** None. **E. Lee:** None.

Nanosymposium

382. Neuroinflammation: Neurotoxicity and Protection

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Presentation Number: 382.01

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Korea Government (MEST) Basic Science Research Program grant 2012R1A1A2040338

Korea government (MSIP) National Research Foundation of Korea (NRF) grant 2012R1A3A2048834

Title: Natural Killer cells target sensory neurons for degeneration after peripheral nerve injury

Authors: *A. J. DAVIES[1], H. KIM[1], J. CHOI[1], S. BACK[2], Y. KIM[3], S. ROH[1], S. KIM[1], Y. BAE[3], H. NA[4], A. LATREMOLIERE[5], M. COSTIGAN[5], S. OH[1];
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Abstract: Axon degeneration is a key process during nervous system development and is also a characteristic of disease or injury to the mature nervous system. However, disparities between different modes of axon degeneration suggest there are likely distinct trigger mechanisms. Natural Killer (NK) cells are cytotoxic lymphocytes typically involved in cell lysis of tumour or virus-infected target cells and their activity is co-ordinated by a balance of activatory and inhibitory signals. We first examined the interactions of NK cells with primary sensory neurons. *In vitro*, DRG neurons acutely isolated from embryonic (E15) mice were highly sensitive to NK cell cytotoxicity compared with adult DRG. Embryonic DRG cytotoxicty correlated with the differential expression of the NK cell activatory ligand, retinoic acid early transcript (*Raet1*), and could be attenuated either by siRNA knockdown of *Raet1* or blockade of the activatory receptor NKG2D. Using time-lapse confocal imaging we show that neurite degeneration requires direct NK cell contact and is concurrent with a transient rise in intracellular Ca^{2+}. We then investigated the response and potential function of NK cells in the context of peripheral nerve injury using mice which express either yellow fluorescent protein (YFP) or the diphtheria toxin receptor (DTR) under the control of the NK cell specific gene NKp46, allowing us to identify and systemically deplete NK cells *in vivo*. NK cells infiltrated peripheral nerve tissues in an injury-dependent manner. Levels of the cytotoxic factor granzyme B were elevated in the injured sciatic nerve and were partially attenuated by NK cell depletion suggesting NK cells act in a cytotoxic capacity. We found that nerve injury drove upregulation of *Raet1* transcripts in DRG tissue and that adult DRG neurons cultured from nerve injured mice were more susceptible to NK-mediated neurite degeneration than sham controls. Systemic depletion of NK cells prior to sciatic nerve crush resulted in the partial retention of peripheral sensation to pin prick stimulation, suggesting that loss of NK function may lead to a greater preservation of sensory axon integrity after injury. Together our results suggest that cytotoxic NK cells may constitute a novel cellular trigger for local axon degeneration of sensory neurons after peripheral nerve injury.

Disclosures: **A.J. Davies:** None. **H. Kim:** None. **J. Choi:** None. **S. Back:** None. **Y. Kim:** None. **S. Roh:** None. **S. Kim:** None. **Y. Bae:** None. **H. Na:** None. **A. Latremoliere:** None. **M. Costigan:** None. **S. Oh:** None.

Nanosymposium

382. Neuroinflammation: Neurotoxicity and Protection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIA Grant AG045034

Bluefield Postdoctoral Fellowship 2014

Title: Ganciclovir suppresses brain inflammation by activating the STING dependent type I interferon response

Authors: *V. MATHUR[1,3], R. BURAI[4], R. T. VEST[2], D. DO[2], K. N. MISTRY[3], H. A. LASHUEL[4], T. WYSS-CORAY[2,3];
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Abstract: The 2'-deoxyguanosine analog, Ganciclovir (GCV), is a highly successful drug routinely used to treat viral infections in patients. We previously showed that GCV reduced symptoms and microgliosis in experimental autoimmune encephalomyelitis model in mice. We found that GCV reduces inflammation and induces a type I interferon response in microglia and monocytes independent of its canonical target, viral thymidine kinase. GCV and more potent GCV-dimers, that we synthesized, require the innate immune adaptor STING to exert these beneficial effects in cultured microglia and in experimental autoimmune encephalomyelitis in mice. Furthermore, inhibition of STING pathway-associated activation of Tbk1 and IRF3 as well as Jak/Stat signaling reduces activity of GCV and its derivatives. Collectively, our findings uncover a remarkable new function of GCV involving a key regulator of cellular immune defense and open potential opportunities to target neuroinflammation.

Disclosures: **V. Mathur:** None. **R. Burai:** None. **R.T. Vest:** None. **D. Do:** None. **K.N. Mistry:** None. **H.A. Lashuel:** None. **T. Wyss-Coray:** None.

Nanosymposium

382. Neuroinflammation: Neurotoxicity and Protection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS-1K08NS094683-01

Title: TGFβ-1 mediates ABCD1 dependent brain endothelial dysfunction

Authors: N. SASIDHARAN, M. C. VISSERS, J. M. T. SNYDER, Y. GONG, F. EICHLER, *P. L. MUSOLINO;
MGH/Harvard, Boston, MA

Abstract: Cerebral X-linked adrenoleukodystrophy (CALD), the most common monogenetic peroxisomal disorder, is a neurodegenerative disorder of childhood that results from mutations in the *ABCD1* gene and manifests as progressive inflammatory demyelination, neurological dysfunction, and death. Several lines of evidence suggest that microvascular endothelial dysfunction is involved in CALD onset: (1) regional perfusion changes preceding blood brain barrier disruption (2) extravasation of blood borne monocytes at leading edge of demyelination and (3) selective brain endothelial dysfunction in the absence of ABCD1. Changes in the expression of Claudin5, ICAM1 and VCAM in mutant endothelium appear to mediate increases in adhesion and transmigration of monocytes. Taking advantage that the *ABCD1*$^{-/-}$ mouse is protected from cerebral inflammatory demyelination we compare human vs mouse microvascular endothelial cells to identify the molecular pathways involved in ABCD1 dependant endothelial dysfunction.
Microarray profiling and *in silico* pathway analysis using Metacore was conducted to characterize novel genetic and protein interactors of ABCD1. TGFbeta1 and c-myc were positive candidates. QPCR, western blot and functional endothelial assays were conducted in siRNA ABCD1 silenced cells for validation of interactions. Experiments were done in parallel with primary brain endothelial cells from mice and humans.
Human endothelial cells displayed a 3-fold upregulation of TGFbeta1 expression upon ABCD1 silencing in contrast with mouse cells which showed no changes. Down regulation of Claudin5 and increased adhesion to monocytes after ABCD1 depletion was observed in human but not mouse brain microvascular endothelium. Strikingly, adding recombinant TGFbeta1 to ABCD1 silenced cells led to decreased Claudin5 levels in both human and mouse endothelium.
Taken together these data suggest a synergistic effect between TGFBeta1 and ABCD1 leading to downregulation of Claudin5 and increased adhesion to monocytes that is selective to human brain endothelium. Continuing this systematic approach comparing human vs mouse will allow us to dissect the mechanisms by which ABCD1 alters TGFbeta1 and its role in the pathogenesis

of inflammatory demyelination. Furthermore, this approach may yield a new model system for identification of novel therapeutic targets.

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Nanosymposium

382. Neuroinflammation: Neurotoxicity and Protection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NHMRC Project Grant

Title: The complement receptor C5aR1 drives NLRP3 inflammasome activation and neuropathology in experimental models of Parkinson's disease

Authors: *R. GORDON[1], E. A. BALMACEDA[2], S. MANTOVANI[3], K. ZHOU[3], A. G. KANTHASAMY[4], M. A. COOPER[2], K. SCHRODER[2], T. M. WOODRUFF[3];
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Abstract: The persistent neuroinflammatory response that accompanies dopaminergic degeneration in Parkinson’s disease has been shown to exacerbate pathology and drive disease progression. However, the precise mechanisms and inflammatory mediators that sustain chronic neuroinflammation remain to be established. Activation of the innate immune complement system has been reported in PD, with early complement fragments upregulated around regions of neuronal death in post-mortem tissue from PD patients. Complement activation in the brain, by either canonical or extrinsic pathways, generates the potent pro-inflammatory molecule C5a, which exerts its major pro-inflammatory effects through its cell surface G-protein coupled receptors, C5aR1 and C5aR2. Herein, we identify elevated C5a levels in the serum of PD patients, suggesting widespread complement activation in PD. We also demonstrate that synuclein fibrils can activate the complement cascade to generate C5a in complement-sufficient blood. In the CNS, we found that complement is activated, and C5aR1 is upregulated on reactive microglia, early in the disease course in multiple pre-clinical mouse models of PD. Crucially, stimulation of primed mouse microglia with C5a (100 nM) caused a time-dependent activation of the NLRP3 inflammasome, which was abolished in NLRP3 deficient cells. Key hallmarks of inflammasome activation including ASC speck formation and secretion of cleaved caspase-1 and

IL-1 beta were evident 24 to 48 h after stimulation with C5a. Significantly, pharmacological inhibition of C5a-C5aR signaling using an orally active and CNS-permeable C5aR antagonist, PMX205, protected against behavioral deficits and dopaminergic degeneration in experimental PD. Taken together, our results suggest that complement activation and persistent C5a generation by misfolded protein aggregates in the CNS can contribute to inflammasome activation and thereby exacerbate disease pathology. Selective inhibition of C5aR1 could therefore be a potential therapeutic strategy to mitigate chronic microglial neuroinflammation, and thereby slow disease progression in PD.

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Nanosymposium

382. Neuroinflammation: Neurotoxicity and Protection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: VA Merit Grant

SCIRF

Title: Targeting the alternative pathway of complement to improve functional recovery after spinal cord injury

Authors: *A. NARANG[1], C. ATKINSON[1], N. L. BANIK[2], M. MEHROTRA[3], S. TOMLINSON[4];
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Abstract: Following injury to the spinal cord, a post-traumatic inflammatory response occurs which is thought to play an important role in secondary neuronal injury and the impairment of functional recovery. The complement system plays an important role in post-traumatic inflammation and the progressive degenerative events that take place. There are three pathways of complement activation; the classical, lectin and alternative pathways. Using complement deficient mice, we previously demonstrated that the alternative pathway plays a key role in driving secondary injury after SCI. Therefore, in this study we investigated the role of the

alternative pathway in a clinically relevant paradigm using an alternative pathway specific inhibitor, factor H (fH). We utilized a site-targeting strategy by linking fH to a fragment of complement receptor 2 (CR2) that binds to complement C3 cleavage products deposited at sites of complement activation. A human counterpart of this construct TT30 has been tested for safety and efficacy. This approach improves efficacy and minimizes systemic complement inhibition and immunosuppression, a benefit to SCI patients since they are prone to urinary tract infection. Administration of CR2-fH to mice after contusion injury to the spinal cord significantly improved locomotor recovery. Using fluorescently labeled CR2-fH and ex-vivo fluorescence tomography, we demonstrated that intravenously injected CR2-fH localized to the site of spinal cord injury and was retained for up to 7 days. Similar levels of CR2-fH were measured in the injured spinal cords whether the inhibitor was administered 30 min or 3 hours after SCI. Furthermore, analysis of locomotor gait recovery over a 21 day period revealed significant and similar levels of improvement in mice given the inhibitor at either 30 min or 3 hour after SCI. Improved recovery correlated with reduced complement deposition at the injury site. Finally, nanostring analysis of gene expression in spinal cord tissue at 3 days post injury revealed that CR2-fH reversed changes in expression of 67% of genes associated with SCI. Key inflammatory pathways modulated by CR2-fH were TLR signaling, cytokine signaling pathways, and macrophage polarization, some of which have been identified as critical players in the immunopathology of SCI. These findings were confirmed by immunohistochemical staining of macrophage/microglial polarization.CR2fH treated mice had increased M2 type macrophages/microglia 3days after injury in comparison to the vehicle treated mice.

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Nanosymposium

382. Neuroinflammation: Neurotoxicity and Protection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: MIRECC

Title: Effects of LPS on expression of mRNA for IL-6, IL-7, and IL-10 and mRNA for IL-6 and IL-7 receptors in CNS and spleen

Authors: ***P. SZOT**[1,4], A. FRANKLIN[1], T. PETRU BEUCA[1], K. BULLOCK[2], K. HANSEN[2], W. BANKS[2,5], D. LATTEMANN[3,4], M. RASKIND[1,4], E. PESKIND[1,4];

[1]MIRECC, [2]GRECC, [3]BSR&D, Puget Sound Hlth. Care Syst., Seattle, WA; [4]Psychiatry and Behavioral Sci., [5]Med., Univ. of Washington, Seattle, WA

Abstract: Neuroinflammation is proposed to be an important component in several central nervous system (CNS) disorders including Alzheimer's disease, Parkinson's disease and traumatic brain injury. The intraperitoneal (ip) administration of lipopolysaccharide (LPS) induces peripheral inflammation and neuroinflammation as evidenced by elevations in blood and brain levels of cytokines. However, the cellular and anatomical sources of these cytokines are not known. Here, we used in situ hybridization to examine in brain and spleen the sources of cytokine production after 3-injection regime previously shown to elevate cytokine levels in brain and blood. Administration of LPS significantly increased the mRNA expression of interleukin (IL)-6 and IL-10 in the spleen, an important peripheral organ for an immune response, consistent with increases in blood levels for these cytokines after ip LPS. IL-6 mRNA was mainly in the germinal centers of the spleen, whereas IL-10 was also in the white pulp. LPS decreased expression of IL-6 receptor mRNA in spleen, but had no effect on IL-7 or IL-7 receptor mRNA. In the CNS, IL-6 mRNA was expressed in neurons prior to LPS in regions that include the cortex, cerebellum, and hippocampus. After LPS, IL-6 mRNA showed a diffuse, extra-neuronal, punctate pattern throughout the CNS. The mRNA for IL-6 receptor showed expression mainly by neurons and neuronal expression was increased except in the cerebellum by LPS. IL-10 mRNA was widely expressed by many specific brain regions, with LPS tending to decrease expression in forebrain and increase it in hindbrain. IL-7 mRNA was limited mainly to the cerebellum. LPS had no effect on IL-7 or IL-7 receptor mRNA expression in the CNS. These studies indicate that LPS induced neuroinflammation has unique effects on the regional and cellular patterns of CNS and splenic cytokine expression.

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Nanosymposium

382. Neuroinflammation: Neurotoxicity and Protection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01NS083410

Title: IL-1beta, a HAND-relevant proinflammatory cytokine, increases MMP-13 release and PAR-1 signaling in astrocytes.

Authors: *K. A. MAGUIRE-ZEISS[1], T. YIN[1], E. WENZEL[2], K. CONANT[1];
[1]Neurosci., [2]Pharmacol., Georgetown Univ. Med. Ctr., Washington, DC

Abstract: Though PAR-1 is a GPCR highly expressed on the cell surface of neurons, astrocytes, and microglia, and expression is increased in HIV associated neurological disorders (HAND), disease relevant sequelae of receptor activation have not been well investigated. PAR-1 is activated by cleavage of its extracellular N-terminal domain revealing a tethered peptide ligand that folds in to activate signaling. This cleavage and activation is mediated by select proteases that are substantially elevated in the setting of HAND. Herein we examine matrix metalloproteinase-13 (MMP-13) a potent PAR-1 agonist that is expressed in the CNS. First, we demonstrate that astrocyte expression and release of MMP-13 is increased in response to IL-1beta, a HAND-relevant proinflammatory cytokine. Furthermore, since GPCR linked intracellular signaling in astrocytes, as well as astrocyte release of chemokines, are thought to play a role in HAND progression, we examined the direct effect of MMP-13 on astrocytes. We find that treatment of astrocytes with catalytic MMP-13 stimulates the expression and release of monocyte chemoattractant protein-1 (MCP-1 / CCL-2), a protein critical for the migration and CNS infiltration of macrophages. Full-length recombinant MMP-13 had no significant effect demonstrating that active protease is required for increased expression of this cytokine. Importantly, pre-treatment of astrocytes with a PAR-1 antagonist attenuated MMP-13 directed MCP-1 release. Taken together this study suggests that HAND-relevant inflammation can incite astrocytes to release the PAR-1 activating enzyme, MMP-13, which in turn augments the release of MCP-1 to potentially perpetuate inflammation. This work is significant because PAR-1 antagonists are now in clinical trials for coronary artery disease and might be considered for treatment of HAND.

Disclosures: K.A. Maguire-Zeiss: None. **T. Yin:** None. **E. Wenzel:** None. **K. Conant:** None.

Nanosymposium

382. Neuroinflammation: Neurotoxicity and Protection

Location: SDCC 25A

Time: Monday, November 14, 2016, 1:00 PM - 4:30 PM

Presentation Number: 382.08

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Comparison of the injury, and consequent inflammatory response, after spinal cord injury in neonates and mature rats may lead to a novel therapeutic avenue

Authors: *T. SUTHERLAND, A. SAPKOTA, C. GORRIE;
Sch. of Life Sci., Univ. of Technol. Sydney, Ultimo, Australia

Abstract: Spinal cord injury (SCI) is a devastating condition that can result in severe loss of tissue, varying degrees of functional impairment and exhibit only limited repair. This can have debilitating effects on the quality of life for SCI patients. Currently there is no cure for SCI, and no proven treatment in the acute phases of SCI. There is much research focused on reducing the degenerative secondary injury phase and promoting tissue repair and regeneration. however, there is nothing currently available to SCI patients. The body's own immune response plays an important role in the progression of SCI, however it is still contentious whether this cascading immune response is beneficial or detrimental to recovery. There exists a trend for a better functional recovery in younger patients, compared to adults, which is also reported for animal studies, however the reasons for this and potential impact on therapies are yet to be elucidated. Using a mild contusion injury model from a NYU impactor adult (9wk), and infant (P7) Sprague-Dawley rats were compared histologically at 24hrs, 1wk, 2wks and 6wks post-injury (n=108) to examine the injury progression. The innate cells in the inflammatory response were examined using neutrophil counts and ED1/IBA1 double labelling for microglia/macrophages. Further animals were assessed using fresh spinal cord tissue for flow cytometry to quantitate different phenotypes of macrophages, neutrophils and T-Cells; as well as spinal cord supernatant and CSF for multiplex cytokine ELISA.
This study found a significantly different injury pattern over time in the infants and a decreased inflammatory response, demonstrated by decreased neutrophil infiltration, macrophage and microglial activation in the neonate groups compared to the mature groups (ANOVA $P<0.05$). This was visible at acute and chronic time points. There were also greater proportions of ramified microglia visible in the neonate groups. The flow cytometry and ELISA findings support the initial immunohistochemistry results and further highlight the cellular and molecular differences in the inflammatory response in young animals.
The results of this study suggest that the inflammatory response is significantly different in developing and mature spinal cords; these differences may contribute to the to the observed better recovery in young patients. If we can manipulate the adult responses to resemble the infants this may hold great therapeutic potential for patients of all ages, however greater exploration into the mechanisms behind these observed differences is required.

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Nanosymposium

382. Neuroinflammation: Neurotoxicity and Protection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No. 2014R1A2A2A01007289)

Title: Treatment of erythropoietin decreases the cognitive and memory dysfunction by regulating inflammatory response in Post-operative cognitive decline

Authors: *B. KOO, J. LEE, E. KAM, S. CHEON, S. KIM, J. KIM, E. KIM;
Yonsei Univ., SEOUL, Korea, Republic of

Abstract: Cognitive decline and memory impairment are symptoms that occur in elderly patients after a surgical operation which can lead to postoperative cognitive decline (POCD) from days to weeks. Excessive pro-inflammatory cytokines are associated with memory deficits after surgery, however, there is no effective therapy for these patients yet. Erythropoietin (EPO) has protective effect on the brain following any kinds of injuries and modulates plasticity in hippocampal neurons. We hypothesized EPO could be involved in surgery-induced memory impairment. Therefore, we evaluated behavioral parameters related to cognition and memory functions after EPO treatment by elevated plus maze, novel object recognition test, and passive avoidance test. POCD was induced by rubbing intestine and clamping superior mesenteric artery in ICR adult mice. We assessed inflammatory cytokine levels (IL-6, TNF-a, IL-1ß) and characterized the changes of microglia M1/M2 polarization in spleen and hippocampus. The results showed that surgery triggered cognitive and memory decline in behavioral test, and upregulated surgery-triggered systemic IL-1ß, IL-6 mRNA levels. In the hippocampus of mice after surgery, pro-inflammatory M1 markers (IL-1ß, TNF-a, Cxcl10) were up-regulated, whereas M2 marker such as arginase-1 and IL-10 expression were decreased significantly. After EPO treatment, mice exhibited an improvement of LTP, better cognitive and memory functions, and efficiently lowered systemic inflammation. EPO treatment also reduced M1 markers and increased M2 markers in the hippocampus. Conclusively, our data proved that EPO administration might be a neuroprotective therapy for cognitive and memory deficits induced by surgery which is associated with the suppression of systemic and hippocampal inflammatory responses.

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Nanosymposium

382. Neuroinflammation: Neurotoxicity and Protection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Linking low dose chronic peripheral LPS injection to neuroinflammation

Authors: *S. N. CAMPBELL, Y. HE, A. BHATTACHARYA, N. C. DERECKI;
Janssen Res. & Develop., San Diego, CA

Abstract: Chronic inflammation and supranormal levels of circulating pro-inflammatory cytokines are associated with central nervous system (CNS) dysfunction and neuroinflammation. Lipopolysaccharide (LPS), a component of bacterial cell membranes, induces well-characterized inflammatory and behavioral responses. While these responses and mechanisms of action are well characterized in the periphery, less is known of the response in the CNS. Moreover, LPS doses utilized in most studies are relatively high, resulting in sepsis-like illness and lethargy. High doses and measurement of acute biological and behavioral responses make it difficult to parse responses that are representative of frank somatic sickness from those that may be reflective of lasting neuroinflammatory changes. By using much lower doses (50 μg/kg, 100 μg/kg) of LPS administered by ip injection to C57Bl/6 mice chronically (every other day for a period of three weeks), we attempted to mimic chronic low-grade inflammation which is associated in clinical literature with CNS pathology. As an additional control, we also examined the response to acute doses (single injection 12 or 24 hours prior to analysis) of LPS in order to compare immediate and long-term immune CNS response to inflammatory stimuli. Using multi-parameter flow cytometry (FACS), Luminex array analysis of inflammatory factors in both periphery (bone marrow, spleen, blood), peri-CNS regions (meninges) and CNS (brain parenchyma), as well as immunofluorescent labeling of CNS glia by endogenous fluorescence (CX3CR1-GFP$^{+/-}$ mice) and antibody labeling, we examined LPS response simultaneously across multiple sites. Even an acute low dose of 50 ug/kg (1/20 of the reported LD 50 dose for C57Bl/6) yielded strong, quantifiable changes in activation phenotype in all tissues, including meningeal myeloid cells and microglia. Importantly, simultaneous analysis of chronically-dosed animals suggested key myeloid cell populations were differentially affected by acute vs. chronic administration. Cells from both peripheral and CNS tissues of acutely- and chronically-dosed animals showed increased intracellular cytokine labeling via FACS analysis. Antibody labeling of neocortex and hippocampus with Iba-1 and GFAP, or native CX3CR1-GFP expression demonstrated robust differences between acute and chronically-dosed animals. Chronically-dosed animals demonstrated increased activation of microglia—by morphological analysis—and activation of astrocytes via increase in GFAP expression. By examining multiple layers of signaling we aim to elucidate underlying mechanisms that connect peripheral inflammation to neuroinflammation.

Disclosures: S.N. Campbell: None. **Y. He:** None. **A. Bhattacharya:** None. **N.C. Derecki:** None.

Nanosymposium

382. Neuroinflammation: Neurotoxicity and Protection

Location: SDCC 25A

Time: Monday, November 14, 2016, 1:00 PM - 4:30 PM

Presentation Number: 382.11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 CA194924

Title: Characterizing the effects of mammary tumor development on neuroinflammation

Authors: *W. H. WALKER, M. M. GAUDIER-DIAZ, J. C. BORNIGER, A. A. ZALENSKI, N. ZHANG, A. DEVRIES;
Neurosci., The Ohio State Univ., Columbus, OH

Abstract: The American Cancer Society estimates that 247,000 women will be diagnosed with breast cancer in 2016. Unfortunately, for these women they are 25-33% more likely to develop a mood disorder relative to the general population, with some studies demonstrating that as many as 50% will develop a mood disorder that presents depressive or anxious symptoms. To date a definitive cause of cancer related affective disorders has not been conclusively established. However, recent studies have suggested a link between peripheral tumor induced neuroinflammation and affective behavior. Still, several critical gaps in knowledge remain regarding the relationship between peripheral tumors, neuroinflammtion, and affective behavior. The purpose of this study is to determine when the development of peripheral tumors, derived from a nonmetatstaic murine breast cancer cell line, results in neuroinflammtion and behavioral deficits. In this study female Balb/c mice received bilateral subcutaneous injections of 67NR cells ($1x10^5$ cells per injection) or vehicle proximal to the 4th and 9th mammary glands. Following inoculation tumors were allowed to develop and mice were euthanized according to their assigned group on days 5, 10, 15, 20, and 25. Mice with tumors displayed a significant increase in spleen mass on day 25 relative to control animals as well as a significant increase in spleen interlukin-6 on day 20. However, our results reveal no neuroinflammation in the hippocampus or cortex (mRNA or protein) following tumor inoculation at any time point. These data are contrary to previous studies. Two possible explanations for these findings are that tumors were not allowed to develop large enough for neuroinflammation to arise (average total tumor burden at 25 days was .76g; 3.2% of body weight), or that the location of tumor development plays a critical role in evolution of neuroinflammation.

Disclosures: W.H. Walker: None. **M.M. Gaudier-Diaz:** None. **J.C. Borniger:** None. **A.A. Zalenski:** None. **N. Zhang:** None. **A. DeVries:** None.

Nanosymposium

382. Neuroinflammation: Neurotoxicity and Protection

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Time: Monday, November 14, 2016, 1:00 PM - 4:30 PM

Presentation Number: 382.12

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Human monoclonal NMDA receptor auto-antibodies are sufficient for encephalitis pathogenesis

Authors: J. KREYE[1], N. K. WENKE[1], M. CHAYKA[1], J. LEUBNER[1], R. MURUGAN[3], N. MAIER[4], *A. G. MEISEL[2], H. WARDEMANN[5], H. PRÜSS[1];
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Abstract: Anti-NMDA receptor (NMDAR) encephalitis is a recently discovered autoimmune syndrome associated with psychosis, dyskinesias, and seizures. Little is known about the cerebrospinal fluid (CSF) autoantibody repertoire. Antibodies against the NR1 subunit of the NMDAR are thought to be pathogenic, however, direct proof is lacking as previous experiments could not distinguish the contribution of further anti-neuronal antibodies. Using single-cell cloning of full-length immunoglobulin (Ig) heavy and light chain genes, we generated a panel of recombinant monoclonal NR1 antibodies from CSF memory B cells and antibody secreting cells of NMDAR encephalitis patients. Cells typically carried somatically mutated Ig genes and had undergone class-switching to IgG, clonally expanded cells carried identical somatic hypermutation (SHM) patterns. A fraction of NR1 antibodies were non-mutated indicating that tolerance induction against NMDAR was incomplete and SHM not essential for functional antibodies. Human monoclonal NR1 antibodies down-regulated NMDAR in primary hippocampal neurons, resulting in impaired NMDAR currents and calcium influx. Most CSF-derived antibodies did not react against NR1, but against other brain-expressed epitopes including neuronal surfaces. Our functional data using primary hippocampal neurons indicate that human CSF-derived monoclonal NR1 antibodies alone are sufficient to cause neuronal surface receptor down-regulation and subsequent impairment of NMDAR-mediated currents, thus providing ultimate proof of antibody pathogenicity. The observed formation of immunological memory might be relevant for clinical relapses.

Disclosures: **J. Kreye:** None. **N.K. Wenke:** None. **M. Chayka:** None. **J. Leubner:** None. **R. Murugan:** None. **N. Maier:** None. **A.G. Meisel:** None. **H. Wardemann:** None. **H. Prüss:** None.

Nanosymposium

382. Neuroinflammation: Neurotoxicity and Protection

Location: SDCC 25A

Time: Monday, November 14, 2016, 1:00 PM - 4:30 PM

Presentation Number: 382.13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: The Swedish Research Council

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Längmanska Kulturfonden

Title: Identifying a link between metabolic signaling, functional changes and inflammation in different brain regions using a unique SNAP-25 mouse model and western diet

Authors: *M. IRFAN[1], I. VALLADOID ACEBES[1], T. DARAIO[1], P. STANTON[3], K. BRISMAR[1], T. HOKFELT[2], C. BARK[1];
[1]Dept. of Mol. Med. & Surgery, [2]Dept. of Neurosci., Karolinska Institutet, Solna, Sweden; [3]Cell Biol. & Anat., New York Med. Col., Valhalla, NY

Abstract: Assembled SNARE core-complexes from SNAP-25, syntaxin and VAMP are required for activity-dependent neurotransmitter and neuro-endocrine exocytosis. We have engineered a mouse in which one splice variant of SNAP-25, SNAP-25b, is removed but replaced by corresponding levels of SNAP-25a. These mice are predisposed to metabolic disease; obesity and type-2 diabetes, conditions aggravated by western diet (WD). We investigated the impact of this substitution, with and without WD, in behavioral assays, on central inflammation, expression levels of other members of the SNARE core-complex, components of down-stream insulin signaling pathway and on the cAMP signal transduction molecule ATF-3 in cerebellum, frontal cortex, hippocampus and hypothalamus. Mutants and wild-type littermates on C57BL/6 background with or without WD were characterized behaviorally and protein expression levels were examined in different brain regions by immunoblotting and immunohistochemistry. Male mutant mice on control diet exhibited upregulation of total SNAP-25, syntaxin and VAMP, an effect enhanced by WD. This diet also caused brain-region-specific upregulation of a novel SNARE protein, SNAP-47, in both wild-type and male mutant mice. Female mutants on control

diet exhibited upregulation of individual SNAREs but, in contrast to male mice, this effect was reversed by WD in cerebellum and frontal cortex. SNARE levels were unaffected in male mutant mice in the hippocampus, but upregulated in female mutants fed either control or WD. We also observed region and sex specific changes in ATF-3 levels, downregulation of BDNF and total plus phosphorylated forms of AMPK, AKT and STAT-3, in response to both mutation and WD. Changes in functional, metabolic and activating transcriptional factor protein expression levels were accompanied by inflammation, as indicated by astrocyte/microglial activation in brain and impaired performance in a battery of behavioral tests. The SNARE-complex without SNAP-25b but with SNAP-25a predisposes for metabolic changes and inflammation in the brain, and alters regulation of SNARE protein levels and insulin signaling pathways in a sex specific manner; many of these changes are aggravated by WD.

Disclosures: **M. Irfan:** None. **I. Valladoid Acebes:** None. **T. Daraio:** None. **P. Stanton:** None. **K. Brismar:** None. **T. Hokfelt:** None. **C. Bark:** None.

Nanosymposium

382. Neuroinflammation: Neurotoxicity and Protection

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Presentation Number: 382.14

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NASA NNX14AI07G (CAL)

HNDC subsized a portion of the behavioral tests at the Harvard Mouse Neurobehavior Lab

Title: Deep space radiation with ^{56}Fe iron has early, sex-specific effects on CNS in WT and Alzheimer transgenic mice

Authors: ***B. LIU**[1,2], E. FITZPATRICK[1], K. LE[1], Q. SHI[1,2], L. TROJANCZYK[3], M.-A. PARK[2,4], S. WANG[2,4], A. BELANGER[2,4], S. DUBEY[2,4], P. HOLTON[2,4], V. REISER[5], W. TRIGG[6], P. J. LORELLO[7], K. M. O'BANION[3], B. CALDARONE[2,7], M. DICARLI[2,4], C. A. LEMERE[1,2];
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Abstract: Little is known regarding sex differences in the response to deep space radiation. To determine whether ^{56}Fe-particle irradiation (IRR) causes early sex (F, M)- and genotype-specific neurobehavioral and neuropathological changes in mice, age- and sex-matched APP/PS1dE9 Tg and WT mice (4 mo) were exposed to a single dose of whole body IRR with 1000 MeV/μ ^{56}Fe ions at 0, 10 or 50 cGy at Brookhaven National Laboratory. Behavioral tests including SHIRPA, Rotarod, Grip Strength (GS), Tail Suspension (TS), Open Field (OF), Elevated Plus Maze (EPM), Y-Maze (YM), and Contextual Fear Conditioning (CFC) were performed 1 mo post-IRR (n=13-16 mice/group). ^{56}Fe IRR had no effect on general health (SHIRPA) or anxiety (OF, EPM), but resulted in significant 3-way interactions (sex x genotype x dose) on motor activities (OF, YM, EPM, TS) wherein F/Tg/10 cGy and M/Tg/50 cGy ^{56}Fe IRR mice were more active. Male, but not female, Tg mice IRR with 50 cGy ^{56}Fe showed a trend for impaired memory on CFC but not YM. Radiation resulted in weakness (GS) in F/Tg mice; however, 50 cGy IRR improved motor learning (Rotarod) in F/Tg mice. Neuroinflammation was assessed by ^{18}F-GE180 TSPO microPET imaging on 4 mice per group (0 vs 50 cGy ^{56}Fe) at baseline and 2 months post-IRR. An overt pre- vs. post-IRR reduction in ^{18}F-GE180 whole brain uptake and hippocampal uptake was observed in both F/Tg/50 cGy and F/WT/50 cGy ^{56}Fe IRR mice. No changes in PET tracer uptake were seen in control female mice (0 cGy) or in any male mice. MSD ELISA was used to quantify cerebral amyloid-β (Aβ) levels. ^{56}Fe IRR significantly lowered ($p<0.01$) guanidine soluble ("insoluble") Aβx-40 and x-42 and increased Tper soluble Aβ in F/Tg mice but had no effect on Aβ in M Tg mice. Immunohistochemical analyses using Aβ antibody, R1282, and Thio S confirmed that IRR reduced Aβ plaque burden in F/Tg mice but not M/Tg mice. In addition, IRR modestly increased microhemorrhages in M/WT/50 cGy mice. Taken together, ^{56}Fe IRR did not affect general health but exacerbated the Tg hyperactivity phenotype in low-dose IRR female Tg mice and high-dose IRR male Tg mice. Individual and combined effects of sex, genotype and IRR dose were observed for strength and motor coordination with female Tg mice showing higher sensitivity to IRR, while high-dose IRR male Tg mice had mild memory impairment. ^{56}Fe IRR lowered cerebral plaque burden and reduced neuroinflammation (^{18}F-GE180 uptake) in female Tg but not male Tg mice. Taken together, our results from behavioral testing, PET imaging, pathological and biochemical analyses suggest strong sex differences in the early response to ^{56}Fe IRR.

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Nanosymposium

383. Brain and Spinal Cord Injury

Location: SDCC 33C

Time: Monday, November 14, 2016, 1:00 PM - 4:00 PM

Presentation Number: 383.01

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant R01NS079339

MDA Grant 25485

Title: Terminal axonal sprouting is augmented in partially injured motor nerves of BACE1 KO mice

Authors: *C. TALLON, M. H. FARAH;
Neurol., Johns Hopkins Univ. SOM, Baltimore, MD

Abstract: One of the interesting characteristics that distinguishes the central nervous system from the peripheral nervous system is the ability for peripheral nerves to regenerate after injury. The mechanisms underlying this innate ability for regeneration are still poorly understood. Our lab has been studying the effects of beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) activity on the regenerative capacity of peripheral nerves in response to injury. We performed a partial nerve transection of the lateral thoracic nerve in BACE1 knockout mice and then 7 days later determined the percentage of intact neuromuscular junctions that had terminal axonal sprouts growing towards denervated areas. In the BACE1 knockout mice, there was a significant increase in the percentage of neuromuscular junctions that had terminal axonal sprouts when compared with their wild type littermates. We also determined the effect of BACE1 inhibition on cultured adult neurons by providing them with a BACE1 inhibitor and monitored neurite outgrowth. Neurons treated with the BACE1 inhibitor had significantly increased neurite length, number of branches from the cell soma and branches along the longest neuronal process. Ongoing experiments are investigating potential pathways that BACE1 might regulate in response to axonal injury. Preliminary data indicated that reduced BACE1 expression alters pathways that are known to be involved in axonal regeneration. Investigating BACE1's role in the regulation of axonal remodeling in response to injury has important implications in understanding peripheral nervous system repair.

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Nanosymposium

383. Brain and Spinal Cord Injury

Location: SDCC 33C

Time: Monday, November 14, 2016, 1:00 PM - 4:00 PM

Presentation Number: 383.02

Topic: C.09. Brain Injury and Trauma

Title: Establishment of the safety evaluation of integration-free human iPS cell-derived neural stem/progenitor cells as a source of cell therapy for spinal cord injury

Authors: *T. IIDA[1], A. IWANAMI[1], J. KOHYAMA[2], N. NAGOSHI[1], M. MATSUMOTO[1], H. OKANO[2], M. NAKAMURA[1];
[1]Dept of Orthop, Sch. of Med, Keio Univ., Shinjyuku-Ku Tokyo, Japan; [2]Dept of Physiology, Sch. of Med, Keio Univ., Shinjyuku-Ku Tokyo, Japan

Abstract: Introduction:
Recently, we have demonstrated the therapeutic potential of transplanting human iPS cell-derived neural stem/progenitor cells (hiPSC-NS/PCs) for spinal cord injury (SCI) models. However, some cell lines of hiPSC-NS/PCs produced neurogenic tumors after transplantation. The purpose of this study is to assess the efficacy and safety of the new integration-free hiPSC-NS/PCs, which are known to be less prone to forming tumors, and to investigate the genetic and epigenetic alterations in hiPSC-NS/PCs that may be related to tumorigenicity through series of high-throughput genetic and epigenetic analyses. Our final goal is to establish evaluation criteria of hiPSC-NS/PCs regarding tumorigenicity for clinical use through the evidence gained from these investigations.
Methods:
Two hiPS cell lines produced by episomal vector were prepared (836B3-hiPSCs, 414C2-hiPSCs), and were induced to hiPSC-NS/PCs (836B3-NS/PCs, 414C2-NS/PCs). Each hiPSC-NS/PCs was transplanted into the injured spinal cord of NOD-SCID mice; PBS was injected instead of cells in the control group (414C2-NS/PCs; n=30, 836B3-NS/PCs; n=30, control; n=10). Tumorigenicity was evaluated histologically and biologically, and motor function was also evaluated by basso mouse scale (BMS) score. For genetic and epigenetic analyses of hiPSC-NS/PCs, single nucleotide variants (SNV) were evaluated using Ion AmpliSeq Cancer Panel. Illumina OmniExpress-24 and Methylation450 BeadChip was used to evaluate genome wide copy number variations (CNV) and DNA methylation analyses of these hiPSC-NS/PCs, respectively.
Results:
Better motor functional recovery was observed in the 414C2-NS/PCs group compared with the control group ($p < 0.05$), without any tumorigenicity. In contrast, transplanted 836B3-NS/PCs formed tumor at a high rate (60%), and long-term observation revealed the deterioration of motor function accompanied by tumor formation. SNVs related to cancer pathogenesis such as

SRGAP3 were detected only in 836B3-NS/PCs but not in 414C2-NS/PCs. Several CNVs related to cancer pathogenesis
were also detected in 836B3-NS/PCs. The DNA methylation status of several tumor suppressor genes such as CNTNAP2 were quite different between two hiPSC-NS/PCs.
Conclusion:
To establish criteria for quality control of integration-free hiPSC-NS/PCs, we evaluated their tumorigenicity and genetic/epigenetic profiles through *in vitro* and *in vivo* analyses. Here, we revealed differences in the SNVs, CNVs and DNA methylation patterns between the safe and tumorigenic hiPSC-NS/PCs, which enable us to establish criteria for quality control of hiPSC-NS/PCs.

Disclosures: T. Iida: None. **A. Iwanami:** None. **J. Kohyama:** None. **N. Nagoshi:** None. **M. Matsumoto:** None. **H. Okano:** None. **M. Nakamura:** None.

Nanosymposium

383. Brain and Spinal Cord Injury

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Presentation Number: 383.03

Topic: C.09. Brain Injury and Trauma

Support: UCLA HSSEAS Laboratory Start-Up Funds

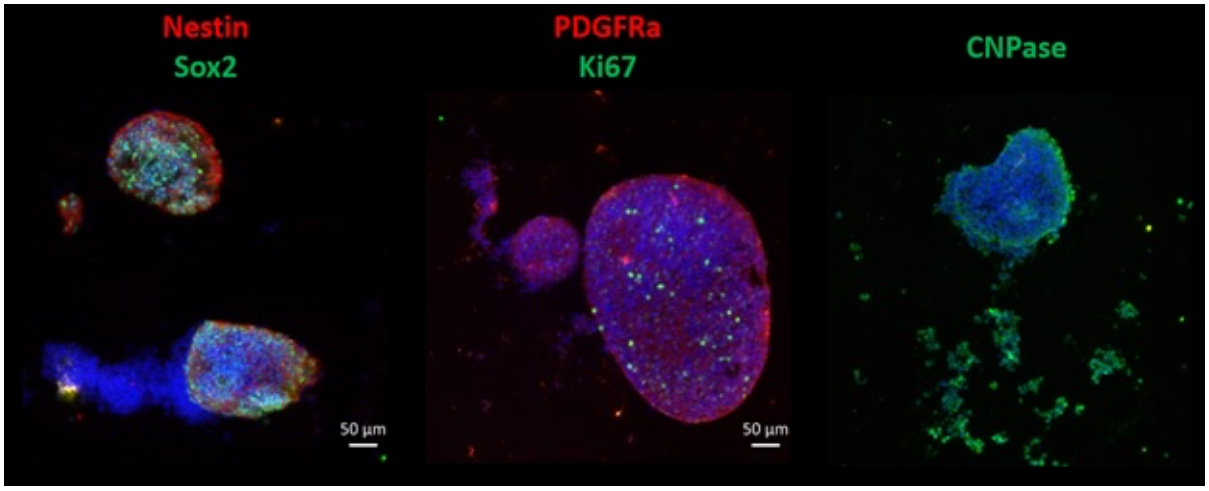
University of California Faculty Career Development Award

Title: Hyaluronic acid hydrogels for spinal cord regeneration

Authors: *C. WALTHERS, J. LIANG, A. EHSANIPOUR, S. SEIDLITS;
Bioengineering, UCLA, Los Angeles, CA

Abstract: In brain injury, multiple sclerosis, and spinal cord injury (SCI), oligodendrocytes die and are irreplaceably lost. Neural stem and progenitor cells (NSPCs) are present in the adult central nervous system (CNS) and have the innate ability to restore function to damaged nerves, yet regrowth of axons has been particularly difficult, as oligodendrocytes do not renew and rarely re-myelinate.
We postulate that a major cause for these difficulties is failure to account for the effects of insoluble, extracellular matrix (ECM)-mediated cues necessary for oligodendrocyte differentiation. Hyaluronic acid (HA) is a major component of ECM in CNS tissue, and has been shown to enhance oligodendrocyte survival in culture, increase oligodendrocyte differentiation, and reduce inflammatory conditions in the CNS. NSPCs cultured in 3D HA hydrogels with pro-

oligodendrocytic peptides have been shown to preferentially differentiate towards oligodendrocyte phenotypes and displayed morphologies similar to those observed in vivo. Our lab has used HA hydrogels cross-linked with multi-branched poly(ethylene) glycol (PEG) chains to create a highly modular polymer platform for stem cell differentiation and delivery. Modulation of various hydrogel parameters (gel stiffness, HA content, peptide content) affected survival, proliferation, and differentiation of encapsulated NSPCs. Specifically, NSPCs grown in 3D hydrogels containing low concentrations of HA showed decreased survival and proliferation compared to NSPCs grown in high HA concentrations. Additionally, NSPC survival and proliferation was increased by RGD, a pro-growth and migration peptide, while pro-differentiation peptides increased oligodendrocyte markers. HA-hydrogels are a potential therapeutic for disease and trauma of the CNS.
NSPCs were encapsulated in HA-hydrogels for 6 weeks and maintained high expression of markers for neural stemness (Nestin, Sox2), proliferation (Ki67), and began expressing markers for oligodendrocytic differentiation (PDGFRa, CNPase). Hoescht (blue) shows nuclear staining.



Disclosures: **C. Walthers:** None. **J. Liang:** None. **A. Ehsanipour:** None. **S. Seidlits:** None.

Nanosymposium

383. Brain and Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

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Natural Medicine and Biotechnology Research of Toyama Prefecture, Japan

Title: Matrine facilitates axonal growth and improves motor function in spinal cord injury in acute and chronic phases

Authors: *N. TANABE, T. KUBOYAMA, C. TOHDA;
Div. of Neuromedical Sci., Inst. of Natural Med., Univ. of Toyama, Toyama, Japan

Abstract: Serious motor dysfunction in spinal cord injury (SCI) is caused by disruption of descending motor tracts. Although reconstruction of spinal tracts by axonal growth must be effective for regaining function, inhibitory factors for axonal growth, such as chondroitin sulfate proteoglycan (CSPG), increase in the lesion site. We previously found that the water extract of dried roots of *Sophora flavescens* (SF) promoted axonal growth even on a CSPG-coated surface. The extract administration improved the axonal density and motor dysfunction in acute SCI mice. In this study, we investigated the effect of matrine, one of the main constituents in SF extract, on axonal growth and SCI mice. Axonal growth activity of matrine was evaluated in primary cortical neurons (ddY mice, E14) cultured on the CPSG. Four days after the treatment, axonal length was quantified by immunostaining for phosphorylated neurofilament-H. Although axonal growth was inhibited on the CSPG, matrine (10 μM) increased axonal growth even on the CSPG. Consecutive oral administrations of matrine (100 μmol/kg/day, for 30 days) or vehicle solution to SCI mice (ddY, female, 8 weeks old) were started from 1h after the injury. Matrine significantly recovered motor function of hindlimbs. Immunohistological analysis indicated that the density of 5-HT positive axons in the lesion site was increased by matrine treatment. Furthermore, the effect of delayed matrine treatment on SCI mice was investigated. Matrine (100 μmol/kg/day) was administered to SCI mice for 154 days from 28 days after the injury. Motor function of matrine-treated SCI mice was significantly recovered during the period. In summary, this study is the first demonstration showing that matrine promoted axonal growth and recovered motor function of SCI mice in acute and chronic phases. Direct binding proteins of matrine were investigated by a drug affinity responsive target stability (DARTS) method using lysate of cultured cortical neurons. Since heat shock protein 90 (HSP90) was identified, a role of matrine-associated HSP90 is under the investigation.

Disclosures: **N. Tanabe:** None. **T. Kuboyama:** None. **C. Tohda:** None.

Nanosymposium

383. Brain and Spinal Cord Injury

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Presentation Number: 383.05

Topic: C.09. Brain Injury and Trauma

Support: Rotary Foundation Global Grants Scholarship

NIH Grant (R01 NS073596)

New York State Spinal Cord Injury Research Board (DOH01-CARTID-2015-00065)

Title: HDAC3 inhibition ameliorates spinal cord injury by modulation of innate immune response

Authors: *T. KUBOYAMA[1,2], Y. HUANG[2], J. WONG[2,3], A. KOEMETER-COX[2], M. MARTINI[2], R. H. FRIEDEL[2,4], H. ZOU[2,4];
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Abstract: Spinal cord injury (SCI) results in neurological deficits that seldom recover. Following injury, the innate immune response of microglia and macrophages adopts a predominantly M1 or pro-inflammatory phenotype, which is detrimental to neural repair and axonal regeneration. Shifting this M1 bias towards a pro-repair, anti-inflammatory, or M2 phenotype is predicted to confer neuroprotection and enhance regeneration; however, the key intracellular driver for global reprogramming of the inflammatory gene network is unclear. Here we show that SCI results in a robust induction of histone deacetylase 3 (HDAC3), and correspondingly, a reduction in global levels of acetylated histones 3 and 4 (AcH3 and AcH4) in M1 macrophages. Remarkably, inhibiting HDAC3 activity after SCI with a specific inhibitor with CNS bio-availability shifts macrophage responses towards an M2 phenotype, along with improved functional recovery and increased axon density at the injury site. Consistently, HDAC3 inhibition results in global inflammatory suppression, as measured by both local cytokine profiling and macrophage-specific nuclear RNA analyses. Mechanistically, we demonstrate that HDAC3 activity is largely responsible for histone deacetylation and sensitization of primary microglia for LPS-induced M1 differentiation. Together, our results reveal a novel HDAC3-mediated epigenetic regulation of the innate immune response after SCI. Reversing the burden of M1 bias and modulating inflammatory gene networks by HDAC3 inhibition represents a promising new direction for immunomodulatory therapy for SCI.

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Nanosymposium

383. Brain and Spinal Cord Injury

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Time: Monday, November 14, 2016, 1:00 PM - 4:00 PM

Presentation Number: 383.06

Topic: C.09. Brain Injury and Trauma

Title: The Neuro-Spinal Scaffold promotes tissue remodeling, axonal sprouting, and Schwann cell myelination following acute spinal cord contusion injury in rats

Authors: ***S. W. MOORE**[1], R. T. LAYER[1], A. B. KUTIKOV[1], P. PODELL[1], A. A. AIMETTI[1], T. R. ULICH[1], J. D. GUEST[2];
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Abstract: The Neuro-Spinal Scaffold (NSS) is a porous PLGA-poly-L-lysine cylinder currently in a clinical trial for the treatment of complete (AIS A) thoracic spinal cord injury (SCI). The NSS is surgically placed within the acute (<96 hours) injury epicenter. Notably, in the first 6 patients treated, four improved from complete to incomplete AIS grades. Here, we examine the histologic consequences of NSS implantation in rat thoracic contusion injuries. In both rats and humans, contusive SCI produces cellular necrosis and secondary tissue loss that generally culminates in a fluid-filled cavity surrounded by a rim of variably preserved tissue. NSS implantation at the lesion epicenter 24 to 72h following rat T10 contusive SCI (IH Impactor 220 kDyn) reduced cavity volume by 86% ($p < 0.05$) and increased spared white matter by 44% ($p < 0.05$) when examined 12-weeks post-implant. Strikingly, we also observed a 111% ($p < 0.05$) increase in remodeled tissue in the lesion epicenter. Immunofluorescence labeling identified abundant laminin, a reduction of GFAP-positive astrocytes, as well as beta-3 tubulin positive axons within the remodeled tissue. This indicates that the NSS supports tissue formation favorable for axon regrowth. Following SCI, Schwann cells (SC) arise from either injured nerve roots or endogenous sources within the CNS. The SC migrate into the injury region, promoting axonal growth and remyelinating segmentally demyelinated axons as they do in the PNS. We observed in NSS-treated animals that SC myelination was extensive within the preserved penumbra white matter and myelination was also detected within the NSS remodeled tissue. Together, these results demonstrate that the NSS, implanted into the acutely injured spinal cord, reduces cavitation, promotes white matter sparing, and increases remodeled tissue supportive to axon sprouting and SC activity. These favorable morphological and cellular modifications may

underlie improvements observed in the clinic. Clinical neuroimaging and electrophysiology may be suitable to assess these consequences in subjects.

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Nanosymposium

383. Brain and Spinal Cord Injury

Location: SDCC 33C

Time: Monday, November 14, 2016, 1:00 PM - 4:00 PM

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Topic: C.09. Brain Injury and Trauma

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Natural Medicine and Biotechnology Research of Toyama Prefecture, Japan

Title: Extracellular neuroleukin improves hindlimb motor dysfunction of spinal cord injury

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Abstract: In the spinal cord injury (SCI), axonal growth in the inhibitory environment is one of essential factors for recovery from motor dysfunction. Neuroleukin (NLK) is a multifunctional protein also known as glucose-6-phosphate isomerase and Autocrine Motility Factor. NLK is a secreted protein and is recognized as a neurotrophic factor. However, a neuroprotective effect was demonstrated, but a neurite outgrowth activity was hardly reported. This study aimed to clarify the axonal growth activity of NLK and its functional significance in an animal model of axonal disruption. Cultured cortical neurons (ddY mice, E14) were treated with recombinant NLK (100 ng/ml) for 5 days, resulting in significant increase in the axonal density. In contusive SCI mice, microinjection of NLK (2 μg/mice, once) to the injured site immediately after injury significantly improved hindlimb motor dysfunction during 20 days. Considering that reactive astrocytes are accumulated in the injured site, we investigated the influence of NLK on astrocytes. Cultured astrocytes (ddY mice, E14) were treated by NLK (100 ng/ml, 6 days), and conditioned medium (ACM) was prepared. The ACM significantly enhanced the axonal density of cultured cortical neurons. Interestingly, the amount of NLK in ACM was increased by NLK stimulation. It may indicate a positive feedback cycle of NLK secretion in astrocytes. In conclusion, we firstly demonstrated that extracellular NLK improved hindlimb motor dysfunction of SCI mice. NLK-elicited axonal growth is perhaps amplified by astrocyte mediated NLK secretion.

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Nanosymposium

383. Brain and Spinal Cord Injury

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Presentation Number: 383.08

Topic: C.09. Brain Injury and Trauma

Title: T2*-weighted MRI provides a novel assessment of spinal cord white matter that correlates more precisely with clinical features of degenerative cervical myelopathy than DTI or MT

Authors: *A. R. MARTIN[1], B. DE LEENER[3], J. COHEN-ADAD[3], D. W. CADOTTE[1], S. KALSI-RYAN[1], S. F. LANGE[1], A. CRAWLEY[2], D. J. MIKULIS[2], H. GINSBERG[1], M. G. FEHLINGS[1];
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Abstract: INTRODUCTION: Diffusion tensor imaging (DTI), magnetization transfer (MT), and T2*-weighted (T2*w) MRI can measure micro- and macro-structural changes, including axonal loss, demyelination, and atrophy. We introduce T2*-white matter (WM)/grey matter (GM) signal intensity ratio (T2*-WM/GM) as a novel measure of tissue injury and compare it against established markers fractional anisotropy (FA), MT ratio (MTR), and cross-sectional area (CSA). We assess how these measures, extracted at the maximally compressed level (MCL), rostral (C1-C3), and caudal (C6-C7) regions, correlate with disability in degenerative cervical myelopathy (DCM).
METHODS: 56 DCM patients (age 56.7; 61% male; 32 mild, 14 moderate, 10 severe) underwent comprehensive clinical assessments and DTI, MT, and T2*w (3T, 13 axial slices, C1-C7). Images were semi-automatically analyzed with Spinal Cord Toolbox (SCT). Metrics extracted at MCL were converted to Z scores to facilitate analysis at varying rostro-caudal levels. Group comparisons against 32 healthy subjects used T tests, univariate correlations used Spearman coefficients, and backwards stepwise linear regression was used for multivariate analysis, including age, sex, height, weight, and neck length as covariates. Metrics from total WM were analyzed against global disability (modified Japanese Orthopedic Association, mJOA score), while metrics from lateral corticospinal tract (LCST) and fasciculus cuneatus (FCun) were analyzed against ipsilateral upper extremity (UE) power (10 myotomes) and UE sensation.
RESULTS: T2*-WM/GM was increased at all cord levels (rostral: $p=5x10^{-7}$, MCL: $p=3x10^{-9}$, caudal: $p=4x10^{-4}$), outperforming FA (rostral: p=0.001, MCL: $p=2x10^{-5}$, caudal: p=0.01) and MTR (rostral: p=0.02, MCL: p=0.006, caudal: p=0.98). T2*-WM/GM also provided stronger correlation with mJOA (rostral: r=-0.62, MCL: r=-0.63) than FA (r=0.39, 0.53) and MTR (r=0.28, 0.38). Rostral T2*-WM/GM predicted ipsilateral weakness (r=0.65) and sensory deficit (r=0.71) better than FA (r=0.57, 0.61) and MTR (r = 0.27, 0.39). Linear regression for mJOA with all variables generated a model ($R^2=0.53$, $p=9x10^{-13}$) with only CSA_{MCL} ($p=1x10^{-7}$) and T2*-WM/GM_{MCL} (p=0.001). A rostral-only regression model ($R^2=0.49$,$p=5x10^{-10}$) retained T2*-WM/GM ($p=2 \times 10^{-4}$), CSA ($p = 4x10^{-4}$), and FA (p=0.004).
CONCLUSIONS: T2*-WM/GM ratio shows stronger correlation with global and focal impairment than FA and MTR. Our multi-parametric approach offers complimentary information to better characterize tissue injury. These methods may be useful for diagnostics, monitoring of progression/recovery, and outcome prediction in DCM and other pathologies.

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Nanosymposium

383. Brain and Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

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Title: Increased TNF/TNFR1 signaling on macrophages in the injured peripheral nerve of BACE1 KO mice

Authors: *J. A. FISSEL[1,2], M. FARAH[1,2];
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Abstract: The recruitment of macrophages to injured peripheral nerves is beneficial for nerve regeneration. However, normal regeneration is insufficient and incomplete to restore full function. Beta site amyloid precursor protein cleaving enzyme 1 knockout (BACE1 KO) mice have increased recruitment of macrophages to the injured peripheral nerve and more robustly clear myelin debris in the distal segment of the injured nerve. In a recent publication (Liu et al 2016) our group has shown that tumor necrosis factor α receptor (TNFR1) expression is up-regulated in injured sciatic nerves of BACE1 KO mice. This increased expression of TNFR1 has functional relevance, as it increases expression and nuclear translocation of NFκB-p65, resulting in increased subsequent downstream signaling in BACE1 KO tissues. Bone marrow transplantation experiments have provided evidence that this increased expression of TNFR1 is being mediated by BACE1 KO macrophages. The effects on TNFR1 expression/signaling can be reproduced in adult WT mice treated with a BACE1 inhibitor, which could also provide clinical relevance.
In order to determine if TNFα/TNFR1 signaling is driving the phenotype observed in BACE1 KO mice, we have bred TNFR1; BACE1 KO and TNFα; BACE1 KO mice. We have injured the sciatic nerves of 3-4 month old double KO mice along with TNFR1 KO, TNFα KO, BACE1 KO, and WT mice. The results and analysis of these experiments will be presented.

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Nanosymposium

383. Brain and Spinal Cord Injury

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Topic: E.09. Spinal Cord Injury and Plasticity

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Research Manitoba

Title: Neuregulin-1 promotes an anti-inflammatory response associated with reduced glial scarring and improved neurological recovery following spinal cord injury

Authors: *A. ALIZADEH, S. M. DYCK, H. KATARIA, D. NGUYEN, T. SANTHOSH, S. KARIMI-ABDOLREZAEE;
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Abstract: Astrogliosis and neuroinflammation play pivotal roles in the secondary injury mechanisms after spinal cord injury (SCI) with both pro- and anti-regenerative effects. Little is known about the endogenous mechanisms that regulate glial and immune cell response in SCI. We previously reported for the first time that expression of Neuregulin-1 (Nrg-1) is dramatically and permanently downregulated in acute SCI. Glial and immune cells express Nrg-1 receptors, ErbB2, 3, 4, suggesting the potential ramifications of Nrg-1 dysregulation on glial activity and neuroinflammation. In this study, we sought to unravel the role of Nrg- 1 in regulating astrogliosis and immune response following SCI. Using complimentary *in vitro* and *in vivo* approaches, we demonstrate that Nrg-1 availability exerts anti- inflammatory and neuroprotective effects that can be culminated to foster recovery of function following SCI. *In vitro,* in a primary mixed culture of rat astrocytes and microglia activated by lipopolysaccharide (LPS), we show that treatment with recombinant human Neuregulin-1 (rhNrg-1β1) attenuates several characteristics of activated glia. We found a reduction in chondroitin sulfate proteoglycans (CSPGs), nitric oxide (NO), interleukin (IL)-1β and tumor necrosis factor (TNF)-α. Nrg-1 also attenuates proliferation and nestin expression of activated astrocytes. In rat compressive SCI, we delivered rhNrg-1β1 intrathecally for up to 6 weeks. We conducted Western blotting, gelatin zymography and stereology-based immunohistology at 1, 3, 7, 14 and 70 days post-SCI. Our analyses revealed that Nrg-1 promotes an anti-inflammatory phenotype in glial and immune cells associated with increased IL-10 and arginase-1 expression and a global decrease in pro-degenerative markers such as IL-1β, TNF-α and matrix metalloproteinases (MMP-2 and 9) after SCI. Nrg-1 also reduces glial scarring by decreasing GFAP expression and deposition of CSPGs in the SCI lesion. Importantly, Nrg-1 treatment significantly improves

neurobehavioural recovery following SCI. Mechanistically, we demonstrate that Nrg-1 effects on activated glia are mediated through ErbB2 tyrosine phosphorylation in an ErbB2/3 heterodimer complex. Intracellualrly, Nrg- 1 exerts its effects through downregulation of Myd88, a downstream adaptor of Toll-like receptors, and phosphorylation of Erk1/2 and STAT3. Our findings for the first time provide novel insight into the promising role of Nrg-1 in modulating activated glial and immune cells to harness their beneficial properties in repair and recovery following SCI. Supported by the CIHR, CPA and RM.

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Nanosymposium

383. Brain and Spinal Cord Injury

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Title: Aging negatively affects axon regeneration in the mammalian central nervous system

Authors: *C. G. GEOFFROY[1], B. J. HILTON[2], M. CHEN[1], W. TETZLAFF[2], B. ZHENG[1]; [1]UCSD, La Jolla, CA; [2]Dept. of Zoology, Intl. Collaboration on Repair Discoveries, Vancouver, BC, Canada

Abstract: Understanding how aging impacts our body's response to disease and injury is of fundamental value in modern medicine. Very little is known about how aging impacts the way our central nervous system (CNS, including the brain and the spinal cord) responds to injury. Here we present the first study on the effect of age in axon regeneration after CNS injury in mammals. Because CNS axons do not naturally regenerate after injury, previously it was not even possible to study this problem. Taking advantage of recent advances in neuron-intrinsic

control of CNS regeneration, we assessed the effect of targeting *Pten*, one of the most potent single molecular manipulations to date, in promoting central axon regeneration in mice of different ages. We found that in both corticospinal and rubrospinal neurons, *Pten* deletion in aging mice remains effective in preventing axotomy-induced decline in neuron-intrinsic growth state as assessed by mTOR activity, soma size and axonal growth proximal to a spinal cord injury. However, regeneration distal to injury is greatly diminished, accompanied by changes of astroglial and inflammatory responses at the injury site, implicating an increasing level of inhibitory influences at the injury site at increasing ages. Thus, *Pten* deletion unmasks an age-dependent decline in CNS axon regeneration. Not only is this finding of fundamental value to basic neuroscience, it has important implications on therapeutic development for spinal cord injury and a variety of other CNS conditions with axonal pathologies.

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Nanosymposium

383. Brain and Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

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East Carolina University Wooten Laboratory for Neurodegenerative Disease Research

East Carolina University Undergraduate Research and Creative Activities Award

Title: Targeting GSK-3beta signaling to prevent maladaptive sensory growth and the development of at and below level spinal cord injury pain

Authors: *S. K. BAREISS[1], M. ROWE[1], B. CONNER[1], A. WONKA[1], J. YOW[2], K. L. BREWER[2];
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Abstract: Neuropathic pain is a common, debilitating consequence following spinal cord injury (SCI). Recent evidence suggests that this pain may be due, in part, to maladaptive growth of afferent fibers at and below the level of injury. We have previously shown that SCI results in inhibition of glycogen synthase kinase-3β (GSK-3β) in both the dorsal root ganglia and dorsal horn, serving as a potential regulator of these growth responses. The objective of this study was

to correlate changes in GSK-3β activity with sensory outgrowth at and below the level of SCI, and determine if the effect of GSK-3β activation on downstream cytoskeletal substrates could block SCI-induced growth and the development of at- and below-level pain. Long-Evans rats underwent intramedullary injection of quisqualic acid (SCI) or saline (sham control) into the dorsal gray matter and allowed to survive for 1, 3, 7, 14, and 22 days (n=5-10 per group). Animals in 7, 14, and 22 day groups were examined for at-level dysesthesias (overgrooming) and below-level pain (thermal hyperalgesia and mechanical allodynia). DRG at and below the level of injury were cultured and analyzed for neurite outgrowth. Spinal cords were analyzed for alterations in GSK-3β signaling. Time course studies show early (1 day) and persistent (22 day) increases in at- and below-level growth responses following SCI with corresponding changes in GSK-3β, δ-catenin, and CRMP-2 expression at multiple time points. These alterations correlated with the presence and severity of pain related behaviors. To determine the effect of GSK-3β activation on afferent growth and pain behaviors, rats received intrathecal delivery of a GSK-3β activator (LY294002) after injury and were sacrificed 22 days later. LY294002 delivery initiated at the time of injury significantly decreased the SCI-induced growth and reduced the development of at-level dysesthesias (overgrooming) and below-level hyperalgesia and allodynia over the 22 day survival period. These data support that alterations in GSK-3β signaling contributes to maladaptive growth responses and the development of at- and below-level pain following SCI. GSK-3β and its downstream cytoskeletal substrates potentially constitute new therapeutic targets to prevent pain following SCI.

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Nanosymposium

384. Representation of Objects and Numbers Across Ventral and Dorsal Pathways

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ITN-316746-PRISM

Title: Neural coding of object shape in the macaque frontal cortex

Authors: I. CAPRARA, E. PREMEREUR, M. C. ROMERO, *P. JANSSEN;
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Abstract: Previous studies showed effective connectivity between the posterior sector of the Anterior Intraparietal area (pAIP) and prefrontal area 45B (Premereur E et al. (2015) PLoS Biology *13: e1002072*). Moreover, neurons in pAIP respond selectively to 2D shapes, contours and small line fragments (Romero MC et al. (2014) Journal of Neuroscience 34, 4006-4021). In the present study, we aimed to investigate the selectivity of single neurons in area 45B and to compare this to its input stage (pAIP). First, we performed electrical microstimulation during functional Magnetic Resonance Imaging (fMRI) in pAIP and observed the activation of area 45B in two monkeys, consistent with previous studies. Then, we recorded single-cell responses in area 45B in the activation induced by microstimulation of pAIP using images of objects during passive fixation. We first mapped the receptive field (RF) for every responsive cell using images of objects (3 deg) in a 12 by 8 deg area during passive fixation in the center of the screen, and then determined the minimum effective shape features evoking selective responses using a stimulus reduction approach. The stimuli consisted of contour shapes derived from images of real-world objects (full outlines; circumference ~16 deg), and line segments obtained by subdividing the full contours into 4, 8 and 16 fragments (length 4, 2 and 1 deg, respectively). We tested 108 responsive neurons in two monkeys. The large majority of those neurons (92%, 100/108) responded significantly to at least one of the 4-fragment stimuli, 98% (106/108) responded to at least one of the 8-fragment stimuli and 100% (108/108) responded to at least one of the 16-fragment level. Moreover, we determined the minimum effective shape feature (MESF) as the lowest level of fragmentation for which we observed a response which was at least 70% of the full outline response and not significantly smaller than the response to the original contour. With this criterion, for 65% of the neurons tested the MESF was one of the 16-fragment stimuli (76/117), while for only 3% of the neurons the MESF was one of the 8- (2/117) or 4-fragment stimuli (1/117). For the remaining neurons (25%, 29/117), the full contour was the minimum effective shape feature. Furthermore, the RF profiles in area 45B showed a very high complexity, ranging from large uniform RFs to small foveal RFs, similar to pAIP**.** In conclusion, EM-fMRI guided single-cell recordings show that area 45B neurons respond selectively to images of objects, and that their minimum effective shape features generally consist of very small line fragments measuring 1deg.

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Nanosymposium

384. Representation of Objects and Numbers Across Ventral and Dorsal Pathways

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Title: Luminance gradient at object borders communicates object location to the human oculo-motor system

Authors: *M. J. KILPELAINEN[1], M. A. GEORGESON[2];
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Abstract: For successful visually guided behaviour, it is essential to know the location of obstacles and other objects in our environment with sufficient precision. The visual processing of location (and other properties) of objects is generally assumed to be dominated by neural representations of the objects' edges. What exactly determines the perceived location of edges is still unresolved, however. One prominent model of human edge localization predicts that people see luminance edges at the points of maximal local phase coherence of the edge's Fourier transform, another that edges are seen at points of steepest luminance gradient. Earlier tests of these models, where subjects have marked the perceived location of the edge, have yielded surprisingly conflicting results. In this study, we test the two predictions with a task that is arguably closer to natural visual behaviour. Instead of consciously inspecting the edges per se, the six subjects (three naïve) moved their gaze from a central fixation point to the perceived centre of a square (width 4 degrees of visual angle), centred at 8 deg eccentricity. To test whether observed shifts in the saccade landing point were caused by shifts in a central luminance mass, rather than the edges of the square, we ran the experiments with two levels of blur, which affects the structure of the edges, but not the central area of the square. When the (horizontal and/or vertical) edges of the square were manipulated so that the luminance gradient peak moved, but the phase coherence peak stayed stationary, the average saccade end point moved, in close agreement with the predictions of the luminance gradient model. The pattern of results was remarkably similar in all 6 subjects. When the amount of blur in the stimuli was halved, the perceived shift of the square also halved, again in close agreement with the luminance gradient model. In contrast, a central luminance mass account predicts that the blur change should have no effect. Our results show, firstly, that edges have a robust effect on the perceived location of an object. Secondly, these edge signals are communicated to the level of conscious perception and oculomotor commands by a neural system that is sensitive to luminance gradients rather than phase coherence.

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Nanosymposium

384. Representation of Objects and Numbers Across Ventral and Dorsal Pathways

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Title: Differential sensitivity to whole vs. scrambled objects in ventral and dorsal pathways.

Authors: *E. FREUD[1], J. C. CULHAM[2], M. BEHRMANN[1];
[1]Carnegie Mellon Univ., Pittsburgh, PA; [2]Brain and Mind Inst., Univ. of Western Ontario, London, ON, Canada

Abstract: The nature of visual object representations in the ventral pathway and their perceptual significance have been characterized in great detail. One of the hallmark properties attributed to these ventral representations is sensitivity to object shape as a whole compared to scrambled objects. Recently, however, accumulating evidence suggests that object-based representations are also subserved by the dorsal visual pathway, although less is known about the characteristics of these representations and their perceptual importance. The present study combines psychophysical, neuropsychological and functional magnetic resonance imaging (fMRI) to address this issue. To examine the sensitivity to the object whole vs. parts, participants completed a fMRI scan while viewing objects with different levels of scrambling (from intact to 256-part decomposition). Object scrambling modulated the activation profiles of the two pathways in a linear fashion, with fMRI activations reduced as a function of the scrambling level. Interestingly, relative to the ventral pathway, dorsal-pathway representations were found to be more resilient to object scrambling and less correlated with recognition performance. These results suggest that, in contrast with ventral pathway representations, dorsal-pathway representations are less sensitive to object shape as a whole. Nevertheless, these results could be accounted for by an alternative explanation in which dorsal-pathway representations are merely a degraded version of the ventral representations that are not qualitatively different. To assess this, we adopted the same gradual scrambling experiment with SM, a patient with visual agnosia following a lesion to the right ventral pathway. Critically, SM's dorsal-pathway activation profile and its correlation with recognition performance were comparable to those observed for the controls. On the other hand, SM's ventral-pathway activation, specifically in the right

hemisphere, was less sensitive to object scrambling and less correlated with behavior compared with the controls. Thus, these findings suggest that the dorsal-pathway representations do not depend upon ventral-pathway representations, encode differential object properties and cannot support intact object recognition. Together, the present study provides novel evidence for the nature of object representations in the dorsal pathway and their fundamental differences from ventral-pathway object representations.

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Nanosymposium

384. Representation of Objects and Numbers Across Ventral and Dorsal Pathways

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Title: Rediscovering the ventral and dorsal pathways of visual information processing

Authors: ***Y. XU**, M. VAZIRI PASHKAM;
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Abstract: The ventral pathway has been traditionally thought to process object feature information whereas the dorsal pathway object position/action-related information. This two-pathway model has played a major role in shaping our understanding of the function of the posterior regions in visual cognition. Over the last two decades, however, both monkey neurophysiology and human brain imaging studies have reported robust object feature responses in the dorsal pathway and object location responses in the ventral pathway. Do similar visual representations exist in the two pathways? Or is the two-pathway distinction still valid? Using fMRI MVPA, here we examined in human observers the decoding of eight natural object categories in topographically and functionally defined occipital and parietal visual processing regions. Observers viewed blocks of images containing different exemplars from the same category and performed a 1-back repetition detection task. We first obtained pairwised decoding for the eight object categories and constructed a category-level representational similarity matrix for each brain region. We then correlated these matrices between pairs of brain regions and constructed a region-wise representational similarity matrix depicting how similar brain regions were in representing these object categories. To visualize the relationship between the different brain regions, we applied multi-dimensional scaling (MDS). Despite higher-level ventral regions and dorsal regions sharing a number of processing characteristics, they were separated from each

other in the MDS plot and were organized systematically according to their locations in the ventral and dorsal pathways. In additional experiments, we show that the emergence of these two pathways could not be due to the low-level image differences between the eight natural object categories or the specific object categories used, as the same separation between the ventral and dorsal regions was observed when we used nine categories of artificial objects. Instead, this separation seemed to reflect a difference in how objects are represented in the ventral and dorsal regions. Our study thus rediscovers the ventral and dorsal pathways of visual information processing, defined not by the type of visual information represented, but by how visual information may be represented.

Disclosures: **Y. Xu:** None. **M. Vaziri Pashkam:** None.

Nanosymposium

384. Representation of Objects and Numbers Across Ventral and Dorsal Pathways

Location: SDCC 23A

Time: Monday, November 14, 2016, 1:00 PM - 4:15 PM

Presentation Number: 384.05

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Support: ERC-2011-Stg-284101

IUAP-P7/11

ZW11_10

Title: Task context overrules action-related representational content in the human frontoparietal areas

Authors: ***S. BRACCI**, N. DANIELS, H. OP DE BEECK;
Lab. voor Biologische Psychologie, KU Leuven, Leuven, Belgium

Abstract: The ventral and dorsal visual pathways are both activated when people process objects, and their specific role is unclear. Two views can be distinguished. First, both pathways contain object representations for different purposes (i.e., action versus object vision). Second, the ventral pathway represents all object properties and the dorsal pathway represents whatever object properties are relevant for the current task context. The current literature contains evidence for both hypotheses, but no studies designed to dissociate between them. A study is needed to target representations that are, most likely, differentially represented in the two pathways (hypothesis 1) while manipulating task context (hypothesis 2). To this end, the reported human imaging study tested a stimulus set which dissociated object category from object action and required subjects to process the objects in two task contexts: one targeting

object category information and the other targeting object action information. The ventral visual cortex represented both nonvisual object properties, largely independent of task context. In contrast, representations in parietal and prefrontal areas represented task-relevant object properties only, with no sign of the irrelevant object property. Most strikingly, there was no evidence for a representation of object action in parietal cortex in the category task. These findings support a distinction between the ventral stream that represents object properties to sustain object recognition and semantics and the dorsal stream that only represents object properties behaviorally relevant in a given moment.

Disclosures: **S. Bracci:** None. **N. Daniels:** None. **H. Op de Beeck:** None.

Nanosymposium

384. Representation of Objects and Numbers Across Ventral and Dorsal Pathways

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Title: Real-world size improves recognition of real objects, not images

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Abstract: Patients with visual agnosia can show improved recognition for real-world objects – a phenomenon known as the 'Real Object Advantage'. Some have argued that this phenomenon is attributable to additional stereo cues present in real objects, but not planar images. It is also the case, however, that real objects have a known distance from the observer, and because most real objects have a physical size that corresponds to their real world size, they convey absolute size information in a way that images do not. In a series of behavioral experiments, we investigated the extent to which absolute size cues influence object recognition. We studied recognition performance in a patient with profound visual form agnosia (D.F.) with bilateral LOC lesions. Recognition performance was also examined in a group of neurologically healthy observers. In both the patient, and healthy observers, recognition was better for real graspable objects (versus size-matched images of the same items shown on an LCD monitor) –particularly for items whose physical size was consistent with real-world size (i.e., shell, apple, spoon) versus objects whose physical size was orders of magnitude smaller than normal size (i.e., toy-sized bus, horse, table).

Follow-up experiments compared recognition performance for 3D-printed objects that were realistically-sized, versus those that were scaled 50% above or below real world size. Performance in the patient, and the healthy observers, was again better for the real-world-sized versus the size-adjusted objects. No performance differences were found in recognition of size-matched 2D images of the stimuli. These patient data suggest that physical size is an important aspect of dorsal stream object representations, and together with the data from controls, our results suggest that absolute size is a fundamental cue that facilitates the identification of graspable objects in the healthy brain.

Disclosures: J.C. Snow: None.

Nanosymposium

384. Representation of Objects and Numbers Across Ventral and Dorsal Pathways

Location: SDCC 23A

Time: Monday, November 14, 2016, 1:00 PM - 4:15 PM

Presentation Number: 384.07

Topic: D.06. Vision

Title: Distinct neural signatures for very small and very large numerosities

Authors: M. FORNACIAI, *J. PARK;
Psychological and Brain Sci., Univ. of Massachusetts, Amherst, MA

Abstract: Behavioral performance in numerical estimation shows distinct response patterns for different ranges of numerosity. When numerosity is very small, there is virtually no variability in the estimates of numerical value. When numerosity is large, the variability of numerical estimates increases linearly with numerosity following the Weber's law. When numerosity is very large so that the stimulus forms a cluttered ensemble, this variability increases with the square root of numerosity. These observations suggest the existence of multiple mechanisms underlying numerosity perception. In this study, we investigate the neurophysiological signatures of such potentially different mechanisms. While their EEG was recorded, participants passively viewed a stream of dot arrays that varied systematically in numerical and non-numerical visual properties, following the design of the previously developed technique (Park et al., 2016). In one case the numerosities were very small (1-4 dots), and in the other case the numerosities were very large (100-400 dots). In both cases, numerosity modulated the visual evoked potentials (VEPs) arising from the right occipitoparietal site around 160 ms, similar to what was previously observed with numerosities between 8 and 32 dots (Park et al., 2016). However, unlike that previous finding, (1) there was no selective modulation of VEPs by very small or very large numerosities in the earlier latency over the medial occipital site, and (2) the modulation of VEPs by very small numerosities around 160 ms was in the opposite direction compared to the

modulation of VEPs by larger numerosities. Overall, the current results, together with the previous finding (Park et al., 2016), provide novel neurophysiological evidence of markedly different mechanisms underlying the processing of different numerosity ranges, thus bolstering recent theories proposing multiple mechanisms underlying numerosity perception.

Disclosures: **M. Fornaciai:** None. **J. Park:** None.

Nanosymposium

384. Representation of Objects and Numbers Across Ventral and Dorsal Pathways

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Support: EU grant

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Department of Health

Title: A generalized sense of number for perception and action

Authors: ***D. BURR**, R. ARRIGHI, G. ANOBILE, I. TOGOLI;
Univ. of Florence, Florence, Italy

Abstract: Much evidence has accumulated to suggest that many animals, including young human infants, possess an abstract sense of approximate quantity: a *sense of number*. A truly abstract sense of number would encode the numerosity of any set of discrete elements, however displayed, and in whatever sensory modality. And as programing movement sequences must utilize numerical information, the number system should also be linked to the generation of movement. Here we use the psychophysical technique of adaptation to study the number sense for items presented both sequentially and simultaneously, and its interaction with motor actions. We show that the numerosity of both auditory and visual sequences is greatly affected by adaptation: adapting to rapid sequences of events causes subsequent displays to appear less numerous; and *vice versa*. Adaptation to visual stimuli was spatially selective (in external, not retinal coordinates), pointing to a sensory rather than cognitive process. Adaptation generalized across modalities, from audition to vision and *vice versa*, and also from touch to vision. Importantly, adaptation generalized across formats: adapting to sequential streams of flashes strongly affected the perceived numerosity of spatial arrays. Finally, we found a clear interaction between self-produced actions and perceived numerosity: a short period of rapid finger-tapping (without sensory feedback) caused subjects to underestimate the number of visual stimuli

presented near the tapping region; and a period of slow tapping caused overestimation. Again the effect was spatially selective, in external not body coordinates, and also occurred across formats, equally strong for sequential (series of flashes) and simultaneous stimuli (clouds of dots). Our results all point towards the existence of a *generalized number sense*, one that transcends vision, audition and touch, as well as motor action, to encode a common, abstract sense of event number in both space and time.

Disclosures: D. Burr: None. **R. Arrighi:** None. **G. Anobile:** None. **I. Togoli:** None.

Nanosymposium

384. Representation of Objects and Numbers Across Ventral and Dorsal Pathways

Location: SDCC 23A

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Presentation Number: 384.09

Topic: D.06. Vision

Title: Integration of Number across separate dot clusters

Authors: *M. J. MORGAN[1], M. KRELLNER[1], J. A. SOLOMON[2];
[1]Max-Planck Inst., Cologne, Germany; [2]Optometry and Visual Sci., City Univ. London, London, United Kingdom

Abstract: Physiological studies indicate the existence of neural populations tuned to difference in number of elements in a texture. However, the numbers involved are mostly quite small (<10), while human observers can reliably discriminate differences between textures involving hundreds of dots, if not thousands. A plausible mechanism of relative numerosity discrimination for large numbers is that spatial samples are extracted containing much smaller number of dots, and discrimination is based on a comparison between these samples in the two patterns. We have investigated the ability of observers to do just that explicitly, using patterns composed of several (4 or 6) clearly separated dot clusters, each of which contained a small number of dots sampled from a binomial distribution (variance $np(1-p)$), with n=2, 4 or 8 in different sessions. The observer's task was to discriminate differences in probability, p between two such patterns in a temporal 2AFC paradigm, over a range of pedestal values for p. Comparison of human performance with that of an ideal observer in the same task showed that at least some observers were reliably using several samples to make the discrimination.
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common visual metric for approximate number and density. *Proceedings of the National Academy of Sciences of the United States of America, 108*(49), 19552-19557. doi: 10.1073/pnas.1113195108
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Raphael, S. & Morgan, M.J. (2014) The computation of relative numerosity, size and density. *Vision Research*, 12,022.

Disclosures: M.J. Morgan: None. **M. Krellner:** None. **J.A. Solomon:** None.

Nanosymposium

384. Representation of Objects and Numbers Across Ventral and Dorsal Pathways

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Title: Coupling of human temporal and parietal neural activity during numerical processing

Authors: *A. L. DAITCH, J. PARVIZI;
Lab. of Behavioral and Cognitive Neurosci. (LBCN), Stanford Univ., Stanford, CA

Abstract: While the ability to approximate or compare rough quantities is present even in human infants and other species such as non-human primates and birds, the association of exact quantities with symbols (such as numerals, e.g. ‘10’) or verbal representations (such as number words, e.g. ‘ten’) is unique to humans exposed to such culturally learned entities. Specific brain regions within the lateral parietal cortex (LPC) and ventral temporal cortex (VTC) have been shown to code for abstract quantity representations, and symbolic numerical representations (numerals), respectively, and current models of numerical processing predict an interaction between these regions when humans interpret and manipulate numerals. However, the fast dynamics of activity within each region, and interaction between them, remain largely unknown, since many imaging methods that have been instrumental in localizing these individual regions lack the temporal resolution to track their fast dynamics. In this study, we took advantage of the high temporal resolution of electrocorticography (ECoG) to track the fast temporal dynamics

within and between the VTC and LPC in 16 human subjects with coverage of one or both of these regions (12 with simultaneous coverage of the VTC and LPC), as subjects read and manipulated visual numerals across several tasks, ranging from simple recognition of visual numerals to more complicated arithmetic computations. We first re-confirmed the existence of numeral/math-selective hubs within the VTC and LPC. Specifically, we observed a small hub within the posterior inferior temporal gyrus (pITG) with selective responses to numerals versus other morphologically similar stimuli (letters, false fonts), surrounded by a larger region not selective for individual numerals but selectively engaged during active mathematical processing. We also observed a region around the anterior intraparietal sulcus (aIPS) selectively engaged in active mathematical processing. These two math-selective regions responded later during mathematical processing than other regions that were also active, but less selective for mathematical processing, suggesting a more high-level, rather than purely sensory-related involvement of these regions. In a few subjects, these pITG and aITG math-selective sites responded nearly simultaneously during numerical processing. Lastly, we demonstrated the first empirical evidence of a selective functional coupling between the pITG and aIPS during numerical processing, which appears to be largely top-down from the aIPS to pITG, allowing us to refine current models of numerical processing in the human brain

Disclosures: **A.L. Daitch:** None. **J. Parvizi:** None.

Nanosymposium

384. Representation of Objects and Numbers Across Ventral and Dorsal Pathways

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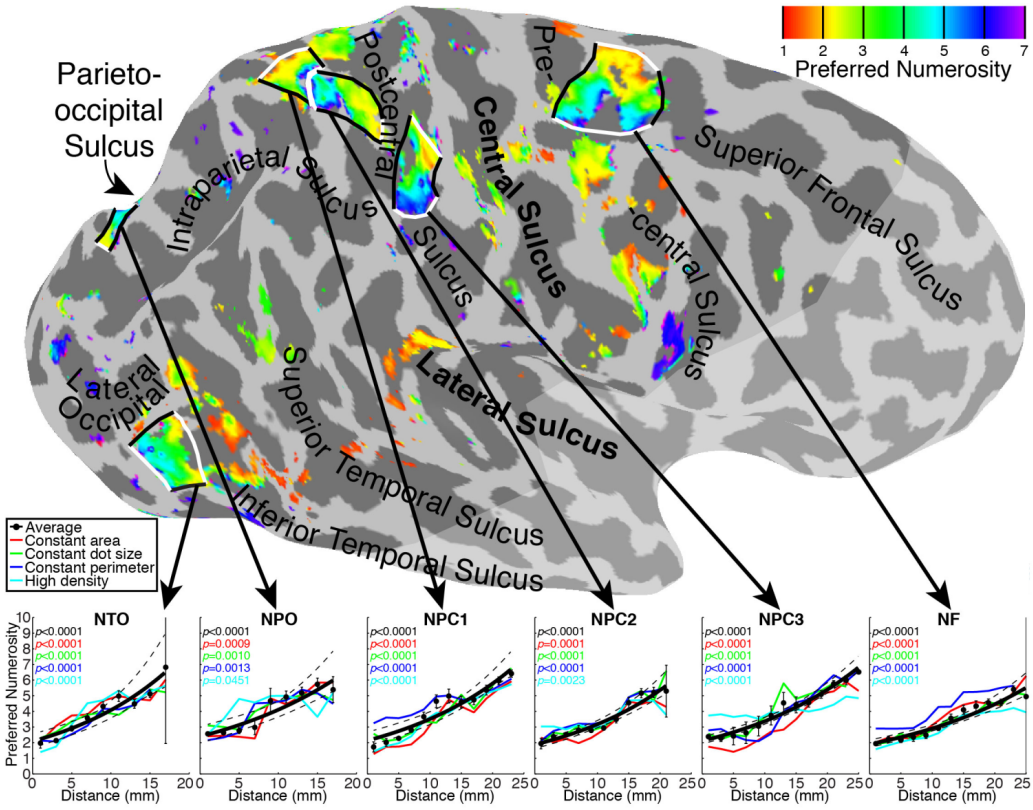
Netherlands Organization for Scientific Research #433.09.223

Title: A network of topographic numerosity maps throughout human association cortex

Authors: *B. M. HARVEY[1], S. O. DUMOULIN[2];
[1]Utrecht Univ., Utrecht, Netherlands; [2]Spinoza Ctr. for Neuroimaging, Amsterdam, Netherlands

Abstract: Perception of numerosity (number of objects in a set) and other quantities is implicated in many cognitive functions including foraging, attention control, decision-making and mathematics. Many parietal and frontal areas are activated during numerosity tasks. We

recently described a topographic map of numerosity-selective neural populations in human right parietal lobe (Harvey et al, Science 2013). Akin to sensory topographic maps, we hypothesize that there is an extensive network of numerosity maps that may allow interactions with multiple cognitive systems. Methods: We acquired ultra high-field fMRI (7T) data while showing stimuli of changing numerosity. We used several stimulus conditions to distinguish numerosity selectivity from selectivity to co-varying stimulus features. We used population receptive field modeling (Dumoulin and Wandell, 2008, Neuroimage) to summarize each recording site's response as a tuning function with a particular preferred numerosity and tuning width. Results: In each hemisphere, we found six maps of gradually changing numerosity. We found two new numerosity maps near the previously described map in the postcentral sulcus. In the occipital lobe, we found one map at the superior end of the parieto-occipital sulcus and another anterior to the lateral occipital sulcus. We also found a frontal numerosity map at the junction of the precentral and superior frontal sulci. Left hemisphere maps typically contained more low numerosity preferences, with more high numerosity preferences in the right hemisphere. Within each hemisphere, anterior maps contained a smaller proportion of high numerosity preferences than posterior maps, and maps differed considerably in size. Unlike sensory topographic maps, numerosity tuning widths were very similar between maps. Conclusion: There is a similar representation of numerosity in brain areas implicated in object recognition, motion perception, attention control, decision-making and mathematics. This suggests a broad role for quantity processing in supporting many perceptual and cognitive functions.



Disclosures: **B.M. Harvey:** None. **S.O. Dumoulin:** None.

Nanosymposium

384. Representation of Objects and Numbers Across Ventral and Dorsal Pathways

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Title: Exploring the neuronal population responses in the human prefrontal cortex during integrated and segmented arithmetic processing

Authors: *X. YANG[1,2], A. DAITCH[2], J. PARVIZI[2];
[1]Stanford Univ., Stanford, CA; [2]Dept. of Neurol. and Neurolog. Sci., Lab. of Behavioral and Cognitive Neurosci. (LBCN), Stanford, CA

Abstract: Extant neuroimaging evidence in humans and electrophysiological studies in non-human primates have demonstrated that arithmetic processing would elicit increased neural activations in the intraparietal sulcus (IPS) and ventral temporal cortical (VTC) regions. However, the role of human prefrontal cortex (PFC) during arithmetic processing and its interactions with other key areas within the mathematical processing network still remain unclear. We recorded electrocorticographic (ECoG) signals in 10 human subjects implanted with chronic intracranial electrodes in the prefrontal cortex. We analyzed high-frequency broadband (HFB) or high-gamma activity during an integrated math experiment, which required retrieval of arithmetic and autobiographical knowledge to make a true or false judgment, followed by a segmented math experiment where arithmetic equations were presented step by step. Our analyses revealed several PFC neuronal populations activated during the overall processing of arithmetic conditions, with a fast and sustained activation at the posterior middle frontal gyrus (pMFG) and a gradually growing activation at the anterior middle frontal gyrus (aMFG) in the left hemisphere; a flipped response pattern was observed in the right pMFG and aMFG. The posterior superior frontal gyrus (pSFG) and inferior frontal gyrus (pIFG) showed a similar activation profile of a fast growing and slowly dying down activation bilaterally. Statistical analyses of the HFB response onset latency indicated that VTC responded first to arithmetic conditions, followed by PFC, and IPS joined at last to complete the mathematical processing network. The results of the segmented math experiment suggested that the prefrontal cortex was also involved in the neural processing of hierarchical structures of arithmetic equations. Our findings are in line with reported MEG and ECoG studies on syntactic structure building during language comprehension where hierarchical structures are also embedded. This study provides a means for both exploring the activity of a specific neuronal population in the human brain during the overall processing of arithmetic conditions and the activity of the same population of neurons

throughout the online parsing and unification of the hierarchical structure with high spatial and temporal resolutions.

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Nanosymposium

384. Representation of Objects and Numbers Across Ventral and Dorsal Pathways

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Topic: D.06. Vision

Support: NIH Grant EY019303

Title: Pharmacological inactivation of intraparietal sulcus reveals a causal role in ordinal comparison in macaque monkeys.

Authors: ***N. K. DEWIND**[1], J.-Y. PENG[3], E. M. BRANNON[1], M. L. PLATT[2]; [1]Psychology, [2]Neurosci., Univ. of Pennsylvania, Philadelphia, PA; [3]Cell Biol. and Neurosci., Rutgers, New Brunswick, NJ

Abstract: The "number sense" describes the intuitive ability to quantify without counting. Single neuron recordings in non-human primates and functional imaging in humans suggest that the intraparietal sulcus (IPS) is an important neuroanatomical locus of numerical estimation. Other lines of inquiry, however, have demonstrated that the IPS is related to numerous other functions, including attention and ordering magnitudes. Here we describe two experiments that directly test the hypothesis that IPS plays a unique role in numerical cognition. We used muscimol to reversibly inactivate the ventral and lateral intraparietal areas, separately, in two monkeys performing a numerical discrimination task and a color discrimination control task matched for difficulty. Overall, VIP inactivation slowed response times and LIP inactivation both slowed response times and impaired accuracy, compared to performance following injections of saline. We found no evidence for a selective impairment in number discrimination. Instead, impairments were similar in both the color and number discrimination tasks, ruling out a specialized role for IPS in number processing. We also ruled out any profound motor or sensory impairments that could have interfered with performance on the tasks. Thus, our experiments support the idea that the IPS contributes more broadly to the comparison of stimuli along ordinal continua, such as line length or motion coherence, rather than the specific discrimination of number.

Disclosures: **N.K. Dewind:** None. **J. Peng:** None. **E.M. Brannon:** None. **M.L. Platt:** None.

Nanosymposium

385. Spatial Attention and Working Memory

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BBSRC (UK) Grant BB/M010732/1

Title: The neural basis of dynamic coding in prefrontal cortex during a spatial working memory task

Authors: *E. SPAAK[1], D. WASMUHT[1], T. J. BUSCHMAN[2], E. K. MILLER[3], M. STOKES[1]; [1]Dept. of Exptl. Psychology, Oxford Univ., Oxford, United Kingdom; [2]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [3]Dept. of Brain & Cognitive Sci., MIT, Cambridge, MA

Abstract: Working memory (WM) provides the cognitive mechanism which allows animals to exhibit intelligent behaviour beyond immediate stimulus-response mappings. WM maintenance is classically assumed to depend on active neuronal firing: a discontinuity in external events is bridged by introducing a literal continuity in neuronal activity. Recently, increasing evidence suggests that such neural stability is the exception rather than the rule; instead, neural states are more properly characterized as highly dynamic, and information can be maintained without elevated firing rates.

During performance of a spatial working memory task, spiking activity was recorded simultaneously from multiple neurons in non-human primates (*Macaca mulatta*). Activity was recorded from several brain regions: lateral prefrontal cortex (PFC), frontal eye fields (FEF), and lateral intraparietal cortex (LIP). We applied several types of multi- and univariate analyses to (1) test for the presence of and (2) characterize the nature of dynamic neural coding during WM maintenance. We found strong evidence for dynamic coding during WM delays in all regions, particularly the PFC. This dynamic code was due to a combination of two factors: individual neurons change their coding preference across time (a specific form of mixed selectivity), and the neural population has a consistent pattern of preferred onset times (the neurons form a 'cascade').

These findings are in line with our earlier reports of dynamic coding during memory-guided saccade execution, and extend this earlier work to more complex, 'human-like', WM-based cognitive tasks. Importantly, we are now able to provide a more detailed view of the neural dynamics underlying dynamic coding, and its prevalence in different cortical regions. Finally, the large-scale nature of the present data allowed us to characterize synaptic connectivity between individual neurons. In addition to the clear results mentioned above, we will present

preliminary evidence compatible with the hypothesis that rapid short-term plasticity can function as a substrate for WM maintenance.

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Nanosymposium

385. Spatial Attention and Working Memory

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Topic: D.06. Vision

Support: CIHR

NSERC

Title: Noise correlation structure shapes ensemble coding of working memory in prefrontal cortex

Authors: ***M. LEAVITT**[1], F. PIEPER[2], A. J. SACHS[3], J. C. MARTINEZ-TRUJILLO[4]; [1]Physiol., McGill Univ., Montreal, QC, Canada; [2]Inst. for Neuro- & Pathophysiology, Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [3]Div. of Neurosurgery, Ottawa Hosp. Res. Inst., Univ. of Ottawa, Otttawa, ON, Canada; [4]Robarts Res. Institute; Dept. of Psychiatry; Dept. of Physiol. and Pharmacol., Univ. of Western Ontario, London, ON, Canada

Abstract: Single neurons in the primate lateral prefrontal cortex (LPFC) are thought to encode working memory (WM) representations of visual space via sustained firing. However, neurophysiological studies of WM typically record from individual neurons, thus we lack an understanding of how *ensembles* of simultaneously-recorded neurons represent WM: we do not know if WM representations are affected by between-neuron phenomena (e.g. noise correlations—r_{sc}), nor how WM coding properties scale with the size and composition of neuronal ensembles. In order to investigate these questions, we used microelectrode arrays to record from neuronal ensembles in LPFC area 8a of two rhesus macaques while they performed a traditional oculomotor delayed-response task, and assessed the information content of the ensembles using a linear classifier. We found that the size and composition of macaque LPFC neuronal ensembles have a profound effect on the amount of information encoded during a spatial WM task. Removing the intrinsic r_{sc} structure of a neuronal ensemble could yield inverse effects on the decoding of WM information depending on the size and tuning properties of the ensemble. However, decoding was consistently impaired after removing the r_{sc} structure from

neuronal ensembles that maximize WM information. Furthermore, neurons that are not WM-selective when examined individually using traditional criteria can still increase the information content of an ensemble by altering the r_{sc} structure. Our results indicate that LPFC neuronal ensembles encode WM information through a synergistic interaction between single neuron coding properties and emergent ensemble-level phenomena.

Disclosures: **M. Leavitt:** None. **F. Pieper:** None. **A.J. Sachs:** None. **J.C. Martinez-Trujillo:** None.

Nanosymposium

385. Spatial Attention and Working Memory

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Title: Neural correlate of visual working memory in the macaque monkey

Authors: ***M. PARE**, C. LI, J. BARBER;
Queen's Univ., Kingston, ON, Canada

Abstract: Persistent activity is regarded as a potential neural correlate of working memory, as it has been observed during the retention interval of tasks probing working memory in both humans and monkeys. In particular, human studies have identified such activity in event-related potentials (ERP) over the posterior cortex. Identifying similar signals in monkeys is essential to bridging the gap between these species and fully understanding the neural basis of human working memory. We previously reported a sustained negativity in rhesus monkeys implanted with electrodes in their skulls over occipital and parietal cortices while they performed a visual sequential comparison task (Li et al., 2014). In this task, monkeys must report with a targeting saccade the location of a color change within an array of colored stimuli following a 1-s retention interval; the number of stimuli in the array (2-5) manipulates memory load. As in human studies, we found that the amplitude and polarity of this signal during the retention interval reflected the spatial location of the target stimulus, was predictive of trial outcome, and scaled with memory load. Here we report on a power spectral analysis of these data. Our main finding is a modulation in the power of oscillations in both alpha (10-13 Hz) and beta (15-30 Hz) bands with the number of items that had to be remembered. These observations are also consistent with local field potentials recorded within the lateral intra-parietal (LIP) area during the same task. In addition, spike spectrograms from LIP neurons reveal a similar power modulation in alpha- and beta-band

frequencies during the delay period of the memory guided saccade task. Altogether, these findings provide a link to the single-neuron mechanisms of working memory in monkeys and further validate the monkey as a model of human visual working memory.

Disclosures: **M. Pare:** None. **C. Li:** None. **J. Barber:** None.

Nanosymposium

385. Spatial Attention and Working Memory

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Support: NIH EY014924

Title: Modulation of neuronal activity in macaque area V4 during spatial working memory and saccade preparation

Authors: ***D. JONIKAITIS**[1], T. MOORE[2];
[1]Howard Hughes Med. Inst., Stanford Univ. Sch. of Med., Stanford, CA; [2]Howard Hughes Med. Inst. and Stanford Univ., Stanford, CA

Abstract: Spatial attention, visual spatial working memory (WM) and oculomotor control are three processes thought to be highly interdependent and brought about by similar neural mechanisms. Although both oculomotor preparation and spatial WM appear to influence the deployment of spatial attention, the relative contributions of the former two functions remain unclear. During spatial attention, neurons in posterior visual cortex exhibit greater sensitivity to visual stimulation, and this modulation is thought to underlie the perceptual benefits of spatial attention. To investigate the relative contributions of oculomotor preparation and spatial WM to this modulation, we measured the influence of the two processes on the responses of neurons in area V4 of monkeys performing a task that dissociates them. During the task, while the monkey fixated a central spot, a briefly flashed peripheral visual cue indicated a location to be remembered. After a delay period (1 - 2 seconds), two stimuli appeared, one at the memorized location, and one at another (randomized) location. The monkey was rewarded for saccades either to the stimulus at the memorized location (‘Look’ blocks) or to the other location (‘Avoid’ blocks). Importantly, in both types of blocks, the monkey was required to remember the cued location, yet only in the ‘Look’ blocks could it prepare a saccade to that location. In the ‘Avoid’ blocks, saccade preparation is eliminated, as indicated by slow saccadic reaction times. During the task, we are recording from neurons in area V4 using linear array microelectrodes and measuring their responses during the delay period. Measurement of delay period activity is

facilitated by the use of a textured display background that is irrelevant to the task, but evokes spiking activity in the recorded neurons. This procedure allows us to contrast the delay activity observed during trials on which the memory cue appears in the neuronal receptive field (RF) to trials on which the cue appears elsewhere. Indeed, we find that during the delay period, the texture-driven responses of V4 neurons are enhanced during trials on which the monkey memorizes the RF location. In addition, this procedure also allows us to contrast delay activity between the 'Look' and 'Avoid' blocks, and thus to determine whether spatial WM is sufficient for the observed delay period modulation, or if instead that modulation depends on the preparation of eye movements.

Disclosures: D. Jonikaitis: None. **T. Moore:** None.

Nanosymposium

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NEI R01EY026924

Title: Spatial working memory enhances visual cortical representations

Authors: *B. NOUDOOST[1], Y. MERRIKHI[3], T. MOORE[4], K. CLARK[1], E. ALBARRAN[4], M. PARSA[2];
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Abstract: Prefrontal cortex is known to modulate sensory signals in posterior visual cortex, possibly via the direct projections from the frontal eye field (FEF) to multiple visual cortical areas. Upon examining the nature of the FEF signal sent to visual cortex, we found that persistent, working-memory related activity is the predominant feature of FEF neurons projecting to extrastriate cortex. This signal was not sufficient to drive spiking activity in extrastriate areas V4 and the middle temporal area (MT); however, we found that during memory maintenance visually driven responses in both these areas were modulated by the content of spatial working memory. Using linear array electrodes, responses of neurons in MT and V4 were

recorded during a memory-guided saccade task. The probe-evoked visual responses and receptive fields (RFs) were compared during the fixation and spatial working memory periods. Although the firing rate of the MT neurons was not altered by the memory location in the absence of any visual stimuli, the visual responses of the same neurons depended upon the content of spatial working memory. The RFs of MT neurons also expanded and shifted toward the remembered location. The net effect of these changes was to increase the number of neurons responding to a probe stimulus near the remembered location, improving their ability to represent the stimulus (as assessed by the performance of an SVM classifier using the population activity). Moreover, we measured the efficacy of visual cortical input into the FEF and found that it was enhanced when remembering a corresponding spatial location. These results suggest persistent signals sent from the FEF as a means to alter representations within posterior visual areas, and imply a positive feedback loop between prefrontal and visual areas to gate the processing of visual signals according to the content of working memory.

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Nanosymposium

385. Spatial Attention and Working Memory

Location: SDCC 7B

Time: Monday, November 14, 2016, 1:00 PM - 4:00 PM

Presentation Number: 385.06

Topic: D.06. Vision

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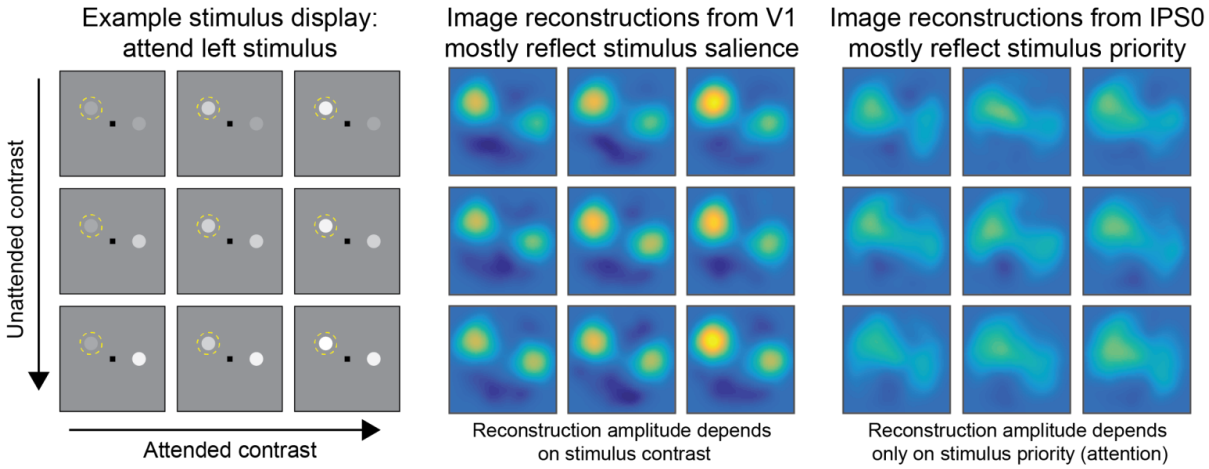
Title: Graded representations of stimulus salience and attentional priority across visually-responsive cortex

Authors: ***T. C. SPRAGUE**[1,2], S. ITTHIPURIPAT[2], V. A. VO[2], J. T. SERENCES[3,2]; [1]Dept. of Psychology, New York Univ., New York, NY; [2]Neurosciences Grad. Program, [3]Dept Psychology, UC San Diego, La Jolla, CA

Abstract: The visual system faces the complex task of selectively processing behaviorally relevant parts of a scene in the context of irrelevant parts, which could each have different

degrees of salience (Itti & Koch, 2001). Neural responses in early visual regions show a strong dependence on contrast (a component of salience), while those measured from higher-order visual regions show a greater sensitivity to attentional demands (Luck et al, 1997). Activity patterns across entire visual regions are thought to support maps of stimulus salience and/or behavioral priority over the entire visual field (Serences & Yantis, 2006). Here, to identify salience and priority maps across the human brain, we simultaneously manipulated salience (contrast) and attentional priority (locus of spatial attention) in a display featuring two stimuli while scanning participants with fMRI. We used activation patterns within several visually responsive ROIs and an image reconstruction technique to visualize maps of the entire visual field and quantify stimulus representations on each trial (Sprague & Serences, 2013). We reasoned that image reconstructions from a pure salience map would reflect only information about stimulus contrast, while those from a pure priority map would reflect only information about the behaviorally relevant stimulus. In early visual regions (V1-hV4), stimulus representations tracked both visual salience and attentional priority: their activation increased with contrast and with behavioral relevance. However, in higher-order regions (IPS0), we primarily observed representations of priority: the attended stimulus appeared most clearly in the reconstructions, with minimal activation associated with the ignored stimulus. Interestingly, representations of ignored high-contrast stimuli were never stronger than those of attended low-contrast stimuli. These results suggest a graded involvement of visual brain regions in integrating information about bottom-up salience and top-down attentional priority even at early stages of cortical visual processing.



Disclosures: **T.C. Sprague:** None. **S. Itthipuripat:** None. **V.A. Vo:** None. **J.T. Serences:** None.

Nanosymposium

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Title: Rhythmic neural activity within the macaque attention network modulates moment-to-moment sampling of the visual environment

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Abstract: Imagine New York's Times Square: tall buildings, blinking lights, and a swarm of people. Given the brain's limited processing resources, this scene represents an overload of sensory information. To sort through it all, the brain uses various selection mechanisms, broadly referred to as *attention*. Spatial selection, one such mechanism, has been compared to a spotlight that continuously scans the visual environment, pausing to illuminate potentially relevant locations. Recent research has shown that the enhanced processing associated with this metaphorical spotlight is not sustained. Instead, it fluctuates rhythmically, creating alternating periods of enhanced and diminished sensory processing. That is, we rhythmically sample our visual environment. These fluctuations in spatial selection predictably modulate behavioral performance in both humans and macaques, by (1) influencing the deployment of attention in response to a spatial cue and by (2) influencing perceptual sensitivity at the moment a subsequent target occurs. To investigate neural sources contributing to rhythmic sampling of the visual environment, we simultaneously recorded from three hubs of the attention network in the macaque: the frontal eye fields (FEF), the lateral intraparietal area (LIP), and the pulvinar nucleus of the thalamus. We specifically investigated how spike rates and oscillatory components of local field potentials (LFPs) correlate with the detection of a low-contrast visual target. Our data reveal local neural activity and network-level interactions that contribute to rhythmic sampling at both cued (more likely) and uncued (less likely) target locations. There is a dissociation of function within the attention network. Contributions to rhythmic sampling from different network hubs shift under different conditions of attentional deployment. LIP and the pulvinar mediate rhythmic sampling at the cued (more likely) target location, while FEF and the pulvinar mediate rhythmic sampling at uncued (less likely) target locations. Our results

demonstrate that *multiple* rhythmic processes *concurrently* modulate moment-to-moment perceptual sensitivity at *multiple* locations across the visual environment.

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Nanosymposium

385. Spatial Attention and Working Memory

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Topic: D.06. Vision

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Title: Neural modulation of visual input at expected distractor locations

Authors: *M. STOKES[1], Y. BAUER[2], A. VON LAUTZ[3], C. SUMMERFIELD[4], M. NOONAN[4];
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Abstract: Recent evidence suggests that flexible top-down mechanisms of cognitive control are specialised for target-related attention, whereas distractor suppression only emerges when the predictive information can be inferred directly from past experience. For example, in the case of visuospatial attention, cueing a specific distractor location is only effective when the distractor location repeats across trials, not when distractor location is cued on a trial-wise basis. This constraint on distractor cueing contrasts with flexible cueing for target locations. We now explore how predictions for target and distractor location build up across trials as well as the time course and neural components involved in target facilitation and distractor suppression respectively. In a speeded target discrimination task subjects are implicitly cued to the location of the target or distractor via manipulations in the underlying predictability of the two stimuli. In different sessions we collected EEG and MEG data from the same subjects. Behaviourally, reaction times were reduced when either stimuli was more spatially predictable. A reinforcement learning model generated trial-wise estimates of the spatial priors for each location independently for targets and distractors. This analysis revealed that learning rates were higher for targets than distractors. Analysis of the EEG data replicated our previous study showing a bilateral reduction in the amplitude of the P1 after distractor stimulus repetitions. This was mirrored by a trend towards an increase in P1 amplitude after target stimulus repetitions in electrodes contralateral to targets. Lateralized N2pc effects were also less pronounced after

distractor repetition than non-repetition trials. This is consistent with a predictive coding model of expectation suppression that is selective for task-irrelevant information.

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Nanosymposium

385. Spatial Attention and Working Memory

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Topic: D.06. Vision

Support: R01 144-AAA6814

NIH IRACDA

Title: Preparatory encoding of the location and scope of human spatial attention

Authors: ***J. SAMAHA**[1], T. C. SPRAGUE[2], B. VOYTEK[3], A. GAZZALEY[4], B. R. POSTLE[1]; [1]UW Madison, Madison, WI; [2]New York Univ., New York, NY; [3]UCSD, San Diego, CA; [4]Univ. of California, San Francisco, San Francisco, CA

Abstract: Humans can dynamically shift the location and scope of their spatial attentional focus. This ability is hypothesized to rely on top-down gain modulation of neural populations selective for the attended feature (e.g., spatial region), prior to the onset of the anticipated stimulus. Using a multivariate encoding model of electroencephalographic (EEG) activity, we show that preparatory encoding of spatial attention is reflected in the topography of pre-target alpha-band (8-13 Hz) oscillations. In experiment 1, a symbolic central cue directed covert spatial attention to one of six visual field locations arranged concentrically around fixation. Beginning around 450 ms post-cue, we could robustly reconstruct the attended location from the topography of posterior alpha power, but not phase. In experiment 2, we designed a novel distributed attention task, wherein a central cue indicated the span of arc along an imaginary circle, from 0 to 360 degrees, along which a target could appear. Response time and accuracy improved for narrower spans, when attention could be more spatially focused. An alpha-based encoding model revealed a linear decline in spatial selectivity as subjects' allocated attention across wider spans of the visual field. The magnitude of this decline predicted the magnitude of accuracy and response time declines across subjects. Together these results suggest that multivariate patterns of alpha-band power can be used to track the onset, location, and spatial scope of covert visual attention.

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Nanosymposium

385. Spatial Attention and Working Memory

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Topic: D.06. Vision

Support: R01MH062349

R01EY016407

Title: Visual field maps constrain working memory precision

Authors: ***C. E. CURTIS**[1], W. E. MACKEY[2], X. DING[3], X.-J. WANG[3], J. WINAWER[3]; [1]Psych & CNS, [2]Psychology, [3]Ctr. for Neural Sci., NYU, New York, NY

Abstract: Working memory (WM) suffers from severe capacity limitations that vary greatly both between individuals and across the lifespan. Recent psychophysical work suggests that WM is a finite resource, and that this resource is unevenly distributed across items stored and maintained in WM. Internal representations of sensory stimuli are corrupted by random noise, and this noise is modulated by the amount of resource allocated to a particular item. In turn, the precision of an item in WM is directly related to how much of this resource is being dedicated to it. Currently, the neurobiological basis of this limitation is unknown. Here, we demonstrate that WM accuracy is limited by the size of visual field maps in early visual and frontoparietal cortex. First, we simulated population activity in a bump attractor network using various population sizes to examine the relationship between the size of the population and the fidelity of the WM representation. Results show that as population size increases, the peak amplitude of the WM representation increases while the variance of the WM representation decreases. This is consistent with our hypothesis that population size may underlie limitations in WM precision. Second, we used nonlinear population receptive-field mapping to identify visual field maps in early visual cortex as well as parietal and frontal cortices in both hemispheres for each subject. This allowed us to quantify the size of specific populations in individual subjects. We then correlated the size of each visual field map with individual performance in the contralateral visual field during a spatial WM task and found that the size of multiple visual field maps predicted WM precision. Finally, we simulated population level activity in each visual field map within each subject. This simulation confirms the predictions of our theoretical model: the variance of the WM representation in the population activity correlates with WM accuracy.

These results strongly support the hypothesis that WM resources are limited by the size of visual field maps, specifically in brain regions known to be critical for WM performance. Our results provide key insights into the neurobiological basis of resource limitations in WM and suggest that such limitations have a structural basis.

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Nanosymposium

385. Spatial Attention and Working Memory

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Topic: D.06. Vision

Support: NSF GRFP (VAV)

NIMH R01-MH092345 (JTS)

Title: Spatial attention modulates voxel receptive fields to boost the fidelity of multi-voxel stimulus representations

Authors: *V. A. VO[1], T. C. SPRAGUE[3,1], J. T. SERENCES[2,1];
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Abstract: Covert spatial attention is known to modulate the response properties of both single units in macaques and single voxels in humans. These unit-level changes can be described as a shift in the center of a receptive field (RF) or a change in its size or amplitude (Connor et al., 1997; Womelsdorf et al., 2006; 2008; Klein et al., 2014; Sprague & Serences, 2013; Sheremata & Silver, 2015; Kay et al. 2015). However, the direction and magnitude of these attentional modulations vary depending on the RF's position in the visual field and its spatial relationship to the attended location (Niebergall et al., 2011; Anton-Erxleben et al., 2009). This makes it difficult to predict how unit-level changes in spatial encoding properties would affect the quality of the neural representation at the population level (e.g., over a group of single cells or in a group of voxels). A leading hypothesis suggests that unit-level attentional modulations in spatial RFs act to jointly increase the overall fidelity of population-level representations of the attended location (Anton-Erxleben & Carrasco, 2013). We evaluated this hypothesis by measuring fMRI BOLD responses while subjects (n = 7) attended to different locations in a display while an irrelevant visual mapping stimulus appeared at different locations on the screen. At the unit level,

we first measured how spatial attention modulated single voxel receptive fields (vRFs). Then at the population level, we used a multivariate spatial encoding model to reconstruct a representation of the mapping stimulus on each trial. This allowed us to quantify how the combined modulations across all vRFs in a visual region influenced the quality of population codes for visual stimuli. At the unit-level, we found that spatial attention leads to a complex pattern of voxel RF modulations, including changes in size, gain, and preferred position in V1 through V4 and IPS0. At the population-level, we found an increase in the amplitude of reconstructed stimuli near the attended target. Finally, we used each voxel's estimated vRF to simulate how different types of unit-level vRF changes affect the quality of the population code. We observed that a combination of gain and preferred position changes in vRFs had the largest impact on attention-driven increases in the fidelity of multivariate stimulus reconstructions. Furthermore, we suggest that the pattern of these changes may be altered by the spatial scope of the subject's attention field (Klein et al, 2014; Womelsdorf et al, 2008; Reynolds & Heeger, 2009). Our approach provides a template for further investigation into the mapping between single units and populations.

Disclosures: V.A. Vo: None. **T.C. Sprague:** None. **J.T. Serences:** None.

Nanosymposium

385. Spatial Attention and Working Memory

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Presentation Number: 385.12

Topic: D.06. Vision

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Title: Handedness-dependent hemispheric asymmetries in parietal spatial attention maps

Authors: *S. L. SHEREMATA[1,2], M. A. SILVER[3,4,5];
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Abstract: Recent studies have demonstrated hemispheric asymmetries in fronto-parietal attention networks involved in visual attention and short-term memory. While handedness has often been associated with brain lateralization, few studies have compared hemispheric asymmetries in right- and left-handed individuals. In this study, we employed fMRI and a spatial attention paradigm to determine the effects of handedness on attention-dependent asymmetries in retinotopically-defined areas of the intraparietal sulcus (IPS). Eighteen participants (nine left-

handed) maintained fixation while attending to either a central fixation square (attend-fixation condition) or a peripheral mapping stimulus (attend-stimulus condition). Population receptive fields (pRFs) were estimated in retinotopically-defined regions of occipital and parietal cortex for each participant while the mapping stimulus traversed locations throughout the visual field. pRF size and center location were estimated for each voxel for both attention conditions. In both left-handed and right-handed participants, attending to the stimulus increased pRF size in both hemispheres of occipital and parietal cortex. In contrast, spatial attention effects on pRF center locations in parietal cortex exhibited hemispheric asymmetries that were modulated by handedness. In right-handed subjects, attending to the stimulus resulted in more peripheral preferred locations of contralateral representations in the left, but not the right, hemisphere, compared with attending fixation. For right-handed subjects, the combined effects of attention on pRF size and preferred location preserved contralateral representations in left parietal cortex. In contrast, attentional modulation of pRF size, but not preferred location, significantly increased representation of the ipsilateral (right) visual hemifield in right parietal cortex. In left-handed subjects, the effects of attention on pRF center were reversed, leading to greater ipsilateral representations in the left than right hemisphere in the attend-stimulus condition. No asymmetries were found in the occipital cortex for either right- or left-handed subjects. These results reveal that handedness modulates attention-dependent hemispheric asymmetries in parietal cortex. Specifically, directing attention to a moving stimulus results in significantly more peripherally-located pRFs in the hemisphere contralateral to the dominant hand. Investigating handedness effects is important for understanding individual differences in hemispheric asymmetries and is relevant for the study of spatial neglect and its neural substrates.

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Nanosymposium

386. Physiological and Pathophysiological Mechanisms of the Blood Brain Barrier

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Presentation Number: 386.01

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Lundbeck Foundation R126-2012-12143

Danish Council for Independent Research Technology and Production Sciences 12–126975

Title: Pericyte modulation by functional antibodies obtained by a novel selection strategy

Authors: *J. JUST[1,2], K. DRASBEK[1,4], S. LYKKEMARK[2,4], C. NIELSEN[2], P. KRISTENSEN[3];
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Abstract: Pericytes surround the endothelial cells of the microvasculature, giving them a unique position to serve as important constituents for vascular homeostasis. The pericyte serve as an active participant in several crucial vascular functions including angiogenesis, vascular stability and permeability. However, pericyte loss or dysfunction has been described in a number of pathologies. The pericyte coverage of tumor vasculature is altered compared to normal vasculature while pericyte loss in several neurodegenerative diseases, and even the natural aging process itself, correlate with a decreased barrier function of the microvessels. Targeting the pericyte could therefore prove instrumental in the further development of vascular therapeutics. In order to therapeutically target the pericyte, we describe a novel single cell selection strategy, ASPIRATION, and show that this method provides an efficient platform for the selection of functional antibodies against single cells. We identified the cognate antigen of one of the selected antibodies, C3, as pericyte-expressed fibronectin. This antibody was shown to be a potent inhibitor of pericyte migration in a scratch wound assay. Furthermore, a pro-angiogenic response was observed when the C3 antibody was added to a pericyte and endothelial cell co-culture tube formation assay. Thus, we identify a distinct fibronectin epitope important for pericyte interaction and functionality. Targeting of this epitope in pathologies where pericytes are affected could potentially prove of therapeutic benefit.

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Nanosymposium

386. Physiological and Pathophysiological Mechanisms of the Blood Brain Barrier

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Presentation Number: 386.02

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01AG039452

Title: Pericyte degeneration causes diffuse white matter dysfunction as assessed by advanced magnetic resonance imaging

Authors: *A. MONTAGNE[1], A. M. NIKOLAKOPOULOU[1], Z. ZHAO[1], G. SI[1], D. LAZIC[1], M. DAIANU[2], A. P. SAGARE[1], R. E. JACOBS[3], S. R. BARNES[3], P. M. THOMPSON[2], B. V.

ZLOKOVIC[1];
[1]USC, Los Angeles, CA; [2]Mark & Mary Stevens Neuroimaging & Informatics Inst., USC, Marina del Rey, CA; [3]Div. of Biol. and Biol. Engin., Caltech, Pasadena, CA

Abstract: White matter (WM) and pericyte degeneration have been observed in many neurodegenerative diseases. Pericytes are vascular mural cells embedded in the wall of small blood vessels. In the brain, they control blood-brain barrier (BBB) integrity and cerebral blood flow (CBF) and they participate in clearance of toxins. Mice with global pericyte degeneration, caused by platelet-derived growth factor receptor-β (*Pdgfrβ*) deficiency in pericytes ($Pdgfr\beta^{F7/F7}$), have been shown to develop aberrant CBF responses and chronic BBB breakdown associated with brain accumulation of serum proteins (*e.g.*, fibrinogen) in grey matter regions (*e.g.*, cortical mantles and hippocampus). However, whether WM is affected by pericyte loss, and whether pericytes are necessary for healthy WM, remain unknown to date. Using diffusion-weighted magnetic resonance imaging (MRI), we found that $Pdgfr\beta^{F7/F7}$ mice have early WM atrophy. Also, diffusion tensor imaging (DTI)-based metrics revealed decreased fractional anisotropy (FA) within different WM regions-of-interest (ROIs) including corpus callosum (CC) and anterior cingulum (AC), as well as significant fiber loss as assessed by 3D-DTI-tractography, confirming a loss of WM structural connectivity in 12- to 16-week-old $Pdgfr\beta^{F7/F7}$ animals. Interestingly, no WM damage was detected in younger 4- to 6-week-old $Pdgfr\beta^{F7/F7}$ mice when compared to age-matched $Pdgfr\beta^{+/+}$ littermate controls. Interestingly, we discovered early vascular dysfunctions in 4- to 6-week-old $Pdgfr\beta^{F7/F7}$ mice prior to WM injury using two advanced gadolinium-based contrast MRI approaches. First, we identified a significant increase in the BBB transfer constant, K_{trans}, in multiple WM ROIs (*i.e.*, CC, AC, internal (IC) and external (EC) capsules) in 4- to 6-week-old $Pdgfr\beta^{F7/F7}$ mice compared to age-matched $Pdgfr\beta^{+/+}$ mice by the use of an innovative dynamic contrast-enhanced (DCE)-MRI method. In addition, we used a novel dynamic susceptibility contrast (DSC)-MRI approach and uncovered that CBF was critically reduced within the CC, AC, IC, and EC areas in 4- to 6-week-old $Pdgfr\beta^{F7/F7}$ mice compared to age-matched $Pdgfr\beta^{+/+}$. Finally, pharmacological depletion of fibrinogen levels rescued WM dysfunctions in pericyte-deficient mice, including a reversal of BBB breakdown (DCE-MRI), restoration of CBF function (DSC-MRI), and reorganization of WM tracts (DTI-MRI) in the CC. Overall, our results show that pericyte-deficiency causes vascular dysfunction at a very early stage in different WM regions, allowing blood-derived neurotoxic fibrinogen to enter the brain, thus locally damaging WM connectivity.

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Nanosymposium

386. Physiological and Pathophysiological Mechanisms of the Blood Brain Barrier

Location: SDCC 1B

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Presentation Number: 386.03

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Validation of CD98hc as a novel blood brain barrier target

Authors: *B. CHIH[1], J. J. ZUCHERO[2], X. CHEN[3], N. BIEN-LY[2], D. BUMBACA[4], R. K. TONG[5], X. GAO[6], S. ZHANG[2], K. HOYTE[6], W. LUK[6], M. A. HUNTLEY[7], L. PHU[5], C. TAN[3], D. KALLOP[8], R. M. WEIMER[8], Y. LU[6], D. S. KIRKPATRICK[5], J. ERNST[5], M. S. DENNIS[3], R. J. WATTS[2];
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Abstract: The blood-brain barrier (BBB) poses a major challenge for developing effective antibody therapies for neurological diseases. Using proteomic analysis of isolated mouse BECs, we identified multiple highly expressed proteins, including basigin, Glut1, and CD98hc. To measure if these targets can be utilized to bring therapeutic antibodies into the brain, antibodies to each of these targets were generated. Antibodies to each of these targets were significantly enriched in the brain after administration in vivo. Bispecific antibodies were generated, pairing with an anti BACE arm to reduce Aβ in the brain in order to further demonstrate that the antibodies are delivered into the brain parenchyma as measured by pharmacodynamic response. In particular, antibodies against CD98hc showed robust accumulation in brain after systemic dosing, and a significant pharmacodynamic response as measured by brain Aβ reduction. We further showed that antiCD98hc antibodies did not block amino acid uptake in cell based assays and did not reduce CD98hc protein level in the brain, demonstrating a good safety profile. The discovery of CD98hc as a robust receptor-mediated transcytosis pathway for antibody delivery to the brain expands the current approaches available for enhancing brain uptake of therapeutic antibodies.

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Phu: A. Employment/Salary (full or part-time): Genentech. **C. Tan:** A. Employment/Salary (full or part-time): Genentech. **D. Kallop:** A. Employment/Salary (full or part-time): Genentech. **R.M. Weimer:** A. Employment/Salary (full or part-time): Genentech. **Y. Lu:** A. Employment/Salary (full or part-time): Genentech. **D.S. Kirkpatrick:** A. Employment/Salary (full or part-time): Genentech. **J. Ernst:** A. Employment/Salary (full or part-time): Genentech. **M.S. Dennis:** A. Employment/Salary (full or part-time): Genentech. **R.J. Watts:** A. Employment/Salary (full or part-time): Genentech.

Nanosymposium

386. Physiological and Pathophysiological Mechanisms of the Blood Brain Barrier

Location: SDCC 1B

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Presentation Number: 386.04

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Regional Heterogeneity of the Blood-Brain Barrier

Authors: *M. BLANCHETTE[1], N. RUDERISCH[3], R. DANEMAN[2];
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Abstract: The blood-brain barrier (BBB) consists of a set of different properties expressed by the brain endothelial cells (BEC), including a high expression of tight junction molecules and specific transporters, low rates of trancytosis and a low expression of leucocyte adhesion molecules. These properties allow a tight regulation of the ions, molecules and cells moving across the BBB in order to protect the brain from endogenous and exogenous blood borne molecules, bacteria and viruses. The specific transporters expressed at the BBB control the entrance of specific nutrients, signaling factors, and waste molecules, mandatory for the maintenance of brain homeostasis to allow proper brain function. The different regions of the CNS are composed of different neuronal types and display different ratios of neuronal versus glial cells. This suggests that different regions of the brain may need different levels nutrients, neurotransmitter precursors or signaling factors to achieve proper neurological functions, however it is not known if there are regional specializations of the BBB required to regulate local properties of the brain. In order to determine if there is a regional specialization of the BBB within the different regions of the CNS, we performed RNA sequencing on endothelial cells isolated from different regions of the mouse CNS: the forebrain, cerebellum, spinal cord. To isolate endothelial cells, we took advantage of the Tie2GFP mice, which express GFP under the Tie2 promoter. Cell suspensions from each CNS region were sorted for GFP positive cells and RNA extraction, purification and then RNA sequencing was performed on each sample. The

different expression of BBB specific genes was compared between the three different isolated CNS regions. We found multiple genes and different pathways enriched at the BBB in each CNS region. We are now exploring their function at the BBB and how they are needed for proper brain function. These data suggest that the BBB has fundamental basic characteristics but also has certain heterogeneity to fulfill the specific needs of each brain regions. This opens a whole new field of research as the BBB was thought to be a specific set of features displayed by all BECs.

Disclosures: M. Blanchette: None. **N. Ruderisch:** None. **R. Daneman:** None.

Nanosymposium

386. Physiological and Pathophysiological Mechanisms of the Blood Brain Barrier

Location: SDCC 1B

Time: Monday, November 14, 2016, 1:00 PM - 3:00 PM

Presentation Number: 386.05

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Clayton Medical Research Foundation

Title: Intranasal delivery of peptidergic corticotropin-releasing factor receptor antagonists is facilitated by cell-penetrating peptides

Authors: *L. A. TAN, J. M. VAUGHAN, K. P. GARIBAY, P. E. SAWCHENKO;
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Abstract: Corticotropin-releasing factor (CRF) and its receptors are mediators of neuroendocrine and other stress response modalities, and have been implicated in the etiology of mood disorders and neurodegenerative diseases. As such, the CRF system is a potential therapeutic target for a variety of disorders, and many CRF receptor small-molecule or peptide antagonists have been synthesized. However, non-invasively targeting the central CRF system has been problematic, as the blood-brain barrier (BBB) excludes larger peptides, and peripherally-administered small molecules that freely penetrate the BBB may exert undesirable peripheral effects in a CRF receptor-dependent or -independent manner. Recently, we have employed an intranasal delivery strategy to non-invasively administer a small-molecule CRF_1 receptor antagonist (R121,919) directly to the brain, which specifically, dose-dependently, and transiently blocked CRF_1 receptors throughout the brain. Unexpectedly, intranasal delivery of R121,919 to mice led to a transient hypothermic effect, lowering core body temperature of treated mice by as much as 10 °C for as long as 12 hours. This effect was not observed following intraperitoneal injections of R121,919 at the same dose, nor was it seen in CRF receptor knockout mice. This led to the exploration of the use of peptide antagonists, whose size (~10-

fold greater molecular weight) may hinder their ability to access the brain, even via the intranasal route. Therefore, we co-administered the cell-penetrating peptide, penetratin, which effectively facilitated entry of the CRF-related peptide, PD-sauvagine, into the brain, as evidenced by displacement of CRF receptor radioligand binding. Current experiments are aimed at testing the effects of intranasal delivery of peptide antagonists on central (e.g., fos expression) versus peripheral (e.g., corticosterone secretion) CRF receptor-mediated endpoints.

Disclosures: **L.A. Tan:** None. **J.M. Vaughan:** None. **K.P. Garibay:** None. **P.E. Sawchenko:** None.

Nanosymposium

386. Physiological and Pathophysiological Mechanisms of the Blood Brain Barrier

Location: SDCC 1B

Time: Monday, November 14, 2016, 1:00 PM - 3:00 PM

Presentation Number: 386.06

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: RO1AG039452

Title: Pericyte ablation leads to disruption of the neurovascular unit

Authors: ***B. V. ZLOKOVIC**, A. M. NIKOLAKOPOULOU, Z. ZHAO, K. KISLER, P. KONG, D. LAZIC, A. P. SAGARE, M. D. SWEENEY, E. J. LAWSON, Y. YANG, A. GO; Zilkha Neurogenetic Inst., Keck Sch. of Med. of the Univ. of Southern California, Los Angeles, CA

Abstract: Brain pericytes are the vascular mural cells located within the basement membrane of blood microvessels. Pericytes maintain blood-brain barrier integrity, regulate cerebral blood flow (CBF) and participate in the clearance of brain toxic byproducts. Much of the knowledge has been gained from studies in mice with inherited pericyte deficiency caused by aberrant signaling between endothelial-derived platelet-derived-growth factor B (PDGF-B) and PDGF-receptor β (PDGFRβ) in pericytes. Here, we report the development of a new murine pericyte-specific Cre line using a double-promoter strategy, and show Cre-dependent dtTomato reporter expression only in brain pericytes, but not in oligodendrocytes, oligodendrocyte progenitor cells, vascular smooth muscle cells, brain endothelial cells, astrocytes or microglia. Next, we developed a model, where pericyte ablation was induced after diphtheria toxin (DT) administration in adult mice expressing the Cre-dependent human DT receptor (DTR) in pericytes. We show that acute global ablation of pericytes leads to neurovascular dysfunction and a series of pathophysiological events that begin with dysregulated cerebral blood flow responses and blood-brain barrier breakdown, causing white matter injury, neuronal dysfunction and functional deficits. These data

suggest that pericytes play an important role in maintaining the integrity of the neurovascular unit and that their loss leads to disruption of the neurovascular unit, causing cerebrovascular disorder and white matter and neurodegenerative changes.

Disclosures: **B.V. Zlokovic:** None. **A.M. Nikolakopoulou:** None. **Z. Zhao:** None. **K. Kisler:** None. **P. Kong:** None. **D. Lazic:** None. **A.P. Sagare:** None. **M.D. Sweeney:** None. **E.J. Lawson:** None. **Y. Yang:** None. **A. Go:** None.

Nanosymposium

386. Physiological and Pathophysiological Mechanisms of the Blood Brain Barrier

Location: SDCC 1B

Time: Monday, November 14, 2016, 1:00 PM - 3:00 PM

Presentation Number: 386.07

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NMSS grant PP-1506-04755

Title: Glymphatic function is suppressed in the experimental autoimmune encephalomyelitis, EAE, model of multiple sclerosis

Authors: ***I. LUNDGAARD**[1], S. O'NEIL[1], E. YANG[1], H. VINITSKY[1], M. NEDERGAARD[1,2]; [1]Univ. of Rochester, Rochester, NY; [2]Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Multiple Sclerosis (MS) is an autoimmune disease targeting myelin in the central nervous system. T-lymphocytes are needed to initiate pathology and peri-vascular immune cell cuffing is often observed in MS patients. The peri-vascular cuffs are located in the anatomical pathways of the glymphatic system, which is a brain-wide clearance system utilizing peri-vascular pathways for convective flow of solutes. It is unknown whether MS affects the glymphatic system. Here we used the experimental autoimmune encephalomyelitis (EAE) mouse model of MS and investigated cerebrospinal fluid (CSF) dynamics. We found that the flow of CSF was reduced to the brain and even more severely diminished in the spinal cord of chronic EAE mice. The distribution of CSF was inversely correlated with the number of lesions, suggesting that EAE tissue pathology affects the glymphatic system in chronic disease. Intriguingly, inhibition of the glymphatic function using two independent methods during the pre-symptomatic and early disease phase significantly ameliorated EAE clinical symptoms. This suggests that the glymphatic system might play a role in transporting T-lymphocytes from the CSF to the parenchyma. Targeting the glymphatic system in the early phase of MS might be a novel mechanism to curb disease by preventing immune cell influx from the CSF to the brain.

Disclosures: **I. Lundgaard:** None. **S. O'Neil:** None. **E. Yang:** None. **H. Vinitsky:** None. **M. Nedergaard:** None.

Nanosymposium

386. Physiological and Pathophysiological Mechanisms of the Blood Brain Barrier

Location: SDCC 1B

Time: Monday, November 14, 2016, 1:00 PM - 3:00 PM

Presentation Number: 386.08

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Microfluidics to model the human blood-brain barrier for studies of barrier function, drug penetration, and leukocyte-endothelial interactions

Authors: ***B. R. OBERMEIER**[1], G. MARSH[1], A. HUANG[1], M. KOLLER[2], K. FISHER[2], A. C. COTLEUR[1], F. SHIMIZU[3], Y. SANO[3], T. KANDA[3], J. DUFFIELD[1], R. M. RANSOHOFF[1]; [1]Biogen, Cambridge, MA; [2]Nortis, Inc., Woodinville, WA; [3]Yamaguchi Univ., Ube, Yamaguchi, Japan

Abstract: In a plethora of neurologic disorders, the blood-brain barrier (BBB) plays a crucial role in initiating or aggravating damage to the neural tissue. Situated right at the interface between the blood and the brain, the BBB has to fulfill its manifold barrier and gateway functions properly to ensure an optimal environment for the neuronal network. To elucidate mechanisms of barrier function and dysfunction, and to explore new therapeutic avenues, cell-based models are needed that recapitulate the BBB's unique anatomic and functional properties in a physiological flow-based setting.

We have established a microfluidic 3D cell culture model of the BBB that closely mimics the multicellular structure of the in vivo BBB. We use human brain microvascular endothelial cells, pericytes, and astrocytes as tri-culture in a chip developed by Nortis, Inc., which allows physical contact between all cells. For functional assays, we perform live cell imaging to assess permeability, to measure transport of therapeutic antibodies, and to study dynamic leukocyte-endothelial interactions under flow. Recovery of cells from the chip allows us to perform transcriptomic and proteomic profiling comparing different culture conditions and treatments. When BBB cells are tri-cultured in the chip, they form various cellular interactions, in which pericytes and astrocytes invest the abluminal aspect of the endothelium. Cells deposit laminin to generate a basement membrane decorating the endothelial tube. Over time, a tight barrier is obtained that discriminates between substrates of different sizes. The presence of pericytes and astrocytes improves barrier tightness as compared to endothelial monocultures. We do not observe any passive leak to antibodies, qualifying the model for transcytosis studies with therapeutic antibodies and potentially predicting brain penetration. Activation with inflammatory

cytokines results in increased barrier permeability as expected and promotes leukocytes to interact with the endothelium. Using this inflammatory in vitro setting as paradigm, we have successfully reproduced leukocyte rolling, arrest, and crawling in discrete steps, as reported by others from intravital two-photon imaging in a rat model of multiple sclerosis.
Our novel 3D microfluidic model of the human BBB represents potent technology to mimic and study molecular mechanisms of barrier (dys)function and other aspects of neurologic disease on a chip, notably in a highly physiological setting. The tissue-like microenvironment incorporating human cells allows studies of human disease without relying on interspecies extrapolation, a key feature of cell-based research.

Disclosures: **B.R. Obermeier:** A. Employment/Salary (full or part-time): Biogen. **G. Marsh:** A. Employment/Salary (full or part-time): Biogen. **A. Huang:** A. Employment/Salary (full or part-time): Biogen. **M. Koller:** A. Employment/Salary (full or part-time): Nortis, Inc. **K. Fisher:** A. Employment/Salary (full or part-time): Nortis, Inc. **A.C. Cotleur:** A. Employment/Salary (full or part-time): Biogen. **F. Shimizu:** A. Employment/Salary (full or part-time): Biogen. **Y. Sano:** None. **T. Kanda:** None. **J. Duffield:** A. Employment/Salary (full or part-time): Biogen. **R.M. Ransohoff:** A. Employment/Salary (full or part-time): Biogen.

Nanosymposium

387. Oxytocin and Social Behavior

Location: SDCC 5B

Time: Monday, November 14, 2016, 1:00 PM - 2:45 PM

Presentation Number: 387.01

Topic: G.02. Motivation

Support: FI Grant

Ministerio de Educación

Title: The endocrinome of social communication

Authors: ***C. THEOFANOPOULOU**[1], C. BOECKX[2];
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Abstract: A growing amount of evidence supports the decisive role of several hormones in the motivational circuits that underlie social communication. Independent studies have highlighted the importance of three hormones: oxytocin, dopamine and serotonin. The aim of this study is firstly, to construct a synthetic framework of the brain circuits where oxytocin, dopamine and serotonin interactions mediate motivation, based mostly on animal studies (mice, prairie voles, songbirds), and secondly, to show that this framework may also account for the human reward

system subserving social communication. For this second goal, we relied on a Pubmed search of studies pertinent to the localization of these hormones in the human brain and the effects they exert at a behavioral level, and backed up the information we found searching in the Allen Brain Atlas for information concerning which brain areas the genes of these hormones and their receptors and transporters are expressed.
Oxytocin, dopamine and serotonin have been independently shown to partake in social-communicative processes. Oxytocin has been proposed to be a key neural substrate for affiliation and copulation (Melis et al. 2007, Wang et al. 2013) and recently has been implicated in auditory processing (Marlin et al. 2015). In humans, oxytocin has been associated with socio-emotional behaviors (Carter 2014) and the disruption of the oxytocinergic system in deficits like Autism, Schizophrenia and Williams Syndrome (Grinevich et al. 2015) is likely to underpin related communicative deficits. Dopamine has been found to be involved both in the social and vocal aspects of communication (Edwards and Self 2006, Hara et al. 2007, Hoffman et al. 2016). Regarding human studies, Ripollés et al. 2014 found that dopamine provides the motivational substrate for word learning. Likewise, serotonin has been demonstrated to take part in the motor, vocal and social aspects of communication in mice and songbirds (Beis et al. 2015, Wöhr et al. 2015, Wood et al. 2011).
The framework proposed is mostly based on the social reward mechanisms observed between the ventral tegmental area (VTA) and the nucleus accumbens (Nacc). Succinctly: oxytocin neurons, targeting the VTA, stimulate the dopaminergic neurons which project to the Nacc (Melis et al. 2009, Succu et al. 2011). Oxytocin and dopamine are colocalized in the striatum and oxytocin neurons also express dopamine receptors (Romero-Fernandez et al. 2013). In turn, social reward in the Nacc requires coordinated activity of oxytocin and serotonin (Dolen et al. 2013) and in human experiments their interaction extends to further brain areas to regulate emotion-based behavior (Mottolese et al. 2014).

Disclosures: **C. Theofanopoulou:** None. **C. Boeckx:** None.

Nanosymposium

387. Oxytocin and Social Behavior

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Presentation Number: 387.02

Topic: G.02. Motivation

Support: Simons Foundation Autism Research Initiative (Grant 305112)

Title: Oxytocin gates VTA dopamine neurons to promote pro-social behaviors

Authors: *L. W. HUNG[1], K. BEIER[1], J. S. POLEPALLI[1], S. NEUNER[1], M. WRIGHT[1], G. DOLEN[3], K. DEISSEROTH[2], R. MALENKA[1];
[1]Psychiatry, [2]Bioengineering, Stanford Univ., Stanford, CA; [3]Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Oxytocin (OT) is often considered a pro-social hormone yet the mechanisms by which it promotes sociability are unclear. One hypothesis posits that oxytocin affects mesolimbic reward circuitry to increase motivation for pro-social behavior. Previous studies have shown that OT increases social drive at least in part via its effect in the nucleus accumbens (NAc). However, the critical role of the ventral tegmental area (VTA) in motivated behaviors and the presence of OT receptors and fibers in the VTA suggest that OT's effects on VTA dopamine (DA) neurons may also contribute to the gating of social behavior. To determine the necessity of OT signaling in the VTA and its relevance for social motivation, we ablated or reduced oxytocin signaling in VTA DA neurons via a number of different strategies. Silencing of paraventricular nucleus (PVN) hypothalamic neurons projecting to the VTA lead to a reduction in social reward as measured by a social conditioned place preference assay. In addition, knocking out OT receptors in the VTA by injection of a Cre virus into the VTA of conditional OT receptor knockout mice as well as from DA neurons by crossing these mice with DAT-Cre mice both reduced social reward. If OT release in VTA and NAc are important for gating social behavior, social interactions should cause OT release in these structures via increases in the activity of OT neurons. Using *in vivo* calcium imaging, we found that novel social interactions increases the activity of PVN OT neurons. To determine the consequences of direct activation of these neurons, we expressed a variation of channelrhodopsin (ChETA) in PVN OT neurons and found that their optogenetic activation was sufficient to elicit conditioned place preference and enhance social interaction. Social reinforcement was also observed during PVN OT neuron terminal stimulation in the VTA; an effect that was blocked by an OT receptor antagonist. To determine mechanistically how OT is affecting VTA DA neurons, we made whole cell patch clamp recordings from a homogeneous population of VTA DA neurons that project to the NAc medial shell (NAcMedS). Application of the OT receptor agonist TGOT on average reduced evoked IPSCs while at the same time modestly enhancing evoked EPSCs, resulting in a net increase in excitation onto these neurons. Together these results demonstrate that the action of OT on VTA DA neurons is necessary and sufficient for enhancing the motivation for social interactions.

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Nanosymposium

387. Oxytocin and Social Behavior

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Presentation Number: 387.03

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R01MH096983

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Title: Consolation behavior in prairie vole is predicted by oxytocin receptor density in anterior cingulate cortex

Authors: *J. P. BURKETT[1], L. KING[3], E. ANDARI[2], L. YOUNG[2];
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Abstract: Empathy for the pain and suffering of others is a widespread mechanism among social animals that provides a motivation for prosocial behaviors. Consolation is one such prosocial response that has been observed in a wide range of animals, including a laboratory rodent, the prairie vole (*Microtus ochrogaster*). Our previous research demonstrated that consolation behavior in the prairie vole is empathy-based and is regulated by oxytocin receptor (OTR) signaling in the anterior cingulate cortex (ACC). OTR density in the ACC varies between individual prairie voles, yet the role of this biological variation in contributing to behavioral variation is unknown. We examined the relationship between OTR density and consoling behavior using data from five experiments, split into a discovery sample (Expt. 1, N=54) and a replication sample (Expt. 2, N=7; Expt. 3, N=12; Expt. 4, N=12; Expt. 5, N=43). Analysis of both samples revealed a negative correlation between consoling response and OTR density in the ACC ($p=0.02$, $r=-0.3$, Hedges' $g=-0.6$) but not OTR density in other brain regions. Voles in both the highest and lowest quartiles of OTR density showed a significant consoling response (high, $p=0.003$; low, $p=0.0002$), but voles in the lowest quartile performed more consoling behavior than those in the highest quartile ($p=0.03$). These results show that the magnitude of the consoling response in individual animals can be predicted by the density of OTR in the ACC, suggesting that OTR density in this region may be behaviorally relevant. High OTR density in ACC may drive an increase in personal distress in response to the distress of others, which is related to lower levels of helping in humans, great apes and rats.

Disclosures: **J.P. Burkett:** None. **L. King:** None. **E. Andari:** None. **L. Young:** None.

Nanosymposium

387. Oxytocin and Social Behavior

Location: SDCC 5B

Time: Monday, November 14, 2016, 1:00 PM - 2:45 PM

Presentation Number: 387.04

Topic: F.02. Behavioral Neuroendocrinology

Support: EU FP7 consortium Fem-NATCD (602407)

Title: Oxytocin and vasopressin inhibit virgin female aggression via V_{1a} receptors

Authors: *T. R. DE JONG[1], V. E. M. OLIVEIRA[2], I. D. NEUMANN[2];
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Abstract: Oxytocin (OXT) and vasopressin (AVP) are known to modulate social behaviors in a sex-specific manner. In rodents, inter-male aggression is typically inhibited by OXT and facilitated by AVP. Aggression among virgin female rats is also inhibited by OXT, but the effects of AVP are not yet known. 4 cohorts of single-housed adult virgin female Wistar rats (n=7-12/cohort) were implanted with ICV cannulae. After a 3-day recovery, they underwent three consecutive daily 10-min female intruder tests (FIT1-3, training phase) resulting in stable high aggression levels. On day 4 and day 6 (day 5 served as washout), the animals were infused once with VEH (Ringer, 5 µl) and once with either OXT (50 ng / 5 µl), AVP (0.1 and 1 ng / 5 µl) or the selective OXT receptor (OXTR) agonist TGOT (100 ng / 5 µl) in a within-subjects cross-over design, and tested in a FIT 15 min after infusion. Both OXT and AVP strongly decreased aggression compared with VEH, specifically reducing “threat” and “keep down” behavior and attack frequency. Surprisingly, TGOT increased aggressive behavior. Since OXT can bind to AVP-preferring V_{1a} receptors (V_{1a}R), we tested whether this pathway is responsible for its anti-aggressive effects in females. Female rats (n=7-12/group) were implanted with ICV cannulae, single-housed and trained as above. Then, females were infused with either a selective OXTR antagonist (OT-A) or a selective $V1_a$R antagonist ($V1_a$R-A) (both 750 ng / 2.5 µl) 15 min prior to OXT (50 ng / 2.5µl) or VEH. Aggression was measured in a FIT 15 min after the last infusion. OXT was again able to inhibit aggression, and this could be blocked by pre-treatment with $V1_a$R-A, but not OT-A. Interestingly, treatment with OT-A alone showed a trend to decrease aggression. Taken together, these data indicate that in contrast to male rats, the anti-aggressive effects of OXT in female rats are mediated by its binding to $V1_a$R rather than OXTR. Selective activation of OXTR appears to have a pro-aggressive effect in females. In ongoing research, we aim to localize the pro- and anti-aggressive effects of OXTR and $V1_a$R activation via targeted infusions of selective (ant)agonists into the central amygdala, lateral septum and prefrontal cortex, amongst others. We will furthermore investigate whether high-aggressive phenotypes, for example induced by post-weaning isolation, are associated with abnormal OXTR and $V1_a$R binding and/or altered OXT and AVP mRNA levels.

Disclosures: **T.R. de Jong:** None. **V.E.M. Oliveira:** None. **I.D. Neumann:** None.

Nanosymposium

387. Oxytocin and Social Behavior

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Time: Monday, November 14, 2016, 1:00 PM - 2:45 PM

Presentation Number: 387.05

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant HD075750

Title: Developmental consequences in offspring following maternal oxytocin treatment at birth

Authors: ***W. KENKEL**[1], A. PERKEYBILE[1], J. R. YEE[1], T. LILLARD[2], C. F. FERRIS[3], S. CARTER[1], J. CONNELLY[2];
[1]Indiana Univ. Bloomington, Bloomington, IN; [2]Univ. of Virginia, Charlottesville, VA; [3]Northeastern Univ., Boston, MA

Abstract: Oxytocin is the most widely used hormone to induce and/or augment labor and is routinely administered in at least 23% of births in the U.S. However, oxytocin is also a centrally active neuropeptide with long-lasting developmental consequences on the neonatal brain. Here, we report several changes in oxytocin-regulated behaviors observed in the offspring of female prairie voles treated with oxytocin (0.25 mg/kg) on the day of delivery as a model for human labor induction. In the neonatal period, we examined ultrasonic vocalizations as a broad measure of response to separation and found that pups born to oxytocin-treated dams vocalized more than saline-treated control offspring. In the adolescent period, we examined anxiety-related behavior in an open field and found no treatment effect. In adulthood, we examined alloparental responsiveness and observed increased caregiving behavior in the offspring of oxytocin-treated dams. Offspring born to oxytocin treated dams were also more gregarious during a test of partner preference formation, spending more time huddling in side by side contact and less time in a solitary cage. In each of these domains, we have replicated these effects and have shown via cross-fostering that they are not due to changes in maternal behavior. We are currently investigating the epigenetic mechanisms for these long-lasting effects of early life oxytocin exposure. Such translational findings have direct implications to human health and behavior

Disclosures: **W. Kenkel:** None. **A. Perkeybile:** None. **J.R. Yee:** None. **T. Lillard:** None. **C.F. Ferris:** None. **S. Carter:** None. **J. Connelly:** None.

Nanosymposium

387. Oxytocin and Social Behavior

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Topic: F.02. Behavioral Neuroendocrinology

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Brain & Behavior Research Foundation

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MSCRF

SR/RUSK

Title: Retinoic acid signaling in the anterior insula: a fail-safe system for social cognition complementary to the oxytocin cascade

Authors: *S.-H. KIM[1,2], M. RANNALS[3], J. R. MOORE[2], M. KONDO[2], T. CASH-PADGETT[2], B. MAHER[3], A. SAWA[2];
[1]Psychiatry and Behavioral Sci., [2]Johns Hopkins Univ., Baltimore, MD; [3]Lieber Inst. for Brain Develop., Baltimore, MD

Abstract: Social cognition is one of the most important behavioral constructs, and oxytocin has been studied extensively as a promising mediator for the process. As social cognition is so fundamental to maintain human society, we have wondered whether multiple mechanisms mediate the process.
One enigma is that, although the anterior insula (AI) has been highlighted as a brain region involved in social and affective behaviors via lesion and imaging studies, the area rarely receives the projections of oxytocin neurons. Thus, the main hypothesis of the present study is that there should be a novel mediator underlying social cognition, equivalent to but independent of oxytocin, in the AI.
To address this question, we looked for molecules that are exclusively enriched in the AI and have focused on Cyp26B1, a retinoic acid (RA)-degrading enzyme. Conditional depletion of Cyp26B1 in knockout model and virus systems has consistently shown that the molecular modulation of Cyp26B1 selectively in the AI impairs social, but not non-social, cognition. The deficits are ameliorated by rescuing expression of Cyp26B1 as well as optogenetic stimulation of pyramidal neurons in the AI *in vivo*. We also report that the RA-mediated alteration in dendritic spine plasticity and the resultant attenuated neuronal activity in the layer 5 pyramidal neurons underlie the AI-oriented social deficits.
Of importance, the social deficits elicited by AI-selective loss of Cyp26B1 are ameliorated by

systemic injection of oxytocin *in vivo*, whereas the administration of oxytocin onto the brain slices with AI-selective loss of Cyp26B1 does not affect (e.g., normalize) the molecular and cellular deficits
associated with RA/Cyp26B1. Considering that the projection of oxytocin neurons to the AI is rare, these results suggest that the AI-mediated social cognition involving RA/Cyp26B1 signaling is mechanistically independent of oxytocin-mediated social cognition. Nonetheless, these two mechanisms may complement each other at the phenotypic levels, like a "fail-safe system" in an airplane, in order to properly maintain our social cognition and social behavior.

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Nanosymposium

387. Oxytocin and Social Behavior

Location: SDCC 5B

Time: Monday, November 14, 2016, 1:00 PM - 2:45 PM

Presentation Number: 387.07

Topic: F.01. Neuroethology

Title: Investigating the genetic basis of natural behavior using topic modeling

Authors: *S. MADLON-KAY[1], M. MONTAGUE[2], N. SNYDER-MACKLER[4], K. WATSON[7], P. SKENE[5], J. HORVATH[8,9], L. BRENT[10], K. HELLER[6], M. PLATT[2,3];
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Abstract: Large bodies of research on both humans and animal models suggest that genetic variation in neurobiological pathways is important for sociality and social behavior, as well as for neurodevelopmental disorders with strong social phenotypes such as ASD. However, traditional laboratory-based transgenic animal approaches are limited in their ability to assess whether and how genetic variation contributes to naturalistic social behaviors in real-world environments, as animals amenable to transgenic research display relatively limited social behaviors and live in laboratory environments with much less varied and dynamic social stimuli than natural environments. An alternative approach to understanding genetic contributions to social behavior is to study free-ranging populations of animals whose biology and social behavior resemble our own. For this purpose, we investigate genetic variation and natural social

behavior in rhesus macaques (Macaca mulatta) in the free-ranging primate colony of Cayo Santiago and develop novel methods for quantifying rich and reliable behavioral phenotypes on the basis of noisy, high-dimensional natural observational behaviors.
Primate behavior in natural environments is both widely varied and highly structured, involving the interplay of many different behaviors at once. While individual behaviors considered in isolation carry limited information, the space of all possible patterns of interactions between behaviors is too large to fully investigate using conventional statistical methods. In order to quantitatively define phenotypes from natural behavior, we adapt a class of unsupervised classification models, commonly known as topic models, to identify and measure patterns of behavior in naturalistic observational data and relate them to genetic variation in neurobiological pathways. Our observational data set contains of over 2300 hours of behavioral observation using 10 minute focal samples across 445 individuals on the island of Cayo Santiago, with an ethogram consisted of 20 point behaviors and 12 behavioral states encompassing both social and self-directed actions. We combine this with a genetic data set of full genome sequences in 217 Cayo Santiago macaques containing over 19 million single nucleotide variants in order to identify patterns of natural social behavior that covary with genetic variation in fundamental neurotransmitter pathways, e.g. dopamine receptors, oxytocin and vasopressin receptors, and serotonin transporters, as well as pathways related to neurodevelopmental disorders such as ASD.

Disclosures: **S. Madlon-Kay:** None. **M. Montague:** None. **N. Snyder-Mackler:** None. **K. Watson:** None. **P. Skene:** None. **J. Horvath:** None. **L. Brent:** None. **K. Heller:** None. **M. Platt:** None.

Nanosymposium

388. Neural Basis of Emotions

Location: SDCC 2

Time: Monday, November 14, 2016, 1:00 PM - 3:30 PM

Presentation Number: 388.01

Topic: G.03. Emotion

Support: NIH Grant R01MH076136

NIH Grant R01DA035484

Title: Distinct brain systems mediate social influence and conditioned cue effects on pain

Authors: ***L. KOBAN**, M. JEPMA, T. D. WAGER;
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Abstract: Both social instructions and experience-based learning can drive expectations and experience of pain. Yet, it is not known whether these different sources of expectations modulate pain via different brain mechanisms. The present study compared how experience-based learning (classical conditioning) and unreinforced social information affect pain-related brain responses and pain experience. In each trial of a learning task, participants were presented with one of two visual cues, serving as conditioned stimuli (CS). One cue (CS_{low}) was followed by low (50%) or medium (50%), the other (CS_{high}) by medium (50%) or high (50%) painful thermal stimulation. These CS were crossed with a social information manipulation, which involved presenting lines that participants were told reflected the pain ratings of other individuals. We measured expectation and pain ratings, as well as brain responses (fMRI) to painful heat. Our results showed significant effects of both CS and unreinforced social information on pain, which were both mediated by self-reported expectations. Brain mediation analyses revealed that learned cue effects were mediated by activity in more posterior regions, including cerebellum and visual areas, whereas social influences on pain were mediated by activity in fronto-parietal regions. Overlapping mediation effects were found in the dorsomedial prefrontal cortex. In conclusion, our results demonstrate strong social influences on pain, and partially distinct brain mechanisms underlying learned versus socially instructed pain regulation.

Disclosures: **L. Koban:** None. **M. Jepma:** None. **T.D. Wager:** None.

Nanosymposium

388. Neural Basis of Emotions

Location: SDCC 2

Time: Monday, November 14, 2016, 1:00 PM - 3:30 PM

Presentation Number: 388.02

Topic: G.03. Emotion

Title: Transgenerational transmission of learned fears via observational conditioning

Authors: *J. A. SILVERS[1], B. CALLAGHAN[2], K. O'SULLIVAN[2], M. VAN TIEGHEM[2], N. TOTTENHAM[2];
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Abstract: We humans do a great deal of learning by observing others, including what to fear and what to trust in our environment. Observational fear learning may be especially important early in life when children turn to their parents to gather information about their world. Yet, the vast majority of empirical research on fear learning in children has thus far focused on firsthand classical conditioning, which may fail to capture one of the primary means by which children acquire fears. To address this gap in the literature, the present study examined observational fear learning in children and adolescents (age range: 6-17 years) as they watched videos of their

parent and an unfamiliar adult undergo fear conditioning. Subsequent to this acquisition learning phase, participants viewed the CS+ and CS- they observed in the videos (test phase). Children and adolescents demonstrated robust observational fear learning, as indicated by changes in their self-reported liking of the CS+ (a geometric shape that was paired with an aversive noise 80% of the observed trials) and CS- (a geometric shape that was never paired with an aversive noise on the observed trials). However, children and adolescents showed enhanced learning via their parent during the test phase. This preferential learning for parent was supported by differential amygdala recruitment. Together, these results suggest that youth preferentially learn fears via observation of their parents and this learning is supported by neural circuitry involved in firsthand (i.e., classical) conditioning.

Disclosures: **J.A. Silvers:** None. **B. Callaghan:** None. **K. O'Sullivan:** None. **M. Van Tieghem:** None. **N. Tottenham:** None.

Nanosymposium

388. Neural Basis of Emotions

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Topic: G.03. Emotion

Support: SNSF 320030_149586/1

Wellcome Trust 091593/Z/10/Z

Title: Dissociating hippocampal contributions to anxiety-like behaviour in human approach/avoidance conflict

Authors: ***D. R. BACH**[1], M. HOFFMANN[2], C. FINKE[2], H. HEEKEREN[3], C. J. PLONER[2]; [1]Univ. of Zurich, Zurich, Switzerland; [2]Charite Univ. Med., Berlin, Germany; [3]Free Univ., Berlin, Germany

Abstract: Anxiety-like behaviour is often modelled with approach-avoidance conflict tasks such as operant conflict, elevated plus maze, or open field. In rodents and humans alike, ventral/anterior hippocampus lesions mimic the effect of anxiolytic drugs in such tasks. This suggests that anxiety-like behaviour is crucially under hippocampal control. However, behavioural patterns in these ethological tasks are complex and correlated, and the - possibly distinct - underlying mechanisms difficult to disentangle. Here, we capitalise on a virtual human computer game that implements a "scoop-and-run" operant conflict test. We have recently validated that this task separately measures passive avoidance (proportion of approach) and

behavioural inhibition (approach latency). Here, we use this task to compare n=3 patients with unilateral surgical hippocampus/amygdala lesions to n=10 healthy control participants, matched for age, gender and education. Lesion patients were more likely than healthy participants to approach when explicitly signalled possible loss was high. Thus, they showed reduced adaptation to increasing loss, i. e. reduced passive avoidance. This replicates our previous findings from a "stay-and-play" spatial approach/avoidance conflict task (Bach et al. 2014, Current Biology). Crucially, however, behavioural inhibition, i. e. approach latency, was unaltered in patients with hippocampal lesions, both in terms of an overall inhibition, and of an increased inhibition when loss is higher. This discrepancy makes it likely that human hippocampus controls passive avoidance, but not behavioural inhibition, in this task. Furthermore, we have previously shown that behavioral inhibition in this paradigm emanates from subjective assumptions on threat-reward correlations (anxiety-like priors). These assumptions can be measured independent from conflict in a "safe predator exposure task" in which participants are taked to press a key to reveal predator status without any potential threat. Rather than uniformly distributing exposure attempts across time, healthy participants prefer to expose a predator shortly after incidental and unobtainable rewards have been displayed. Patients with hippocampus lesions are not only unimpaired in this task but show slightly more pronounced anxiety-like priors. This underlines that behavioural inhibition and the mechanism controlling it are intact after hippocampus lesions while passive avoidance is impaired. Our results suggest that behavioural components may arise from different underlying neural mechanisms, possibly implemented in macroscopically distinct regions.

Disclosures: **D.R. Bach:** None. **M. Hoffmann:** None. **C. Finke:** None. **H. Heekeren:** None. **C.J. Ploner:** None.

Nanosymposium

388. Neural Basis of Emotions

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Presentation Number: 388.04

Topic: F.04. Stress and the Brain

Support: NIMH Grant MH093650

NIMH Grant MH091945

NIDA Grant DA030425

Title: Chronic social defeat stress produces profound alterations in both behavior and in the brain pituitary adenylate cyclase-activated polypeptide (PACAP)/PAC1 receptor system.

Authors: *M. SEIGLIE, C. VELÁZQUEZ-SANCHEZ, A. FERRAGUD FAUS, P. COTTONE, V. SABINO;
Dept. of Pharmacol. and Psychiatry, Boston Univ. Sch. of Med., Boston, MA

Abstract: Stress is one of the leading predisposing factors for the onset of anxiety and depression. Interestingly, only certain individuals are vulnerable to the effects of stress and develop psychopathologies, while others remain resilient. The mechanisms underlying stress resilience and vulnerability remain not fully understood and this gap significantly delays the advancement of biomedical sciences. Identifying the role of specific extrahypothalamic neuropeptides in the pathological response to stress could lead to novel treatments for either enhancing resilience or mitigating susceptibility. Pituitary adenylate cyclase-activating polypeptide (PACAP), a 38-amino acid peptide, and its receptor PAC1R, have been proposed to regulate the stress response in the central amygdala (CeA) and there dysregulation may contribute to the etiology of anxiety, trauma-related and depressive disorders. Here we used the chronic social defeat paradigm which produces a depressive-like symptomatology in a subset of rats, therefore named “susceptible”. Susceptible rats showed heightened anxiety-like behavior, reduced social interaction and reduce consumption of a sweet solution. Rats subject to chronic social defeat also showed significant reductions in body weight gain, increases in immobility time in the forced swim test, and alterations in fear extinction learning. Using immunohistochemistry, we found that PACAP expression levels were significantly increased in the central nucleus of the amygdala (CeA) and decreased in the paraventricular nucleus of the hypothalamus of chronic social defeat rats, compared to control rats. Interestingly, preliminary results obtained using qRT-PCR seem to show that PAC1R mRNA in the CeA is increased only in the susceptible population, compared to control unstressed rats. Studies are currently being performed to test whether knocking down PAC1R in the CeA may prevent the depressive- and anxiety-like symptoms caused by exposure to chronic social defeat stress. Our data suggest that a dysregulation of the PACAP/PAC1R system following chronic stress may represent a neuroadaptation that confers to the behavioral and physiological symptoms of stress susceptibility.

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Nanosymposium

388. Neural Basis of Emotions

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Time: Monday, November 14, 2016, 1:00 PM - 3:30 PM

Presentation Number: 388.05

Topic: G.03. Emotion

Support: Swedish Research Council

Title: Rewarded approach of threatening spiders engages areas of the mesolimbic dopamine system

Authors: *F. AHS[1,2], J. BJÖRKSTRAND[1], M. FREDRIKSON[1,2];
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Abstract: In daily life, it is often necessary to confront threats to obtain rewards. The neural circuitry that controls the decision to approach or avoid threat during such motivational conflicts has been delineated in rodents and includes the mesolimbic dopamine system. Using functional magnetic resonance imaging (fMRI), we tested whether areas of the mesolimbic dopamine system were engaged in 44 spider-fearful individuals that received a variable amount of money, ranging from 1 cent to 50 cents, to view threatening spider pictures or neutral pictures. As expected, participants chose to view highly rewarded spiders more frequently than spiders associated with low reward. This motivational conflict also induced heightened autonomic activity relative to approach of neutral stimuli. As compared to rewarded approach of neutral stimuli, incentivized approach of spiders showed robust activation of the dorsomedial prefrontal cortex, midbrain and the ventral striatum. Of these areas, the ventral striatum tracked the value of reward. We conclude that actively choosing to confront fear-inducing stimuli to receive a reward is associated with increased activity in parts of the mesolimbic dopamine system.

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Nanosymposium

388. Neural Basis of Emotions

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Support: the National Science Centre grant 2013/11/B/NZ3/01560 to E. Knapska

Title: Central amygdala mediates socially transferred fear

Authors: *E. A. KNAPSKA, K. ROKOSZ, A. HAMED, K. KONDRAKIEWICZ;
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Abstract: In its simplest form empathy can be characterized as the capacity to share the emotional state of another being (emotional contagion). Tuning one's emotional state to that of

another increases the probability of similar behavior, which thereby allows for a rapid adaptation to environmental challenges. Emotional contagion, commonly observed in animals, including rodents, is well described at the behavioral level, but the neuronal circuits necessary for sharing emotions are not well understood. The central nucleus of the amygdala (CeA) is critical in fear learning and controls both active and passive defensive responses. To directly test the hypothesis that CeA neuronal circuits are also involved in socially transferred fear, we optogenetically activated ”social fear” neurons in CeA. To induce c-fos-driven expression of channelrhodopsin in ”social fear” neurons, we used a simple model of socially transferred fear. In this model, rats are housed in pairs and one member of the pair (the “demonstrator”) is removed and subjected to fear conditioning. After the fear-conditioning episode, the conditioned animal is allowed to interact with its naïve cage mate (the “observer”). We showed that the subsequent activation of ”social fear” neurons in CeA of the observers enhances exploratory behavior of the familiar environment but inhibits social interaction and ultrasonic communication. The activation of these “social fear” neurons in CeA in the novel environment resulted in increased anxiety reflected by shortened exploration of anxiogenic stimuli. Thus, by activation of CeA neurons involved in social interaction with a fearful partner we were able to reproduce behavioral changes observed as a consequence of the real interaction.

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Nanosymposium

388. Neural Basis of Emotions

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Presentation Number: 388.07

Topic: G.03. Emotion

Support: National Basic Research Program of China (no. 2015CB856400)

Title: Inducing human fear memory extinction during sleep

Authors: ***J. HE**[1,2], J. YUE[1], J. BAN[1], P. LI[1], L. SHI[1], L. LU[1,2,3];
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Abstract: Introduction: Emotional memories are regarded as the root of certain mental disorders, such as post-traumatic stress disorder (PTSD), which are traditionally treated by exposure therapy. However, this therapy may elicit adverse responses, and possibly lead to

relapse or exacerbation. Numerous studies suggested that sleep is beneficial for emotional memory modulation. The aim of the current study was to investigate the effects of exposure to fear-related cues during slow wave sleep (SWS) on human fear memory extinction and the underlying neural mechanisms.
Methods: The volunteers underwent a visual contextual fear conditioning, during which geometric figure (conditioned stimuli, CS^+) were paired with mild electric shocks (unconditioned stimuli, US) together with specific ambient light, one of which was represented during SWS or wakefulness to reactivate the associated memory. The extinction effect was assessed after awake and 7 days later by skin conductance recording. We collected image data during fear conditioning and two test phases and further analyzed the alterations of targeted brain region activation between different stages.
Results: All of the participants exhibited undisturbed sleep. Exposure to an unreinforced fear context (ambient light) during SWS selectively elicits the activated fear memory extinction without altering non-activated fear memory and this
extintion effect may persist 7 days. Besides, we observed the correlation between light exposure duration and specific fear extinction during immediate fear memory test and 7 days post-conditioning test. We found that light stimulus-evoked patterns of fMRI ensemble activity in pre-sleep, post-sleep and 7 days post-conditioning deviated for the reactivated CS^+ and analysis revealed that the activated fear memory extinction and its retention effect may be mediated by hippocampus.
Conclusion: The main finding of the present study was that exposure to the visual cues related to fear memory during SWS reduced fear responses without altering sleep profiles. The deactivation of hippocampus evoked by $tgCS^+$ in the fear memory test after daytime nap was involved in the fear extinction and its long term effect induced by visual stimulus exposure during sleep. Because the subjects were unaware of manipulation conducted during sleep and may avoid certain disadvantages of traditional “extinction therapy” applied during wakefulness. Thus, our study introduces an alternative approach that may safely reduce fear in patients and have potential clinical value.
Keywords: slow wave sleep, fear memory, extinction, hippocampus

Disclosures: **J. He:** None. **J. Yue:** None. **J. Ban:** None. **P. Li:** None. **L. Shi:** None. **L. Lu:** None.

Nanosymposium

388. Neural Basis of Emotions

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Topic: G.03. Emotion

Support: NICHD Grant P01HD075750

Title: Dampened BOLD activation to life threat fear in prairie vole fathers

Authors: *J. R. YEE[1], W. M. KENKEL[2], A. PERKEYBILE[2], K. MOORE[1], P. KULKARNI[1], S. W. PORGES[2], C. CARTER[2], C. F. FERRIS[1];
[1]Ctr. for Translational Neuroimaging, Northeastern Univ., Boston, MA; [2]Indiana Univ., Bloomington, IN

Abstract: In cooperatively breeding species, the father, unencumbered by direct infant care while the mother is on nest, is poised to fulfill many of the protective functions of parenthood. However, a lack of laboratory animal models in which fathers take part in parental care has led to a gap in understanding whether parental fathers experience a transformation in fear regulation that would support protective functions of fatherhood. This research seeks to better understand potential changes in fear regulation that accompany fatherhood by studying neural responses to fearful stimuli in the socially monogamous prairie vole (Microtus ochrogaster). Prairie voles were presented with the scent of a predator (i.e. a live sable ferret) while immobilized for awake functional neuroimaging in order to assess the neural response to life threat fear. Contrary to expectations, experienced fathers showed a dramatically dampened blood oxygen level-dependent (BOLD) response to the scent of the predator as compared to virgin males. Dampened neural responses were observed in areas of the brain associated with fear, olfaction, and pain. While previous work has led to the recent conclusion that parenthood may not have pronounced effects on stress responsiveness or emotionality in fathers, our preliminary findings using high-resolution functional neuroimaging of the entire brain in awake animals, has shown that the transition to fatherhood may be accompanied by dramatic changes in the regulation of fear.

Disclosures: J.R. Yee: None. **W.M. Kenkel:** None. **A. Perkeybile:** None. **K. Moore:** None. **P. Kulkarni:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Animal Imaging Research. **S.W. Porges:** None. **C. Carter:** None. **C.F. Ferris:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Animal Imaging Research, Ekam Solutions.

Nanosymposium

388. Neural Basis of Emotions

Location: SDCC 2

Time: Monday, November 14, 2016, 1:00 PM - 3:30 PM

Presentation Number: 388.09

Topic: G.03. Emotion

Support: Trust for the Meditation Process

Title: The need for neutral speaking controls in laboratory-induced emotional states

Authors: S. GRIMLEY[1], C. KO[2], F. GRACE[3], *L. E. OLSON[1];
[1]Biol. Dept., [2]Psychology Dept., [3]Dept. of Religious Studies, Univ. of Redlands, Redlands, CA

Abstract: Various stress, anxiety, or anger-producing tasks are used in the laboratory to measure physiological responses in emotional states. Examples include the cold pressor test, video stimuli, cognitive tasks, the Trier Social Stress Test, and recall of a previous event. The latter tasks can require the subject to speak during the emotion induction. Typically, the physiological measures during the emotional state are compared to a silent, resting baseline period. This does not allow for differentiation between the stress that is induced by emotion versus the stress due to the physical act of vocalization. We have modified two commonly used laboratory provocations to include a control period of neutral speaking. Physiological data include blood pressure, galvanic skin response, respiration rate, and high frequency heart rate variability. In all physiological measures, there were significant changes during the neutral speaking period compared to a silent baseline, demonstrating the need for this control. When calculating the magnitude of physiological response which would have previously been attributed to emotion, we discovered that 39 - 77% of the response (depending on the measure) could be attributed to vocalization alone. In examining the previous literature using emotion-inducing tasks that require speaking, care should be taken to consider the physiological impact of speech in interpreting results.

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Nanosymposium

388. Neural Basis of Emotions

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Topic: G.03. Emotion

Support: Singapore Ministry of Education Academic Research Fund (Tier 2: MOE2012-T2-1-051)

Title: Behavioral and neurobiological aspects of cultural attachment

Authors: *W. YAP[1,2,3], G. I. CHRISTOPOULOS[1,2,3], B. CHEON[4,5], Y.-Y. HONG[6];
[1]Nanyang Business School, Nanyang Technological Uni, Singapore, Singapore; [2]Culture Sci. Institute, Nanyang Business School, Nanyang Technological Univ., Singapore, Singapore;

[3]Decision and Organizational Neurosci. Lab, Nanyang Business School, Nanyang Technological Univ., Singapore, Singapore; [4]Div. of Psychology, Nanyang Technological Univ., Singapore, Singapore; [5]Clin. Nutr. Res. Ctr., Singapore Inst. for Clin. Sci. (A*Star), Singapore, Singapore; [6]Business Sch., Chinese Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: The concept of cultural attachment (Hong et al., 2006; 2012) suggests that an individual's bond with her own culture provides a secure base when under threat. Here, culture refers to the social group that individuals share beliefs, values and norms with as well as the related abstract symbolic representations. The mechanisms and the associated neural signals underlying this secure character of cultural symbols are untested. Here we adapt Hong et al's (2012) affective transfer task combined with functional MRI. 29 Chinese participants currently living in US underwent functional MRI while being shown a series of threatening images followed by random geometric patterns. Cultural images, either home (China) or host (US), were shown subliminally using the threat images as forward and backward masks. During each trial, participants had to judge the orientation of an arrow, followed by an aesthetic judgment of the random geometric patterns. We expect that the source (home vs. host) of the subliminally shown images will mediate the behavioural and neural responses to the threatening stimulus. Brain areas associated with social cognition, such as medial prefrontal cortex (mPFC), amygdala and parietal areas, are predicted to represent these differential responses. Behavioural results show that participants had slower reaction times when shown threat (vs non-threat) images ($p < 0.05$) and when making negative (vs. positive) aesthetic judgments ($p < 0.01$). Threat images were followed by lower aesthetic ratings ($p < 0.01$). Neural activity at the mPFC (12, 50, -7; Fig 1) revealed an interaction (p(unc.) = .011; $F(1,112) = 6.70$) between levels of threat (low vs. high) and cultural symbol (home vs. host), signalling higher activation towards the home than host cultural symbols when under threat, and the reverse when not under threat. This preliminary analysis suggests that cultural symbols have an effect on quick motor and aesthetic judgments. mPFC signals could separate home vs. host cultural symbols, notably as a function of the situation (threat vs. no threat), suggesting the role of social cognition.

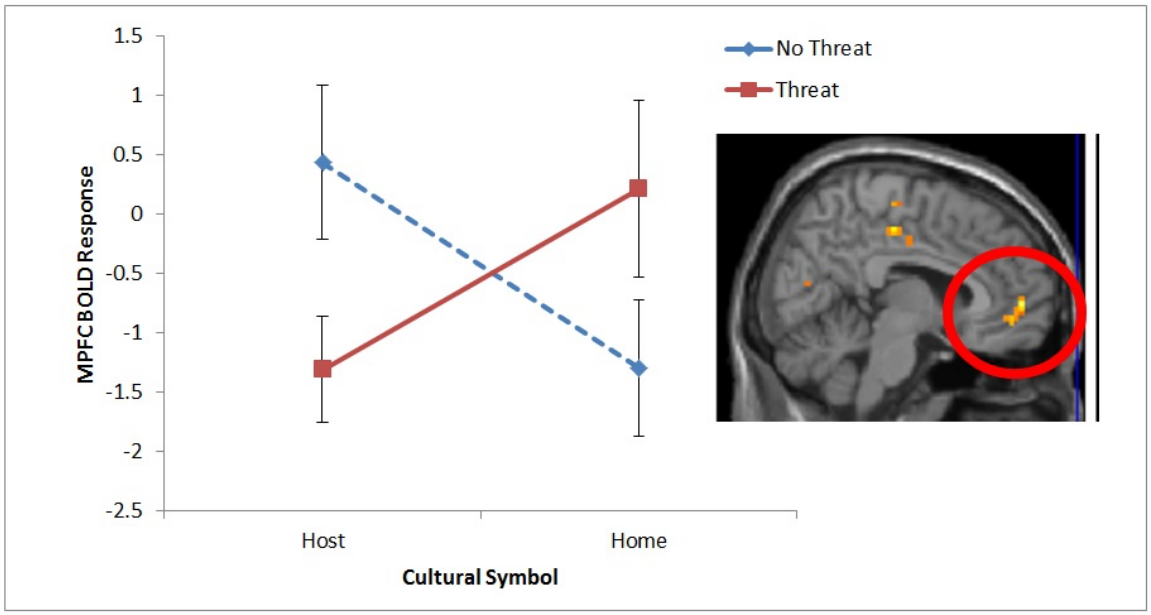

Figure 1

Medial Prefrontal Cortex (12,50,-7; p(unc.) = .011; *F(1,112)*= 6.70) response to cultural symbols (US [Host] vs. Chinese [Home]) as a function of state (threat vs. no threat).

Disclosures: W. Yap: None. **G.I. Christopoulos:** None. **B. Cheon:** None. **Y. Hong:** None.

Nanosymposium

389. The Role of Neuromodulators in Attentional Processing

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Time: Monday, November 14, 2016, 1:00 PM - 3:15 PM

Presentation Number: 389.01

Topic: H.01. Animal Cognition and Behavior

Support: Wellcome Trust

BBSRC

Title: Cholinergic, glutamatergic and attention induced modulation of oscillatory activity in macaque area V1 and frontal eye field

Authors: *A. THIELE[1], J. HERRERO[2], M. GIESELMANN[1], C. BRANDT[1], M. DASILVA[1], S. GOTTHARDT[1];

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Abstract: Attention alters oscillatory activity. Acetylcholine and glutamatergic receptors contribute to attentional signals, and glutamatergic modulation affects attention induced oscillatory activity in V1 (1, 2). The effect of cholinergic neuromodulation on attention induced oscillatory activity in the V1 and higher cortical areas is poorly understood. Here we investigate how attention affects oscillatory activity in V1 and FEF, and how this is altered by cholinergic and glutamatergic receptor activation/inactivation. In V1, attention reduced low frequency (~alpha band) oscillatory spectral power, and it reduced gamma band oscillatory spectral power (1, 3). Local iontophoretic acetylcholine application significantly reduced the gamma band power (broad band from ~45-100Hz), without differential effects on attentional components. Moreover, it increased an attention induced reduction of alpha band spectral power. Muscarinic blockade increased low frequency (delta/theta) band spectral power, and it significantly reduced the effect of attention on alpha band spectral power. Surprisingly, muscarinic and nicotinic blockade significantly increased gamma band power (irrespective of the attentional conditions). NMDA receptor blockade significantly reduced delta/theta\alpha and gamma band power, and increased beta band spectral power. AMPA receptor blockade significantly reduced delta/theta and gamma band power, with no effect on alpha/low beta band power. In the FEF attention significantly increased broad band gamma spectral power, but no distinct increase in narrow band (~35-45 Hz) power with attention was found. Muscarinic and nicotinic blockade strongly increased low frequency (delta/theta/alpha) spectral power, but did not affect the attentional modulation thereof. Acetylcholine application increases beta and broad band gamma power in FEF. The increase in broad band gamma power was significantly stronger in the attend away condition compared to the attend away condition, as if ACh application mimicked attention conditions. These results demonstrate that acetylcholine and glutamatergic receptors affect oscillatory activity in different ways, and a single neurotransmitter even affects it in an area dependent manner. 1.Herrero JL, Gieselmann MA, Sanayei M, & Thiele A (2013) Neuron 78(4):729-739. 2.van Kerkoerle T, et al. (2014) Proc Natl Acad Sci U S A 111(40):14332-14341. 3.Chalk M, et al. (2010) Neuron 66:114-125.

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Nanosymposium

389. The Role of Neuromodulators in Attentional Processing

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NIH Grant EY014924

HHMI

Title: Dopamine affects attention-related activity of neurons in the macaque FEF

Authors: *A. L. MUELLER, T. MOORE;
Stanford Univ., Stanford, CA

Abstract: Dopamine is known to play a key role in attention and attention-related disorders.The frontal eye field (FEF) is an area of the prefrontal cortex causally implicated in the control of visual spatial attention. Recently, we demonstrated that small dose injections of a D1R antagonist into the FEF modulates visually driven signals in posterior visual cortex in a way that closely resembles attentional modulation. We next wanted to examine how local application of dopamine agonists and antagonists affects attention-related activity within the FEF. We trained a rhesus macaque on an attention task and then used iontophoretic electrophysiology to record from FEF neurons while testing the effects of dopamine D1-receptor agonists and antagonists on neuronal activity while the animal performed the task. In a single trial of the attention task, the monkey initially maintains fixation while holding down a lever. Next, two targets appear as well as a central cue indicating which target to attend to. After a delay, the targets are briefly blanked (300 ms). After the blank, both targets reappear and the cued target reappears at either the same orientation, or at a different orientation. The monkey reports a detected change in target orientation by releasing the lever and reports ‘no change’ by keeping the lever depressed. While the animal performed this task, we recorded from FEF neurons and used iontophoresis to locally apply either a D1R agonist (SKF81297) or a D1R antagonist (SCH23390) with currents of either 20nA or 50nA, and retained with -15nA. We examine the visually-driven signals of FEF neurons during the period between cue presentation and target-blanking. We obtain a measure of attentional modulation by contrasting this activity between trials in which the receptive field stimulus is cued (attended) or not cued (unattended). We then examine the effect of dopamine agonists and antagonists on these attentional signals. Thus far, we have observed significant changes in attentional modulation during iontophoretic application of dopaminergic drugs. Our results therefore suggest that dopamine modulates the attention-related activity of FEF neurons.

Disclosures: **A.L. Mueller:** None. **T. Moore:** None.

Nanosymposium

389. The Role of Neuromodulators in Attentional Processing

Location: SDCC 4

Time: Monday, November 14, 2016, 1:00 PM - 3:15 PM

Presentation Number: 389.03

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant AG046580

Title: Cholinergic compromise on attentional function and cortical reorganization in aging

Authors: *B. YEGLA, S. JOSHI, J. A. FRANCESCONI, J. C. FORDE, V. PARIKH; Psychology and Neurosci. Program, Temple Univ., Philadelphia, PA

Abstract: Age-related shifts in neural recruitment from occipital to frontal cortical regions, characterized as the posterior-to-anterior shift in aging (PASA), is hypothesized to serve a compensatory role in aging. However, the underlying neural mechanism of PASA remains poorly understood. In the present study, we first assessed whether PASA patterns noted in human neuroimaging studies also occur in a rodent model of spontaneous aging during attentional task performance. As attention relies on the integrity of cortical cholinergic inputs, we also investigated the necessity of prefrontal and parietal cholinergic innervation to age-related shifts in cortical activation patterns. Young and aged Wistar rats were trained in an operant signal detection task, which required discrimination of signal and non-signal trials. Once rats maintained ≥70% correct responses on both trial types, they underwent infusions of a cholinergic immunotoxin targeting either the prefrontal cortex (PFC) or parietal cortex (PC) for mild cholinergic deafferentation. Control animals received saline infusions into the respective regions. Animals were assessed for attentional capacities 4 weeks later. Additionally, c-fos expression, a marker of neural activity, was examined in the PFC, PC and visual cortex using unbiased stereology. Attentional capacity of aged rats declined after PFC infusion regardless of infusion type ($F_{1,28}$=12.51, p<0.01). Evaluation of PASA recruitment indicated higher PC/visual c-fos ratios (p=0.03) but not PFC/visual c-fos ratios (p>0.14) in aged rats. Additionally, PASA recruitment of the PC network remained unaffected by PFC cholinergic lesion. On the other hand, both young and aged PC-infused rats exhibited cholinergic lesion-specific attentional impairments ($F_{1,28}$=4.48, p=0.04). Interestingly, control aged rats infused with saline into the PC displayed significantly lower c-fos counts in the PFC (p=0.02) and this effect was not observed in aged rats that received parietal cholinergic lesions (p>0.05). In general, c-fos activation in the right attentional network (PFC and PC) of aged rats negatively correlated with performance under conditions of higher cognitive load (p=0.008). Together, these data indicate that aged rats rely heavily on the PFC for attentional function. However, increased recruitment of the cortical attentional network in aged rats is *not* indicative of compensation but may be due to reduced neural efficiency. Additionally, prefrontal and parietal cortical cholinergic signaling is not

essential for PASA induction, although this signaling is required for the optimization of attentional performance irrespective of age.

Disclosures: **B. Yegla:** None. **S. Joshi:** None. **J.A. Francesconi:** None. **J.C. Forde:** None. **V. Parikh:** None.

Nanosymposium

389. The Role of Neuromodulators in Attentional Processing

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Topic: H.01. Animal Cognition and Behavior

Support: Wellcome Trust 093104

Title: Dopaminergic contribution to attentional signals in parietal cortex

Authors: ***J. VAN KEMPEN**[1], C. BRANDT[2], M. A. BELLGROVE[3], A. THIELE[1];
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Abstract: Selective attention facilitates the prioritisation of task-relevant sensory inputs over those which are irrelevant. Although cognitive neuroscience has made great strides in understanding the neural substrates of selective attention, our understanding of its neuropharmacology is incomplete. Recently cholinergic and glutamatergic contributions have been demonstrated, but emerging evidence also suggests an important influence of dopamine. Dopamine has historically been investigated in the context of frontal/prefrontal function arguing that dopaminergic receptor density in the posterior/parietal cortex is rather sparse. However, this notion was derived from rodent data, while in primates DA innervation is comparatively strong, matching that of many primate prefrontal areas (Berger et al. (1991) Trends in Neurosciences). Using recently developed techniques, we recorded single- and multi-unit activity whilst iontophoretically administering dopaminergic agonists and antagonists for specific receptor subtypes to posterior parietal cortex of awake, behaving non-human primates. Across the population, we demonstrate 1) general modulations in firing rate, 2) modulation of the visual response, independent of attentional signals and 3) modulation of attentional signals induced by drug application. More specifically, we show that D1 receptor agonists diminish spike rates and attentional signals whereas D1 receptor antagonists increase firing rates. Out of 50 neurons, selected for being visually responsive, we found 37 that show modulation of activity induced by drug administration, out of these 7 show an interaction effect between drug and attention. These data show that dopamine plays an important role in shaping neuronal responses in macaque

parietal cortex, and contribute to attentional processing. References: Berger B, Gaspar P, Verney C (1991) Dopaminergic innervation of the cerebral cortex: Unexpected differences between rodents and primates. *Trends in Neurosciences* 14:21-27.

Disclosures: J. Van Kempen: None. **C. Brandt:** None. **M.A. Bellgrove:** None. **A. Thiele:** None.

Nanosymposium

389. The Role of Neuromodulators in Attentional Processing

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Topic: H.01. Animal Cognition and Behavior

Support: NIH RO1 MH093354

Title: The contribution of acetylcholine to working memory circuits in primate prefrontal cortex

Authors: *V. C. GALVIN[1], Y. YANG[2], T. C. LIGHTBOURNE[1], S.-T. YANG[1], C. PASPALAS[1], A. F. T. ARNSTEN[1], M. WANG[1];
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Abstract: The prefrontal cortex (PFC) subserves our highest order cognitive abilities, generating the mental representations needed for working memory. Recordings from the dorsolateral PFC (dlPFC) of awake behaving primates uncovered “Delay cells” that generate spatially-tuned, persistent firing across the delay period when there is no sensory stimulation. This firing of Delay cells is considered the neural basis of working memory and arises from recurrent excitation within pyramidal cell microcircuits in deep layer III of primate dlPFC, through NMDA receptor (NMDAR) glutamatergic synapses on dendritic spines. Acetylcholine (ACh) modulates PFC working memory function through actions at both nicotinic and muscarinic receptors. Our previous study revealed that in primate dlPFC, nicotinic α7 receptors (α7R) are concentrated within the postsynaptic density (PSD) of NMDAR synapses on layer III dlPFC spines, and ACh stimulation of α7R is permissive for NMDAR actions and essential for dlPFC Delay cell firing. Among muscarinic receptors, M1 receptors (M1R) are of particular interest, as M1Rs are also in the PSD of glutamate synapses in layer III of dlPFC, . KCNQ “M” channels are also in the synapse, and may depolarize the synaptic membrane following M1R stimulation. This hypothesis was tested in the present study, to determine whether M1R enhances dlPFC Delay cell firing by closing KCNQ channels. Our preliminary data indicate that stimulation of M1R, like α7R, markedly enhances dlPFC Delay cell firing. As both α7R and M1R expressions are can

be reduced in many patients with schizophrenia, our research may help to identify new treatment strategies for patients with cognitive disorders.

Disclosures: **V.C. Galvin:** None. **Y. Yang:** None. **T.C. Lightbourne:** None. **S. Yang:** None. **C. Paspalas:** None. **A.F.T. Arnsten:** None. **M. Wang:** None.

Nanosymposium

389. The Role of Neuromodulators in Attentional Processing

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Topic: H.01. Animal Cognition and Behavior

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Title: Muscarinic M1 receptor modulation of abstract rule representation in primate dorsolateral prefrontal cortex

Authors: ***A. J. MAJOR**, S. VIJAYRAGHAVAN, S. EVERLING;
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Abstract: The neuropharmacology of muscarinic cholinergic receptors in modulation of the executive functions of the dorsolateral prefrontal cortex (DLPFC) has recently been of considerable interest. Integrity of the muscarinic system has been implicated in psychiatric disorders manifesting cognitive and executive dysfunction, such as schizophrenia and Alzheimer's disease. Cholinergic dysfunction impairs working memory performance, and muscarinic receptor antagonist scopolamine disrupts working memory activity related to spatial delayed response and maintenance of abstract rules. We have previously shown that muscarinic blockade with scopolamine using microiontophoresis disrupts task-related selectivity of monkey DLPFC neurons engaged in a rule-guided pro- and antisaccade task. Further, we have explored the physiological consequences of several muscarinic orthosteric agonists with weak subtype specificity on DLPFC neurons (Major, Vijayraghavan, and Everling, Soc. Neurosci. abstract, 2015, #334.10). We found that non-selective muscarinic agonists both excited and inhibited neuronal activity. Some of the complexity of the effects of muscarinic activation on neuronal physiology could be a consequence of heterogeneity in pre- and postsynaptic expression of muscarinic receptor subtypes, including the M1 and M2 receptors that are expressed in DLPFC. Here, we examined the neuropharmacology of muscarinic M1 receptor on monkey DLPFC during performance of rule-guided pro- and antisaccades by microiontophoretically applying a highly selective allosteric enhancer of M1 receptors, VU0357017, and an antagonist with greater preference for M1 receptors, pirenzepine. We found, contrary to expectations based on prior

studies and anatomical inference suggesting M1 receptor stimulation would excite DLPFC, selective M1 receptor stimulation predominantly inhibited DLPFC neurons engaged in task performance. Moreover, pirenzepine blockade of M1 receptors frequently excited prefrontal units. These results have interesting implications for the physiological and behavioural role of muscarinic receptor modulation of prefrontal circuitry.

Disclosures: **A.J. Major:** None. **S. Vijayraghavan:** None. **S. Everling:** None.

Nanosymposium

389. The Role of Neuromodulators in Attentional Processing

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Presentation Number: 389.07

Topic: H.01. Animal Cognition and Behavior

Support: DFG-CRC-889

Title: Cholinergic influences on spatial attention modulation in area MT of primate visual cortex

Authors: *C. QUIGLEY, V. K. VEITH, S. TREUE;
Cognitive Neurosci. Lab., German Primate Ctr., Goettingen, Germany

Abstract: Visual attention enhances the neuronal representation of attended stimuli across different visual areas. There is growing evidence of cholinergic participation in this modulation. Here we investigate the role of acetylcholine in spatial attention modulation of neuronal responses in extrastriate visual cortex.
We recorded single units in MT, a mid-level visual area with well-understood sensory and attention-related properties, in two rhesus monkeys. They performed a spatial attention task, attending one of two random dot patterns (RDPs), one within and one outside the cell's receptive field (RF). The attentional modulation was quantified by comparing firing rates when the stimulus in the RF was attended vs. unattended. A sensory condition required a luminance change detection at fixation while ignoring the RDPs. During recordings, we used pressure injection of cholinergic agents (Veith et al., 2016). The competitive antagonist scopolamine was used to block the muscarinic acetylcholine receptor, or the agonist acetylcholine to increase endogenous extracellular concentration. The pattern of attentional modulation during injection blocks was compared to baseline blocks recorded before injection.
The observed attentional modulation of neuronal responses during baseline blocks replicated previous results from MT. As we aimed to investigate the influence of the substance on the endogenous cholinergic circuit, we analyzed only those cells that showed a significant change in responses with scopolamine injection during sensory condition trials (34/117). If attentional

modulation is mediated by muscarinic receptors, as suggested by previous results in primate V1 (Herrero et al., 2008), a decrease in attentional modulation strength would be expected with injection. However, we found the opposite: scopolamine injection led to a significant increase in attentional modulation. Because the analyzed neurons displayed heterogeneous effects of scopolamine in the sensory condition, we further divided cells into subgroups with increased (16) and decreased (18) firing rates. Both groups showed an increase in attentional modulation during injection.
This increase is inconsistent with a direct involvement of the muscarinic acetylcholine receptor in mediating the firing-rate increases associated with attention in extrastriate visual cortex. A synergistic effect with other neurotransmitters such as glutamate seems more likely.

Disclosures: **C. Quigley:** None. **V.K. Veith:** None. **S. Treue:** None.

Nanosymposium

389. The Role of Neuromodulators in Attentional Processing

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Topic: H.01. Animal Cognition and Behavior

Support: NIDA Grant PO1 DA031656

Title: Poor attentional control as a trait in sign-tracking rats: cortical cholinergic-GABAergic mechanisms

Authors: *Y. KIM, C. R. RIVET, C. LUSTIG, M. SARTER;
Univ. of Michigan, Ann Arbor, MI

Abstract: In addicts, drug cues take on reward value and become powerful draws for involuntary attention. Such attribution of incentive salience to a conditioned stimulus (CS) is assessed by a Pavlovian approach test and rats prone to approach the CS are termed “sign trackers” (ST). In contrast, goal trackers (GT) are less attracted to the CS and preferentially approach the reward delivery site. We previously demonstrated that ST also exhibit poor attentional control, indicated primarily by unstable, highly variable performance over time in an operant sustained attention task (SAT). This unstable performance is linked to low levels of cortical cholinergic neuromodulation (Paolone et al., 2013). Here we test the hypothesis that STs’ unstable task set renders them more vulnerable to disruption by attentional challenge, and that this vulnerability is mediated by failure to increase cholinergic neuromodulation. We measured extracellular acetylcholine (ACh) and several monoamines and amino acids in 4-min cortical dialysates in ST and GT rats performing the SAT under standard conditions and

attentional challenge (distractor/disruptor consisting of flashing houselight). Challenge disrupted performance in all animals. GT post-challenge performance recovered to initial levels, but in ST post-challenge performance remained below baseline. GT showed the typical spike in ACh levels in response to the challenge condition (c.f., St. Peters et al., 2011); ST did not. Instead, ST showed a trend towards increased levels in post-challenge blocks, suggesting a reactive (vs. proactive) response to the challenge and slow re-instatement of the task set. Neither group showed a correlation between basal (pre-task) ACh and GABA levels. In GT, increased ACh and GABA levels during task performance in the standard SAT were correlated. ST did not show ACh-GABA correlations in any condition. These results suggest that poor attentional control in ST is mediated via a generally unresponsive cholinergic system and the absence of a proactive top-down response to distractors. Furthermore, the potential phenotype- and condition-specific relationships between ACh and GABA release indicate that attentional control is mediated by complex network interactions, rather than variations in single neurotransmitter systems.

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Nanosymposium

389. The Role of Neuromodulators in Attentional Processing

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VISN 22 MIRECC

Title: Amphetamine-induced improvement in rat 5-choice continuous performance test (5C-CPT) in poor performers and irrespective of concurrent haloperidol treatment

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Abstract: Introduction: Cognitive impairments mediate functional outcome in brain disorders, including schizophrenia and attention deficit hyperactivity disorder (ADHD). Developing methods to improve cognition while delineating the mechanism(s) of these effects is of critical importance, but is hampered by a discontinuity of testing methods across species. The 5-choice

Continuous Performance Task (5C-CPT) has been developed for analogous assessment of attention in both rodents and humans. Consistent with other CPT variants, 5C-CPT performance is impaired in psychiatric populations.
Hypothesis: We investigated whether the indirect dopamine agonist amphetamine (AMP) enhances rat 5C-CPT performance, and whether such AMP effects are prevented by pretreatment with haloperidol (HAL), a potent dopamine D_2 antagonist.
Methods: Male Long Evans rats (n=25) were trained in the 5C-CPT, then treated with: Exp. 1) AMP (0.1, 0.3, or 1.0 mg/kg) or vehicle, and then Exp. 2) AMP vs. HAL (0.1 mg/kg or 8 μg/kg respectively), or vehicle in cross-over designs before being challenged in the extended-session 5C-CPT. Drug effects were compared in groups with low vs. high baseline (BL) performance.
Results: In Exp. 1, AMP exhibited numerous effects including a drug X baseline interaction on hit rate (target detection; $F_{(3,51)}=4.7$, $p<0.01$) and tended to increase D' [vigilance $F_{(3,51)}=2.6$, $p<0.1$]. *Post hoc* analyses revealed that AMP (0.3 mg/kg) improved hit rate and D' in low BL rats, while 1.0 mg/kg impaired these measures in high BL rats. In Exp. 2, a HAL/AMP X performance interaction was observed on hit rate [$F_{(3,54)}=5.4$, $p<0.005$], whereby AMP improved performance compared with vehicle in low BL rats irrespective of HAL-treatment ($p<0.05$), while HAL impaired high BL rats compared with vehicle ($p<0.05$), an effect blocked by AMP treatment.
Conclusion: These findings reproduce patterns of: 1) AMP-induced improvement of human 5C-CPT performance driven by improved target detection; 2) Low rat BL performance-dependent improvement in the 5C-CPT as with other ADHD treatments such as methylphenidate; and 3) AMP-enhanced attention in healthy humans with low BL attentional performance. Furthermore, these data indicate that such AMP-induced improvements were not prevented by concurrent dopamine D_2 antagonism, suggesting that these AMP effects might be mediated via non-D_2 mechanisms, and also that they might be clinically relevant to schizophrenia patients despite antipsychotic-induced D_2 blockade.

Disclosures: J.W. Young: None. **M.R. Breier:** None. **N.R. Swerdlow:** None.

Nanosymposium

480. Mechanisms of Synapse Formation

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Presentation Number: 480.01

Topic: A.06. Synaptogenesis and Activity-Dependent Development

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Frontiers of Innovation Scholars Program

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Title: Role of an apical-basal polarity protein, Lgl1, in glutamatergic synapse formation and plasticity

Authors: *J. T. SCOTT, M. YE, S. THAKAR, T. YU, H. HEJRAN, Y. ZOU;
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Abstract: Lgl1 is a component of the highly conserved apical-basal polarity signaling pathway, interacting with the basal proteins, such as Discs Large, while antagonizing the apical proteins, such as atypical Protein Kinase C. We found that mouse Lgl1 is localized in the postsynaptic density in developing glutamatergic synapses. Following conditional deletion of Lgl1 at the beginning of synaptogenesis, excitatory synapse number in the hippocampus increases by one week after gene deletion, while inhibitory synapses are not affected. This increase persists into adulthood. In addition, the expression of ionotropic receptors at the synapse is altered. When Lgl1 is deleted in adult animals, a similar increase in excitatory synapse number is observed in addition to impairment in synaptic plasticity. Using genetic and viral tools to delete Lgl1, we observe behavioral phenotypes related to changes in synapse number and synaptic plasticity. Mice with conditional deletion of Lgl1 show increased locomotor activity, cognitive impairment, and abnormal social behavior, indicating that Lgl1 plays an important role in glutamatergic synapse formation and function.

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Nanosymposium

480. Mechanisms of Synapse Formation

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Title: Neuregulin 1, independent of ebb receptors, is a synaptic organizer for central cholinergic synapse

Authors: *K.-F. LEE[1], F. DE WINTER[2], I. PANEK[3], L. SERVILIO[1], B. DOMINGUEZ[1], J. XU[1], T. W. GOULD[4], Z. CHEN[1], C.-T. LEE[1], Y. ZHANG[3], L. TESSAROLLO[5], R. M. BROWNSTONE[3];
[1]Salk Inst., La Jolla, CA; [2]Netherlands Inst. for Neurosci., Amsterdam, Netherlands; [3]Departments of Surgery, Anat. and Neurobio., Dalhousie Univ., Halifax, NS, Canada; [4]Univ. of Nevada Sch. of Med., Reno, NV; [5]Ctr. for Cancer Res., Natl. Cancer Inst., Frederick, MD

Abstract: Central cholinergic synapses play important neuromodulatory roles in neural network integration. But the mechanisms of cholinergic synapse formation remain unknown. At the mammalin cholinergic synapses on motor neurons (MNs), the postsynaptic apparatus is characterized by closely aligned clusters of m2 muscarinic receptor (M2R), small-conductance calcium-activated potassium (SK) channels, structural and signaling molecules. M2R activation increases the excitability of MNs by reducing the afterhyperpolarization (AHP). Previous studies demonstrate that neuregulin 1 (NRG1) and its erbB receptors play multiple roles in neural development and function by forward or reverse signaling pathways. Here we show that NRG1 is clustered on MN postsynaptic membrane and forms a complex with M2R and SKs. We provide genetic evidence that NRG1 is required for postsynaptic specialization, the muscarine-evoked reduction of AHP and increase of MN excitability. Surprisingly, erbB receptors are dispensable. These results suggest a new unexpected NRG1-dependent mechanism for central cholinergic synapses formation and function.

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Nanosymposium

480. Mechanisms of Synapse Formation

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SFARI Research Award / Simons Foundation

Title: Cortical interneuron maturation and integration are mediated by the autism spectrum disorder-associated splicing regulator Rbfox1

Authors: *X. JAGLIN, B. WAMSLEY, G. QUATTROCOLO, B. GAMALLO-LANA, M. NIGRO, A. MAR, B. RUDY, G. FISHELL;
NYU Neurosci. Inst., New York Univ. Sch. of Med., New York, NY

Abstract: What are the molecular mechanisms establishing cell-type specific connectivity and neuronal maturation? This question warrants further investigations because defects in synapse development are widely recognized as a proximal cause of autism spectrum disorder and intellectual disability. We are currently addressing this problem in cortical interneurons (cINs), an excellent system since they present a striking diversity of connectivity patterns and a wide array of genetic tools are now available to specifically manipulate distinct interneuron subtypes. Interestingly, recent work has shed light on the general requirement for neuronal activity in cIN integration into circuits. Although the molecular connection between activity and cIN maturation remains to be elucidated, activity-dependent alternative splicing (AS) of transmembrane proteins and ion channels has recently emerged as a potent post-transcriptional contributor to synaptic development and plasticity. Therefore, we hypothesize that AS tailors the transcriptome to promote the maturation and integration of specific interneuron subtypes into developing cortical circuits.
Notably, we recently identified the RNA binding protein, Fox-1 homolog (Rbfox1) to be developmentally enriched in the precursors that give rise to Parvalbumin (PV)- and Somatostatin (SST)-expressing cINs. Rbfox1 is an activity-regulated splicing regulator that has been shown to modulate the AS of an array of ion channels, neurotransmitter receptors and transmembrane proteins, affecting neuronal excitability and synaptogenesis. We found that *Rbfox1* interneuron-specific conditional knockout presents increased population of cortical interneurons. Paradoxically, *Rbfox1* mutants develop higher seizure susceptibility by the adult age supporting our hypothesis that Rbfox1-mediated AS controls the maturation and integration of cortical interneurons. Using mouse genetics, neuroanatomical and electrophysiological approaches, we are investigating the role of Rbfox1-dependent AS in the establishment of PV- or SST-interneuron synaptic connectivity. In addition, using RNA-seq we are determining Rbfox1-dependent splice variants directing the maturation and connectivity of these interneuron types. Our work uncovers the fundamental role of alternative splicing in directing the integration of interneuron subtypes, a process that can go awry in neuropsychiatric conditions such as autism spectrum disorders and intellectual disability.

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Nanosymposium

480. Mechanisms of Synapse Formation

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

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Title: The molecular mechanism of astrocyte secreted glypican 4 in functional synapse formation

Authors: *I. FARHY TSELNICKER[1], A. C. M. VAN CASTEREN[1], A. LEE[2], A. R. ARICESCU[2], N. J. ALLEN[1];
[1]The Salk Inst. For Biol. Studies, LA Jolla, CA; [2]Wellcome Trust Ctr. for Human Genet. Div. of Structural Biol., Univ. of Oxford, Oxford, United Kingdom

Abstract: Astrocytes play a crucial role in synaptogenesis by secreting factors that regulate many different aspects of synapse formation and function. We identified a family of astrocyte-secreted proteins, glypicans 4 and 6, which induce the formation of functional excitatory synapses between neurons by increasing dendritic clustering of the GluA1 type AMPA receptors (AMPARs) (Allen et al., 2012). Mice lacking glypican 4 (Gpc4) have deficits in excitatory synaptic transmission in the hippocampus at early developmental stages, a time when majority of synapses are formed. Gpc4 acts on a slow time scale (18 hours), which led to the hypothesis that its mechanism of action involves changes in gene expression and/or activation of signaling pathways in neurons via interaction with neuronal receptors. To determine if Gpc4 regulates gene expression in neurons we performed microarray analysis of cultures of rodent retinal ganglion cell neurons (RGCs) treated with purified Gpc4 for 12 hours, a time point which precedes a significant increase in GluA1 clustering. Using this method we identified 50 candidate genes that are upregulated in RGCs by Gpc4. To narrow down the list of candidates we tested which side of the synapse (pre- or post) Gpc4 interacts with, using a Cos7 cell-RGC co-culture assay. We found that Gpc4 interacts with the pre-synaptic axonal side to induce post-synaptic clustering of GluA1. Based on this we focused on candidate genes that are expressed presynaptically, and asked whether manipulating their expression or function in RGCs altered the ability of Gpc4 to induce functional synapse formation. Using these methods we identified the neuronal receptor Gpc4 signals through and the downstream signaling cascade that is induced to cluster GluA1 at the synapse. We have further confirmed our in vitro findings using immunostaining of brain sections from Gpc4 KO mice. These findings reveal a previously unknown mechanism of action of astrocyte secreted Gpc4, and provide mechanistic insight into how functional synapses are formed.
Allen, N. J., Bennett, M. L., Foo, L. C., Wang, G. X., Chakraborty, C., Smith, S. J. and

Barres, B. A. (2012). Astrocyte glypicans 4 and 6 promote formation of excitatory synapses via GluA1 AMPA receptors. *Nature* **486**, 410-4.

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Title: Semaphorin sema4D induces stabilization of inhibitory boutons through c-Met activation and actin rearrangement

Authors: C. P. FRIAS, T. BRESSER, P. VAN BERGEN EN HENEGOUWEN, C. C. HOOGENRAAD, *C. J. WIERENGA;
Utrecht Univ., Utrecht, Netherlands

Abstract: The establishment and plasticity of synapses is an essential process for proper circuit development and function. Inhibitory presynaptic boutons are very dynamic structures that can appear, disappear and reappear along the axon on a timescale of minutes to hours. These dynamics are thought to be essential for inhibitory synapse formation and plasticity, but the underlying molecular mechanisms are still poorly understood.
Here, we use two-photon laser-scanning microscopy in organotypic hippocampal slices from GAD65-GFP mice to examine the molecular mechanisms underlying GABAergic bouton dynamics. We show that actin depolymerization by nanomolar doses of latrunculinB specifically promotes the stabilization of a subset of non-persistent boutons, while leaving overall bouton dynamics generally intact. This stabilization effect was mimicked by treatment with a soluble form of the cell-adhesion molecule Sema4D, suggesting a common underlying mechanism. Sema4D-induced stabilization of inhibitory boutons was associated with an increase in presynaptic VGAT content and postsynaptic gephyrin puncta. c-Met, an autism-linked gene and a known co-receptor of the Sema4D receptor PlexinB1, was found in a subset of inhibitory boutons. We found that inhibition of c-Met completely blocked the Sema4D-induced stabilization of inhibitory boutons.

Our findings show that Sema4D rapidly stabilizes a specific subset of inhibitory boutons, by rearrangement of the actin cytoskeleton. The requirement for c-Met in this process suggests that defects in inhibitory synapse stabilization may contribute to neurodevelopmental disorders such as autism.

Disclosures: C.P. Frias: None. **T. Bresser:** None. **P. van Bergen en Henegouwen:** None. **C.C. Hoogenraad:** None. **C.J. Wierenga:** None.

Nanosymposium

480. Mechanisms of Synapse Formation

Location: SDCC 30B

Time: Tuesday, November 15, 2016, 8:00 AM - 10:30 AM

Presentation Number: 480.06

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: EMBO Long-term Fellow (ALTF_70-2015)

Title: Control of neuronal synapse specification by a highly dedicated alternative splicing program

Authors: *A. M. GOMEZ, L. TRAUNMÜLLER, T.-M. NGUYEN, P. SCHEIFFELE; Univ. of Basel, Basel, Switzerland

Abstract: Alternative RNA splicing represents a central mechanism for expanding the coding power of genomes. Individual RNA-binding proteins can control alternative splicing choices in hundreds of RNA transcripts, thereby tuning amounts and functions of large numbers of cellular proteins. We found that the RNA-binding protein SLM2 is essential for functional specification of glutamatergic synapses in the mouse hippocampus. Genome-wide mapping revealed a markedly selective SLM2-dependent splicing program primarily consisting of only a few target messenger RNAs that encode synaptic proteins. Genetic correction of a single SLM2-dependent target exon in the synaptic recognition molecule neurexin-1 was sufficient to rescue synaptic plasticity and behavioral defects in Slm2 knockout mice. These findings uncover a highly selective alternative splicing program that specifies synaptic properties in the central nervous system.

Disclosures: A.M. Gomez: None. **L. Traunmüller:** None. **T. Nguyen:** None. **P. Scheiffele:** None.

Nanosymposium

480. Mechanisms of Synapse Formation

Location: SDCC 30B

Time: Tuesday, November 15, 2016, 8:00 AM - 10:30 AM

Presentation Number: 480.07

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: CIHR Grant MOP-130526

Title: The X-linked intellectual disability gene, DHHC9, regulates neural circuit formation

Authors: *J. J. SHIMELL[1], D. B. JOVELLAR[1], G. S. BRIGIDI[1], I. TATARNIKOV[2], D. BECCANO-KELLY[2], A. J. MILNERWOOD[2], S. X. BAMJI[1];
[1]Cell. and Physiological Sci., [2]Ctr. for Applied Neurogenetics, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The family of DHHC proteins, which encode palmitoyl acyltransferase (PAT) enzymes, have been implicated in a number of neurodegenerative and neurodevelopmental disorders. Loss-of-function mutations in DHHC9 have been identified in patients with X-linked Intellectual Disability, however its role in the development and function of neural circuits is still unknown. Here we demonstrate that DHHC9 is localized to both excitatory and inhibitory neurons where it plays an important role in promoting and maintaining dendritic outgrowth and arborisation, as well as modifying synapses. Our data suggests that this is palmitoylation dependent and is mediated through Ras GTPase. Together these results show that DHHC9 targets Ras to the plasma membrane where it plays an important role in regulating neuronal growth and synaptic density.

Disclosures: **J.J. Shimell:** None. **D.B. Jovellar:** None. **G.S. Brigidi:** None. **I. Tatarnikov:** None. **D. Beccano-Kelly:** None. **A.J. Milnerwood:** None. **S.X. Bamji:** None.

Nanosymposium

480. Mechanisms of Synapse Formation

Location: SDCC 30B

Time: Tuesday, November 15, 2016, 8:00 AM - 10:30 AM

Presentation Number: 480.08

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH 5T32AG000216-22

NIH 5T32NS007220-33

Title: Genetic pathways regulating excitation and inhibition balance in the *C. elegans* motor circuit

Authors: *K. MCCULLOCH, S. J. CHERRA, S. TAKAYANAGI-KIYA, Y. B. QI, Y. JIN; Div. of Biol. Sci., Univ. of California San Diego, La Jolla, CA

Abstract: Study of the model organism *C. elegans* has contributed greatly to our understanding of the development and function of neural circuits. Its powerful genetic tools and mapped connectome make it an ideal system to identify conserved mechanisms that regulate neural function. Locomotion in *C. elegans* requires the balanced activities of excitatory cholinergic neurons and inhibitory GABAergic motor neurons. In this circuit, an ionotropic acetylcholine receptor, ACR-2, is expressed in dendrites of the cholinergic motor neurons and regulates their excitability. A Valine-to-Methionine missense mutation in the highly conserved TM2 domain of ACR-2 results in a gain-of-function mutation [*acr-2(gf)*] and causes the worms to spontaneously contract body muscles, or "convulse". This convulsion behavior is the result of both increased cholinergic excitation and decreased GABAergic inhibition in the motor circuit. Such physiological defects are reminiscent of those described for seizures. Interestingly, mutations similar to *acr-2(gf)* in the human acetylcholine receptor subunit CHRNB2 have been associated with human familial epilepsies. To identify genes that regulate motor circuit function, we isolated genetic suppressors of *acr-2(gf)* convulsions. Analyses of these suppressors have identified a major class of genes that are known to regulate pre-synaptic release, such as *unc-13*/Munc13, which is required for synaptic vesicle priming. In addition, we identified that loss of function in the gene *sphk-1*/Sphingosine kinase suppressed convulsion of *acr-2(gf)*. Sphingosine kinase is a critical enzyme that regulates sphingolipid metabolism by phosphorylating the lipid sphingosine to generate sphigosine-1-phosphate, a signaling molecule that regulates extra- and intra-cellular responses. Vertebrate genomes have two Sphingosine kinases, Sphk1 and Sphk2. In humans, Sphk1 has been studied for its role in apoptosis and cancer. Although there are also studies suggesting a role in neurodegenerative disease, how Sphk1 functions in the mammalian nervous system is much less well understood. In *C. elegans*, *sphk-1* has been implicated in promoting neurotransmission in response to cholinergic activity through a g-protein signaling pathway. However, we found that the roles of *sphk-1* in *acr-2(gf)* mediated excitation and inhibition imbalance are independent of the known upstream genes of *sphk-1*. We will present our on-going studies that highlight a novel role for sphingolipid metabolism in modulating neural circuit function.

Disclosures: K. McCulloch: None. **S.J. Cherra:** None. **S. Takayanagi-Kiya:** None. **Y.B. Qi:** None. **Y. Jin:** None.

Nanosymposium

480. Mechanisms of Synapse Formation

Location: SDCC 30B

Time: Tuesday, November 15, 2016, 8:00 AM - 10:30 AM

Presentation Number: 480.09

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: MRC PhD Studentship

Wellcome Trust

Title: Formation and maturation of axo-axonic synapses at the axon initial segment

Authors: *A. PAN VAZQUEZ, W. WEFELMEYER, J. BURRONE;
King's Col. London, London, United Kingdom

Abstract: The axon initial segment (AIS) is a structure at the start of the axon with a high density of sodium and potassium channels that defines the site of action potential generation. It is also the target of inhibitory synapses formed by a specific type of GABAergic interneuron, the Chandelier cell. Previous work in the lab has focused on activity-dependent forms of plasticity of the AIS and its synapses. Here, we present data characterising the changes these structures undergo during postnatal development in the rodent neocortex.
We used a recently developed inducible Cre mouse line, Nkx2.1-CreER, to label Chandelier cell interneurons (Taniguchi et al. Neuron 2011). By visualising Chandelier cell interneurons and their boutons in somatosensory cortex of fixed brain preparations at different developmental stages, we uncovered a critical temporal window of synapse formation at the AIS (P14-P16), which corresponded with the gross morphological maturation of the Chandelier cell axonal arbour. We are currently investigating the electrical excitability of the cell during that time period. Surprisingly though, innervation of the AIS continued after this early synaptogenesis period in a late maturation phase, beyond P22. Here, both presynaptic and postsynaptic compartments gradually increased in number along the AIS. We are currently performing *in vivo* imaging to visualise these processes as they occur in the brain.

Disclosures: **A. Pan Vazquez:** None. **W. Wefelmeyer:** None. **J. Burrone:** None.

Nanosymposium

480. Mechanisms of Synapse Formation

Location: SDCC 30B

Time: Tuesday, November 15, 2016, 8:00 AM - 10:30 AM

Presentation Number: 480.10

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH 5T32MH020016

Title: An *In vivo* model of axonal transport and de novo pre-synapse formation

Authors: *D. LIPTON[1], C. MAEDER[1], S. NIWA[1,2], K. SHEN[1];
[1]Howard Hughes Med. Inst., Stanford Univ., Stanford, CA; [2]Frontier Res. Inst. for Interdisciplinary Sciences, Tohoku Univ., Sendai, Japan

Abstract: Formation of pre-synapses in the brain requires several major steps. 1. Synthesis of pre-synaptic materials, including pre-synaptic proteins and synaptic vesicle precursors, in the cell body. 2. Transport of these materials down the axon, mostly by molecular motors such as kinesin and dynein, along microtubule tracks. 3. Deposition of transport packets and assembly of pre-synapses. There are several unanswered questions about how pre-synapses form from these transport packets. How quickly do synapses form in vivo? What types of pre-synaptic materials 'arrive' at the synapse first? Which components of pre-synapse assembly are dependent on kinesin/dynein-mediated axonal transport? And finally how is transport regulated in order to deposit cargos at the synaptic sites? I will present an in vivo model of de novo synapse formation using a post-embryonically born neuron in C. elegans. Here, synapse formation occurs in a matter of minutes. Synaptic transport vesicles and active zone proteins arrive at the synapse simultaneously, with most synaptic components being dependent on UNC-104/kinesin-3. And finally, transport is specially regulated at sites of synapse formation in order to form synapses.

Disclosures: **D. Lipton:** None. **C. Maeder:** None. **S. Niwa:** None. **K. Shen:** None.

Nanosymposium

481. Molecular and Cellular Mechanisms in Autism Models and Other Developmental Diseases

Location: SDCC 25A

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 481.01

Topic: A.07. Developmental Disorders

Support: 5R21NS087077-02

5R01NS052325-07

5T32MH017168-32

Title: A potential role for SAP97 in psychiatric disorders

Authors: *P. GUPTA[1], L. ZHANG[2], R. G. KALB[2];
[1]Neurosci. Grad. Group, Univ. of Pennsylvania Perelman Sch. of Medi, Philadelphia, PA;
[2]Neurol., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Aberrant brain circuitry is implicated in various neuropsychiatric disorders such as autism spectrum disorders (ASD), schizophrenia (SCZ), and intellectual disability. Both ASD and SCZ are classified as "synaptopathies," as a majority of the genes mutated in these disorders play a critical role in synaptic development, function, and neuronal connectivity. Consequently, the genes regulating dendritic growth and arborization are imperative for the formation of functional neuronal networks. Synapse-associated protein of 97kDa (SAP97) is one such gene that is implicated in regulating glutamatergic synaptic transmission within the nervous system and is involved in mediating dendritic morphogenesis. Additionally, whole exome sequencing data suggests SAP97 may play a risk-determining role in the onset of ASD and SCZ. In order to determine whether SAP97 directly contributes to the behavioral phenotypes associated with ASD and SCZ, we have studied mice lacking neuronal SAP97. We have subjected these mice to a battery of behavioral paradigms to screen for a ASD or SCZ phenotype. We have found that male SAP97 conditional knockout mice display an increase in freezing behavior in the cued fear conditioning paradigm. Our findings will determine whether SAP97 plays a direct, causative role in the symptomology associated with ASD and SCZ, as well as elucidate the molecular pathways involved in the development of proper neuronal connectivity.

Disclosures: P. Gupta: None. **L. Zhang:** None. **R.G. Kalb:** None.

Nanosymposium

481. Molecular and Cellular Mechanisms in Autism Models and Other Developmental Diseases

Location: SDCC 25A

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 481.02

Topic: A.07. Developmental Disorders

Support: Dr. Hans- and Liselotte Ritz-Stiftung

Deutscher Akademischer Austauschdienst

Title: Regulation of Shank proteins by intramolecular interactions

Authors: *H.-J. KREIENKAMP[1], H.-H. HOENCK[2], F. HASSANI NIA[2], V. MARTENS[2]; [2]Human Genet., [1]UKE Hamburg, Hamburg, Germany

Abstract: Shank proteins (Shank1-3) are major scaffolding protein of the postsynaptic density of glutamatergic synapses. Through multiple protein interactions, Shank proteins provide an interface between several F-actin associated proteins of the cytoskeleton of the dendritic spine, and neurotransmitter receptors at the postsynaptic plasma membrane. Mutations in the *SHANK3* gene are associated with autism in human patients. So far little is known how individual missense mutations in *SHANK3* affect the function of the protein. We have addressed this by investigating how mutations in the N-terminal portion affect intra- and intramolecular interactions of Shank3. We have previously reported that the N-terminal SPN domain of Shank3 and Shank1 inhibits binding of the cytoskeletal protein alpha-fodrin to the ankyrin repeat region through an intramolecular interaction; a mutation found in an autistic patient disrupted this regulatory interaction. Here we further analyze how interactions of Shank proteins are regulated. We observe that access to the N-terminal SPN domain is regulated by parts of the central portion of the protein, involving the PDZ domain and parts of the long proline rich region. Direct binding assays support the notion that this part of Shank3 may bind to the N-terminus, suggesting that the protein exists in a closed conformation inaccessible to several interaction partners. In addition, we show that mutations found in autism patients disrupt regulation of the transition between open and closed conformations.

Disclosures: H. Kreienkamp: None. **H. Hoenck:** None. **F. Hassani Nia:** None. **V. Martens:** None.

Nanosymposium

481. Molecular and Cellular Mechanisms in Autism Models and Other Developmental Diseases

Location: SDCC 25A

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 481.03

Topic: A.07. Developmental Disorders

Support: NSERC Discovery Grant 2016-06035

International Rett Syndrome Foundation

Ontario Rett Syndrome Foundation

University of Manitoba Research Grant Program

Title: Investigating the role of miR132 in the homeostatic regulation of MeCP2 isoforms in neural stem cells and Rett syndrome patients

Authors: *M. RASTEGAR[1], C. OLSON[1], S. PEJHAN[1], S. AMIRI[1], V. SIU[3], L.-C. ANG[4], M. DEL BIGIO[2];
[1]Dept. of Biochem. and Med. Genet., [2]Dept. of Pathology, Univ. of Manitoba, Winnipeg, MB, Canada; [3]Dept. of Biochem., [4]Dept. of Pathology, Western Univ., London, ON, Canada

Abstract: MicroRNAs (miRNAs) are small non-coding RNA molecules that control gene expression by transcript degradation or translational inhibition. Deregulation of miRNAs is suggested to be involved in MeCP2-associated neurological disorders. MeCP2 is an important epigenetic factor that controls brain development and function. Accordingly, *MECP2* gene mutations cause Rett Syndrome (RTT), a severe form of Autism Spectrum Disorders in young females. RTT patients appear normal at first, but display developmental decline by 6-18 months of age. These patients experience loss of speech, repetitive hand movements, seizures, mental disability and autistic behaviors.
Research by others and us have shown that MeCP2 has two protein isoforms (E1 and E2) with unique N-termini sequences, but sharing the same functional domains. We reported the cell type- and brain region-specific expression and regulation of E1 and E2 isoforms in the brain and brain-derived neural stem cells. MeCP2 isoforms have redundant and non-redundant functions and differently impact RTT. In the brain cells, the MeCP2 homeostasis is tightly controlled by MeCP2-BDNF-miR132 genetic network. The microRNA 132 (miR132) is a neuronal-specific miRNA that inhibits MeCP2, but its own expression is induced by BDNF, controlled in turn by MeCP2. Currently, the involvement of E1 and E2 in the MeCP2 homeostatic network is unclear. The aim of this present study is to investigate the molecular mechanisms that control the cell type-specific MeCP2E1/E2-miR-132-BDNF in the human brain, and the impact in RTT patients. To study the regulatory mechanisms that control MeCP2 homeostasis in the brain, we modulate each of these gene(s)/factor(s) in primary neural stem cells and established human brain cell lines. We use different brain regions of RTT post-mortem brain tissues with different genetic mutation(s) to study the potential deregulation of MeCP2 homeostatic network in RTT brain. Currently, RTT has no cure and its pathobiology is not fully understood. The outcome of our research is expected to provide insight towards understanding how molecular deficiencies at the cellular levels cause compromised brain function in RTT.

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Nanosymposium

481. Molecular and Cellular Mechanisms in Autism Models and Other Developmental Diseases

Location: SDCC 25A

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 481.04

Topic: A.07. Developmental Disorders

Support: IRSF Awatd

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Title: Agap1 regulates endosomal trafficking and dendritic spine morphology downstream of dysbindin.

Authors: *M. ARNOLD[1,2];
[1]Agnes Scott Col., Acworth, GA; [2]Agnes Scott Col., Decatur, GA

Abstract: Arf1 GTPase activating protein (AGAP1) interacts with the vesicle associated Biogenesis of Lysosome Related Organelles Complex-1 (BLOC-1) and adaptor protein 3 (AP-3). In non-neuronal cells, overexpression of AGAP1 results in a build up of endosomal content suggesting it regulates endosome-dependent trafficking. In GWAS studies, AGAP1 has been implicated two neurodevelopmental disorders, Schizophrenia (SZ) and Autism Spectrum Disorder (ASD). However, AGAP1's localization or function within neurons has yet to be reported. In this study, we demonstrate AGAP1 localizes in axons, dendrites, dendritic spines and synapses, and also colocalizes preferentially with markers of early and recycled endosomes. Through both down regulation and overexpression of AGAP1, neuronal endosomal trafficking and spine morphology are affected. Additionally, AGAP1 mRNA and protein exhibited a decrease in the hippocampus of mice lacking dysbindin, which is associated with risk of SZ. We propose that endosomal trafficking regulated by BLOC-1 and AGAP1 contributes to the synapse morphology of neurodevelopmental disorders.

Disclosures: M. Arnold: None.

Nanosymposium

481. Molecular and Cellular Mechanisms in Autism Models and Other Developmental Diseases

Location: SDCC 25A

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 481.05

Topic: A.07. Developmental Disorders

Support: CNHS IDDRC Pilot Grant

Title: Altered BDNF signaling dynamics in a novel mouse model of autism and intellectual disability

Authors: *A. W. OAKS, S. DI COSTANZO, M. ZAMARBIDE, M. MANZINI;
George Washington Univ., Washington, DC

Abstract: Establishment of brain connectivity occurs during late embryonic and early postnatal development, and depends on the formation of specific morphological features in neurons. These include highly branched dendrites, appropriately dense dendritic spines and an adequate number of functional and plastic synapses. Neurodevelopmental disorders, including intellectual disability (ID) and autism spectrum disorder (ASD), involve disruption of this process, leading to the aberrant neuronal morphology and brain connectivity that characterize these conditions. We have recently described loss of function mutations in *CC2D1A* (coiled-coil and C2 domain containing 1A) that cause ID, ASD, and seizures. Here, we examine the role of CC2D1A in the differentiation of cortical neurons.

CC2D1A is a scaffold protein that regulates intracellular signaling, and has been shown to determine how cells respond to external stimuli in the NF-κB, PKA, and AKT pathways, among others. We have hypothesized that CC2D1A enables the formation of signaling complexes with context-specific roles in different cell types and developmental stages. CC2D1A thereby acts as an integrator of extrinsic stimuli, with outputs to multiple signaling pathways. The involved pathways can be activated by many such stimuli and modulate a diverse array of downstream targets, many of which have been implicated in ID and ASD. These functions can also be recruited by brain-derived neurotrophic factor (BDNF), an important modulator of dendrite morphology and synaptic plasticity.

Our studies have shown that Cc2d1a knockout neurons have dysregulated responses to several extracellular ligands, including BDNF, that are important for controlling neuronal differentiation and plasticity. Spine and dendrite morphology of Cc2d1a-deficient neurons is impaired and we found that genetic removal of Cc2d1a causes dysregulation of AKT signaling responses in BDNF-treated cortical neurons. These results suggest that removal of Cc2d1a from neurons disrupts the regulation of the events downstream of BDNF. The signaling scaffold functions of Cc2d1a may therefore be a key determinant of BDNF-AKT signaling specificity, with essential

roles during neuronal differentiation, plasticity, or both. To further probe the role of Cc2d1a in BDNF-AKT signaling, we have employed diverse approaches, including AKT biosensors, live-cell imaging of endosome trafficking, and identification of Cc2d1a-dependent signaling complexes in neurons.

Disclosures: **A.W. Oaks:** None. **S. Di Costanzo:** None. **M. Zamarbide:** None. **M. Manzini:** None.

Nanosymposium

481. Molecular and Cellular Mechanisms in Autism Models and Other Developmental Diseases

Location: SDCC 25A

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 481.06

Topic: A.07. Developmental Disorders

Support: NIMH R00MH102244

Title: Activity-dependent interactions among Autism candidate-gene products at glutamatergic synapses

Authors: ***A. WILLIAMS**, E. A. BROWN, S. E. P. SMITH;
Ctr. for Integrative Brain Res., Seattle Children's Res. Inst., Seattle, WA

Abstract: Autism spectrum disorders (ASDs) are a heterogeneous group of developmental disabilities with shared impairments in social interaction, communication, and stereotyped behaviors. ASD etiology and pathogenesis is complex and not well understood; thus far, over 100 genes encoding diverse protein products have been associated with ASD. Due to the large number of ASD-linked gene products regulated by neuronal activity, it is hypothesized that ASD may develop, at least in part, from dysregulation of activity-dependent synapse development and function. Neuronal activity triggers local changes at the synapse, and can alter shape, composition, and synaptic strength. While changes in gene expression and protein translation are known to mediate long-term activity dependent effects, the local and immediate activity-dependent changes within protein complexes at the glutamatergic post-synapse are not well understood. We have developed a novel flow-cytometry based technique to monitor network-level protein-protein interactions (PPIs) of ASD-associated gene products at the glutamatergic synapse: quantitative multiplex immunoprecipitation (QMI). Using QMI, we are able to simultaneously detect and quantify over 100 protein-protein interactions (PPIs) following neuronal stimulation. As previously reported by others, we find consistent dissociation between ASD-linked mGluR5 and Homer1 following brief stimulation with high potassium in both

primary neuron culture and fresh slice preparations. In addition to this known activity-dependent interaction, we have used QMI to identify novel synaptic PPIs modified by neuronal activity. Our data suggest that ASD gene-products at the glutamatergic synapse are involved in the dynamic short-term response to activity and provides a framework to uncover how ASD-linked mutations may disrupt this process.

Disclosures: **A. Williams:** None. **E.A. Brown:** None. **S.E.P. Smith:** None.

Nanosymposium

481. Molecular and Cellular Mechanisms in Autism Models and Other Developmental Diseases

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Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

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Topic: A.07. Developmental Disorders

Support: NARSAD Young Investigator Award

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Title: GPCR associated proteins - GPRASPing mGluR signalling dysfunction in autism spectrum disorders

Authors: M. EDFAWY, A. L. CARVALHO, ***J. M. PEÇA**;
Ctr. For Neurosci. and Cell Biol., Coimbra, Portugal

Abstract: Autism spectrum disorders (ASDs) are neurodevelopmental conditions diagnosed based on a triad of criteria: deficits in communication, impaired social interactions, and repetitive or restricted interests and behaviors. ASDs pose an immense burden to society and are thought to afflict 1 out of each 68 children. Recent genetic and genomic studies have identified a large number of candidate genes for ASDs that encode for synaptic proteins, indicating synaptic dysfunction may play a critical role in these disorders. Disease susceptibility proteins, such as those in the Neurexin/Neuroligin/PSD-95/SAPAP/SHANK macromolecular complex, converge on ionotropic and metabotropic glutamate signaling. Dysfunction in these genes has been shown to disrupt neuronal morphology, dendritic complexity and synaptic communication. Presently,

several lines of evidence suggest that metabotropic glutamate receptors (mGluRs) play an important role in ASD pathophysiology. Nevertheless, research work centering on the proteins that directly regulate the trafficking and surface availability of mGluRs has not been widely explored. The G Protein-Coupled Receptor Associated Sorting Protein (GPRASP) family regulates the trafficking of diverse classes of G-protein coupled receptors, including the mGluR1 and mGluR5 receptors. More specifically, these proteins are involved in the endocytic sorting of G-coupled protein receptors towards lysosomal degradation. Additionally, GPRASP2 in particular has been recently proposed as an autism susceptibility gene.
To better understand the role of this protein in ASDs we are characterising the expression of GPRASP2, its localization in subcellular compartments, different brain regions and across development. Our data also suggests that the perturbation of the GPRASP2 levels has an impact in the regulation of neuronal complexity, spine density and spine maturation. We are also using mouse molecular genetics to understand the functional, cellular and behavioral consequences of disrupting this candidate gene and its role in the regulation of metabotropic glutamate receptors.

Disclosures: **M. Edfawy:** None. **A.L. Carvalho:** None. **J.M. Peça:** None.

Nanosymposium

481. Molecular and Cellular Mechanisms in Autism Models and Other Developmental Diseases

Location: SDCC 25A

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 481.08

Topic: A.07. Developmental Disorders

Support: MR/K022377/1 (AB & CF)

SFARI 344763 (AB)

Ontario Brain Institute POND programme (JPL)

MRC PhD studentship (CPS)

Title: Neurodevelopmental, craniofacial and behavioural abnormalities in mice deficient for the autism-associated gene Chd8

Authors: ***P. SUETTERLIN**[1], K. L. H. RIEGMAN[1], A. CARUSO[4,5], C. MOHAN[1], S. HURLEY[1], C. P. SCHOTT[2], J. ELLEGOOD[6], I. CRESPO-ENRIQUEZ[1], C. MICHETTI[7], R. ELLINGFORD[1], P. FRANCIS-WEST[1], J. P. LERCH[6], M. SCATTONI[4], L. C. ANDREAE[2], C. FERNANDES[3], A. BASSON[1];
[1]Ctr. for Craniofacial Develop. and Stem Cell Biol., [2]Dept. of Developmental Neurobio., [3]MRC

Social, Genet. & Developmental Psychiatry Ctr., King's Col. London, London, United Kingdom; [4]Neurotoxicology and Neuroendocrinology Section, Dept. of Cell Biol. and Neurosci., Inst. Superiore di Sanità, Rome, Italy; [5]Sch. of Behavioural Neuroscience, Dept. of Psychology, Sapienza Univ., Rome, Italy; [6]Dept. of Med. Biophysics, Univ. of Toronto, Hosp. for Sick Children, Toronto, ON, Canada; [7]Ctr. for Synaptic Neurosci. and Technol., Inst. Italiano di Tecnologia, Genova, Italy

Abstract: *CHD8* haploinsufficiency is associated with autism, developmental delay, macrocephaly and craniofacial dysmorphology, but the specific molecular perturbations underlying these phenotypes are not known. Therefore, we set out to identify the role of CHD8 in brain development in a range of relevant transgenic mouse lines, and to study the behaviour of *CHD8* haploinsufficient mice. Here we show that conditional, pan-neuronal deletion of mouse *Chd8* results in extreme neocortical hypoplasia, caused by a combination of altered proliferation, differentiation and apoptosis of early cortical progenitor cells. Transcriptomic, cellular and genetic analyses further identify CHD8 as an obligatory repressor of p53-regulated pathways during early cortical development. By contrast, $Chd8^{+/-}$ mice exhibit brain overgrowth and craniofacial anomalies reminiscent of humans with *CHD8* mutations. Juvenile $Chd8^{+/-}$ mice presented with growth and motor delay and hyperactivity. Intriguingly, adult $Chd8^{+/-}$ mice displayed a heightened interest in social cues and marked hypo-activity in novel environments. MRI identified hyperplasia of specific regions in the neocortex, hippocampus and cerebellum, providing potential neural substrates of the human neurodevelopmental phenotype that warrant further scrutiny. Electrophysiological recordings demonstrated that *Chd8* heterozygosity was sufficient to alter excitatory drive onto deep-layer cortical pyramidal neurons hinting at disrupted neural network activity. Moreover, quantitative reverse-transcription PCR identified disturbed developmental expression profiles of autism-associated genes in the neocortex. These findings link *Chd8* heterozygosity to brain overgrowth as well as behavioural, electrophysiological and epigenetic dysregulation. Moreover, this validates the $Chd8^{+/-}$ mouse as an appropriate model to study *CHD8* haploinsufficiency syndrome. Future studies will aim to establish causal association between the described physical and behavioural phenotypes and the specific molecular pathways affected by *Chd8* heterozygosity that may be responsible.

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Nanosymposium

481. Molecular and Cellular Mechanisms in Autism Models and Other Developmental Diseases

Location: SDCC 25A

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 481.09

Topic: A.07. Developmental Disorders

Support: 2014CB964600

Title: Visualizing the dynamic of CA1 local circuits in mouse model of autism

Authors: *L. LI[1], L. SUN[2], X. WANG[2];
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Abstract: Duplications of methyl-CpG binding protein 2 (MECP2)-containing genomic segments in human lead to severe autistic symptoms and exhibit high penetration to human male patients, thereby named the MECP2 duplication syndrome. Despite major efforts over the past two decades to elucidate the molecular and functions of MeCP2, the mechanism of that underling the social behavior and cortical information processing are still unclear. Here, we performed cellular-resolution microendoscopic Calcium imaging in freely moving animals while they are performing social interaction; we identified individual social-related neurons in HPC with events triggered Ca^{2+} responses. We found individual social neurons activity preferentially response to social -related cues in wild type mice but not in Mecp2 duplication mice, which can be rescued with gene mutation in vivo. These results suggest that CA1 region involved in the social interactions, and provide a potential target for autism therapeutic interventions.

Disclosures: L. Li: None. **L. Sun:** None. **X. Wang:** None.

Nanosymposium

481. Molecular and Cellular Mechanisms in Autism Models and Other Developmental Diseases

Location: SDCC 25A

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 481.10

Topic: A.07. Developmental Disorders

Title: Pathophysiological role of MUNC18-1 in early infantile epilepsies

Authors: *K.-I. NAGATA, N. HAMADA, H. TABATA;
Inst. For Developmental Research, Aichi Human Service Ctr., Kasugai, Japan

Abstract: Objective: While Munc18-1 is essential for presynaptic vesicle fusion in developed neurons, this molecule is likely to be involved in brain development since gene abnormalities in *MUNC18-1* (*STXBP1*) cause early infantile epileptic encephalopathy with suppression-burst (Ohtahara syndrome), neonatal epileptic encephalopathy and other neurodevelopmental disorders. We analyzed physiological and pathophysiological relevance of Munc18-1 during the cortical development.
Methods: With acute knockdown and expression with the *in utero* electroporation technique, we performed *in vivo* and *in vitro* investigation, including confocal laser microscope-associated live-imaging, to clarify the role of Munc18-1 and its epilepsy-causing mutants in the mouse corticogenesis.
Results: Munc18-1-knockdown caused abnormal migration of cortical neurons during corticogenesis. The phenotype was rescued by an RNAi-resistant Munc18-1. Protein kinase C, but not Cyclin-dependent kinase 5, was likely to be implicated in the migration. Notably, Munc18-1-binding partner, Syntaxin1A but not B, rescued the knockdown phenotype. Time-lapse imaging revealed that the radial migration step was hampered in the cortical plate. Although functional synapses are not formed in the neocortex during the embryonic stage, these results suggest that Munc18-1 has a specific role in Syntaxin1A regulation, which is modulated by Protein kinase C, in the radial migration during the corticogenesis. In addition, disruption of N-Cadherin localization by hampered vesicle trafficking appeared to be involved in the migration defects.
Interpretation: Functional abnormalities of MUNC18-1 may induce aberrant cortical neuron migration leading to functional defects of the cerebral cortex, and consequently contribute to the pathophysiologies of epilepsies and other disorders with *MUNC18-1* abnormalities.

Disclosures: K. Nagata: None. **N. Hamada:** None. **H. Tabata:** None.

Nanosymposium

481. Molecular and Cellular Mechanisms in Autism Models and Other Developmental Diseases

Location: SDCC 25A

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Presentation Number: 481.11

Topic: A.07. Developmental Disorders

Support: ANR-10-IDEX-0002-02

ANR-10-LABX-0030-INRT

ANR-10-INBS-07 PHENOMIN

BPI-TRIAD

Fondation Jerome Lejeune

Title: Elucidating the role of DYRK1A in neurons <and> the consequences of its overdosage in mouse models for tomorrow's treatment of Down syndrome

Authors: *Y. HERAULT[1,2], T. NGUYEN[1,3], A. DUCHON[1], V. BRAULT[1], A. DUBOS[1], H. MEZIANE[2], M. SELLOUM[2], M.-C. BIRLING[2], A. MANOUSOPOULOU[4], S. D. GARBIS[4], L. MEIJER[3];
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Abstract: DYRK1A encodes a dual-specificity tyrosine phosphorylation-regulated kinase that is overexpressed in DS and plays a key role in neurogenesis, outgrowth of axons and dendrites, neuronal trafficking and aging. DYRK1A is one of the current target to improve cognition in Down syndrome (DS) patients. With the Epigallocatechin-3-gallate, an inhibitor of Dyrk1a, the rescue of learning and memory phenotypes has been observed in DS mouse models and patients[1-3]. In order to better understand the molecular and cellular mechanisms that are perturbed by the overexpression of DYRK1A, we planned to rescue the normal dosage of Dyrk1a in the adult brain of the Dp1Yey trisomic models. We used a conditional approach with a conditional allele of Dyrk1a and the Camk2 Cre driver[4] to target the glutamatergic trisomic neurons. Interestingly with such a glutamatergic rescue Dp1Yey mouse learning and memory phenotype were rescued in a way similar to the effect of Dyrk1a inhibitor treatment in other DS models. Then we wanted to identify the molecular consequence of inhibiting DYRK1A kinase activity in DS mouse models. We used two DS mouse models, a single BAC transgenic for Dyrk1a[5] and the Ts65Dn mouse model[6] and the L41 specific Dyrk1a inhibitor[7]. We showed that L41 treatment was able to rescue behaviour and cognition and then we analysed the changes in the phosphoproteome after L41 treatment in DS models. We identified a series of proteins and pathways that are regulated by phosphorylation depending on the L41 treatment, identifying new mode of actions and interactants of DYRK1A at the glutamatergic synapse. This study leads to a better understanding of the role of DYRK1A in neurons, how the cognition and behaviour is perturbed in DS mouse models and certainly in human, and gives us a mode of action for the inhibition of DYRK1A in DS. 1 Souchet, B. *et al. Front Behav Neurosci* **9**, 267 (2015). 2 De la Torre, R. *et al. Mol Nutr Food Res* **58**, 278-288 (2014). 3 Guedj, F. *et al. PLoS One* **4**, e4606 (2009). 4 Mantamadiotis, T. *et al. Nat Genet* **31**, 47-54, (2002). 5 Guedj, F. *et al. Neurobiol Dis* **46**, 190-203 (2012). 6 Reeves, R. H. *et al.* N*at Genet* 1**1,** 177-184 (1995). 7 Tahtouh, T. *et al.* J *Med Chem* 5**5,** 9312-9330 (2012).

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Nanosymposium

481. Molecular and Cellular Mechanisms in Autism Models and Other Developmental Diseases

Location: SDCC 25A

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 481.12

Topic: A.07. Developmental Disorders

Support: NICHD/NIH Grant 1R01HD067731

Title: Parsing the genetic brain connectome using rare identical twins discordant for Down syndrome

Authors: *L. DAI[1], J. ANDERSON[2], M. BURBACK[3], J. EDGIN[5], G. GERIG[6], J. R. KORENBERG[4];
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Abstract: Down syndrome (DS) is a major cause of mental retardation and congenital anomalies. Understanding the genetic or neurocognitive effects of trisomy 21 has always been severely limited by the heterogeneity of genes located on chromosome 21 and by the enormous variations in the remainder of the 30,000 genes located on the other chromosomes. Identical twins are therefore nature's unique opportunity to see the dramatic effects of identical genes on phenotype. Here we report the structural and functional MRI, DTI, and extensive cognitive and genetic measures of a pair of identical twins discordant for DS and compared them to the typical DS and TC cohort. We determined chromosomal features of DS in one, >96% trisomy 21 in cord blood, blood and fibroblasts, and in the other a normalized phenotype, >92% normal karyotype in these tissues. Structural MRI showed distinguishable patterns in most but not all regions between the identical twins. Normalized volumes of the 96% mosaic twin were very close to the DS group, and the 8% mosaic twin volumes were similar to controls. An exception was DS-like volume in the 8% mosaic twin of the right fusiform gyrus, involved in visual memory and language. Only the 96% mosaic twin demonstrated increased internetwork synchrony across networks seen in typical DS during functional MRI. The twins are a graphic vision of how a

simply trisomy for 21 disturbs brain wiring and the results implicate the hippocampal and fusiform gyrus formation in the neural systems responsible for visual-spatial and linguistic deficits of DS.

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Nanosymposium

482. Alzheimer's Disease: Therapeutics in Animal Models

Location: SDCC 33C

Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 482.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH

NIH RO1, MIT

Title: Tfp5, a peptide inhibitor of aberrant and hyperactive cdk5/p25, attenuates pathological phenotypes & restores synaptic function in ck-p25tg mice

Authors: ***H. C. PANT**[1], V. SHUKLA[1], J. SEO[2], B. BK[1], N. AMIN[1], P. REDDY[1], P. GRANT[1], J. STEINER[1], S. SKUNTZ[1], S. KESAVAPANY[4], L.-H. TSAI[3];
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Abstract: It has been reported that cyclin-dependent kinase 5 (Cdk5), a critical neuronal kinase, is hyperactivated in Alzheimers disease (AD) & may be, inpart responsible for the hallmark pathology of amyloid plaques & neurofibrillary tangles (NFTs). It has been proposed by several laboratories that hyperactive cdk5 results from the overexpression of p25, (a truncated fragment of p35, the normal cdk5 regulator), which, when complexed to Cdk5, induces hyperactivity, hyperphosphorylated tau/neurofilament tangles (NFTs), A-beta plaques & neuronal death. It has previously been shown that intraperitoneal (i.p.) injections of a modified truncated 24-aa peptide (TFP5), derived from the Cdk5 activator p35, penetrated the blood-brain barrier & significantly rescued AD-like pathology in 5XFAD model mice. The principal pathology in the 5XFAD mutant, however, is extensive amyloid plaques; hence, as a proof of concept, we believe it is essential to demonstrate the peptide’s efficacy in a mouse model expressing high levels of p25, such as the inducible CK-p25Tgmodel mouse that overexpresses p25 in CamKII positive neurons. This is important because overexpression of p25 in human AD brains (& in some animal models) has been questioned in several laboratories. Using a modified TFP5 treatment,

here we show that peptide i.p. injections in these mice decrease Cdk5 hyperactivity, tau, neurofilament-M/H hyperphosphorylation, & restore synaptic function (LTP) & behavior (i.e., spatial working memory). It is noteworthy that TFP5 does not inhibit endogenous Cdk5/p35 activity, nor other Cdks *in vivo* suggesting it might have no toxic side effects, & may serve as an excellent therapeutic candidate for neurodegenerative disorders expressing abnormally high brain levels of p25 & hyperactive Cdk5.

Disclosures: **H.C. Pant:** A. Employment/Salary (full or part-time): NIH. **V. Shukla:** A. Employment/Salary (full or part-time): NIH. **J. Seo:** A. Employment/Salary (full or part-time): MIT. **B. Bk:** A. Employment/Salary (full or part-time): NIH. **N. Amin:** A. Employment/Salary (full or part-time): NIH. **P. Reddy:** A. Employment/Salary (full or part-time): NIH. **P. Grant:** A. Employment/Salary (full or part-time): NIH. **J. Steiner:** A. Employment/Salary (full or part-time): NIH. **S. Skuntz:** A. Employment/Salary (full or part-time): NIH. **S. Kesavapany:** A. Employment/Salary (full or part-time): Singapore University, Singapore. **L. Tsai:** A. Employment/Salary (full or part-time): MIT.

Nanosymposium

482. Alzheimer's Disease: Therapeutics in Animal Models

Location: SDCC 33C

Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 482.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1RO1NS079637

NIH Fellowship F31NS092202

Title: Cerebrovascular consequences of an anti-Aβ immunotherapy in a co-morbidity mouse model

Authors: ***E. M. WEEKMAN**[1], T. L. SUDDUTH[1], O. W. PHILLIPS[1], D. K. POWELL[2], A. H. ANDERSEN[3], D. M. WILCOCK[1];
[1]Physiol., [2]Biomed. Engin., [3]Anat. and Neurobio., Univ. of Kentucky, Lexington, KY

Abstract: Alzheimer’s disease (AD) and vascular cognitive impairment and dementia (VCID) are the two most common forms of dementia. Furthermore, it is estimated that 40% of AD patients also have some form of VCID. Immunotherapy targeting amyloid-beta (Aβ) remains one of the leading therapeutic approaches in development to treat AD. However, in clinical trials and mouse models, anti-Aβ immunotherapy has significant cerebrovascular adverse events in some individuals, which manifest as vasogenic edema and microhemorrhages. We hypothesize that

VCID co-morbid with AD will worsen the cerebrovascular consequences of anti-Aβ immunotherapy. We used our hyperhomocysteinemia (HHcy)/APP/PS1 mouse model of VCID and amyloid pathology for our co-morbidity model. We placed 9 month old wildtype or APP/PS1 mice on a control diet or a diet that induces HHcy. After 3 months, mice began receiving weekly intraperitoneal injections of control antibody or 3D6. Cognition was assessed with the two-day radial arm water maze. Longitudinal MRI for microhemorrhages was performed. Prussian blue staining was also used to determine microhemorrhages in the brain tissue. Matrix metalloproteinases and neuroinflammatory markers were assessed by qPCR. Microglia and Aβ were quantified using immunohistochemistry. APP/PS1 mice on the HHcy diet with immunotherapy showed a muted immune response and no cognitive benefits, despite significant total Aβ deposition reductions. We did observe significantly increased microhemorrhage induction in the HHcy/APP/PS1 mice receiving immunotherapy relative to the APP/PS1 mice on control diet receiving immunotherapy. Also, there was a significant increase in CAA deposition in the HHcy/APP/PS1 mice as result of immunotherapy relative to the APP/PS1 mice on control diet receiving immunotherapy. In conclusion, anti-Aβ immunotherapy is ineffective in its actions on cognition when VCID is co-morbid with the amyloid pathology and also the prevalence of cerebrovascular adverse events is much greater. These data suggest that anti-Aβ immunotherapy may be contraindicated in AD individuals with cerebrovascular pathologies.

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Nanosymposium

482. Alzheimer's Disease: Therapeutics in Animal Models

Location: SDCC 33C

Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 482.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA AG046139-01

BrightFocus Foundation Fellowship

Title: Soluble TLR5 decoy receptor as a novel therapeutic for Alzheimer’s disease

Authors: ***P. CHAKRABARTY**[1], A. LI[2], T. LADD[2], E. J. KOLLER[2], M. R. STRICKLAND[2], P. E. CRUZ[2], J. BURGESS[3], C. C. FUNK[4], M. ALLEN[3], X. WANG[3], C. YOUNKIN[3], J.

REDDY[3], B. LOHRER[3], L. MEHRKE[3], B. MOORE[2], X. LIU[2], C. CEBALLOS-DIAZ[2], A. M. ROSARIO[2], C. MEDWAY[3], C. JANUS[2], H. LI[4], D. W. DICKSON[3], N. D. PRICE[4], S. G. YOUNKIN[3], N. ERTEKIN-TANER[3], T. E. GOLDE[2];
[1]Neurosci., [2]Univ. of Florida, Gainesville, FL; [3]Mayo Clin. Jacksonville, Jacksonvile, FL; [4]Inst. of Systems Biol., Seattle, WA

Abstract: An invariant feature of the pathological cascade in Alzheimer's diseases (AD) is reactive gliosis, reflecting underlying alterations in the innate immune activation state within the brain. In the brain, Toll-like receptors (TLR) are a major class of immune receptors that sensitize innate immunity to environmental stresses, such as protein aggregates. Our RNA sequencing of human brains showed upregulation of multiple TLRs in the temporal cortex of AD patients. Analysis of co-expression patterns of TLR expression using pairwise correlations in temporal cortex or cerebellum further showed upregulation of select TLRs in human AD compared to healthy controls. Based on previous data that TLRs may interact directly with amyloid β (Aβ) in AD brains, we explored whether a decoy receptor strategy using the extracellular domain of TLR could have therapeutic potential in AD. Intracranial, recombinant adeno-associated virus (rAAV) mediated expression of human TLR5 ectodomain (soluble TLR5, sTLR5) alone or fused to human IgG4 Fc domain (sTLRFc), results in robust attenuation of Aβ accumulation in an APP transgenic mouse model. Further studies demonstrate that sTLR5Fc i) binds to oligomeric and fibrillar Aβ with high affinity ii) forms complexes with Aβ in vivo and iii) blocks Aβ toxicity in primary neuroglia. Both oligomeric and fibrillar Aβ modulate flagellin-mediated activation of human TLR5, but we find little evidence that these directly act as canonical TLR5 signaling ligands in vitro. Analysis of protein coding variants in GWAS data showed that TLR5 H4 haplotype (p.L478I-F616L-I644F-D846G) showed significant association with reduced risk of AD (p=0.0083; OR=0.53, 0.33 to 0.85). Taken together with this GWAS analysis indicating TLR5 may be associated with reduced risk of AD, our data suggests that TLR5 decoy receptor based biologics represent a novel and safe Aβ-selective class of immunobiotherapy for targeting Aβ in AD.

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Nanosymposium

482. Alzheimer's Disease: Therapeutics in Animal Models

Location: SDCC 33C

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Presentation Number: 482.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG18454

Title: Combination of pro-inflammatory IL6 and anti-inflammatory IL10 with anti-A β immunotherapy affects amyloid deposition in AD mouse model

Authors: *T. E. GOLDE, Y. LEVITES, P. CHAKRABARTY, F. BURG, X. LIU, B. BRAMBLETT;
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Abstract: It has been established in the Alzheimer's disease (AD) field that manipulations of immunoprotheasis affect Aβ accumulation and clearance. We have shown that overexpression of proinflammatory IL6 cytokine limits Aβ deposition early in the disease by enhancing glia-mediated amyloid plaque clearance from the brain. On the contrary, expression of antiinflammatory IL-10 leads to increased amyloid loads, worsened cognitive behavior and reduced microglial Aβ phagocytosis. Immunotherapy targeting fibrillary Ab has become a well-established approach with several antibodies in clinical trials. We have shown that passive immunization with pan-Ab antibody prevents plaque formation and reduces amyloid loads in CRND8 mouse model.
The goal of this study is to evaluate the effects of pro- and anti-inflammatory “preconditioning“ of the CNS on the efficacy of anti-Ab immunotherapy. For these studies we chose to combine experimental paradigms we have extensively utilized: anti-Aβ passive immunotherapy and rAAV mediated delivery of cytokines to the mouse brain. Newborn CRND8 mice were transduced with rAAV2/1-Il-6 to induce a pro-inflammatory environment or rAAV2/1-Il-10 to induce an anti-inflammatory environment. Beginning at 2 months of age, 0.5mg anti- Aβ1-16 mAb5 (IgG3) or control mouse IgG was administered IP, biweekly, until 6 months. Amyloid accumulation, Aβ levels and inflammatory markers were assessed by ELISA, SDS-PAGE and immunohistochemistry methods.
Preliminary results demonstrate increased massive gliosis and clearance of amyloid deposits in IL-6 expressing mice, combined with reduction of diffuse amyloid in immunized mice. Combination of the two resulted in even more robust reduction in amyloid levels – 70% reduction in SDS-soluble Aβ 40 levels and 40% reduction in SDS-insoluble, FA- soluble Aβ 42 levels.
Our data will shed light into the role inflammatory processes play in immunotherapy as well as potentially lead to a more effective combinatorial therapy for Alzheimer’s disease.

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Nanosymposium

482. Alzheimer's Disease: Therapeutics in Animal Models

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Presentation Number: 482.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: a Grants-in-Aid for Scientific Research (C) (23500439) from the Ministry of Education, Culture, Sports, Science and Technology of Japan

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Title: Reduction of HSC70 relates to diosgenin-induced memory improvement in a mouse model of Alzheimer’s disease.

Authors: *X. YANG, T. KUBOYAMA, C. TOHDA;
Inst. of Natural Med., Univ. of Toyama, Toyama, Japan

Abstract: We previously found that diosgenin, a constituent of Dioscorea Rhizoma, restored axonal degeneration and improved memory function in a mouse model of Alzheimer’s disease (AD), 5XFAD. In this study, we wanted to investigate diosgenin-elicited expression change of intracellular molecules, which are involved in axonal regrowth and memory recovery. Vehicle solution or diosgenin (0.1 μmol/kg/day, p.o.) was administered to wild-type or 5XFAD (male, 24-27 weeks old) for 15 days. The diosgenin-administered 5XFAD mice showed significant improvement in object recognition memory. After the behavioral test, protein expressions in cortical lysates were compared on 2D-PAGE. We focused several proteins showing drastic changes in the expression level and analyzed those by MALDI-TOF/MS. Heat shock cognate 70 (HSC70) was identified as the protein decreased by diosgenin administration in 5XFAD. Next, cultured cortical neurons (ddY, E14) were treated by $A\beta_{25\text{-}35}$. $A\beta_{25\text{-}35}$ treatment for 3 days increased the expression level of HSC70 and decreased the axonal density. Post treatments by diosgenin (0.1, 1 μM) or a HSC70 inhibitor, VER-155008 (0.05, 0.5 and 5 μM) significantly decreased HSC70 expression and increased density of axons. Administration of VER-155008 (10 μmol/kg/day, i.p.) for 18 days improved object recognition memory in 5XFAD (female, 32-38 weeks old) similar to diosgenin. In these mice, amyloid plaques, PHF-tau and degenerated axons

were decreased in the brain. These results suggest that diosgenin-induced reduction of HSC70 may relate to axonal regrowth and memory recovery. We are now investigating specific functions of HSC70 in signaling for axonal growth and memory enhancement.

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Nanosymposium

482. Alzheimer's Disease: Therapeutics in Animal Models

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Presentation Number: 482.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CNPq

FAPERJ

INNT

HFSP

ISN

Title: Neuroprotective actions of physical exercise in mouse models of Alzheimer’s disease

Authors: ***M. V. LOURENCO**, R. L. FROZZA, D. BECKMAN, S. T. FERREIRA, F. G. DE FELICE;
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Abstract: Physical exercise has been long known for its mental and body health benefits. In the brain, exercise has been shown to activate several signalling pathways that contribute to neuronal health, such as those mediated by BDNF and PGC-1α, and to avoid harmful consequences initiated by occasional injuries. More recently, brain benefits of exercise have been shown in Alzheimer’s disease (AD) patients, but molecular hints underlying neuroprotective properties of physical activity in disease remain to be uncovered. Here, we aimed to understand whether exercise could counteract amyloid toxicity in animal models of AD and obtain novel molecular insights on how these effects could be triggered. Adult mice were subjected to a chronic swimming protocol and then intracerebroventricularly injected with Aβ oligomer preparations. Behavioral assays were performed and mouse hippocampi were analyzed by immunoblotting and ELISA. To obtain mechanistic insights into the role of exercise-derived factors, we used an in vitro model consisting of cultured hippocampal neurons exposed to amyloid-β (Aβ) oligomers,

recognized as central AD toxins, and the APP/PS1 mouse model of AD for biochemical analyses. We found that chronic swimming protects against Aβ oligomer-induced memory deficits. We further identified an exercise-derived factor that could mediate such neuroprotective properties in AD mice. Consistently, this factor appears to prevent synaptotoxic mechanisms and memory impairment in AD mice. Our results demonstrate that Aβ oligomer-induced memory impairment was prevented by physical activity, and offer novel hints into possible strategies of neuroprotection in AD. These observations could also provide novel grounds for prescribing exercise as a preventive or adjuvant approach against AD-related memory decline.

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Nanosymposium

482. Alzheimer's Disease: Therapeutics in Animal Models

Location: SDCC 33C

Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 482.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG051086

AG10133

ISDH13609

Title: Endothelium derived miR200b may prevent Alzheimer’s amyloid-β (Aβ) precursor protein (APP) and Vascular Endothelial Growth Factor (VEGF) in diabetes

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Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, synaptic loss, and proteinaceous deposits, specifically aggregated Aβ peptides in neuritic plaques and hyperphosphorylated microtubule-associated protein τ in neurofibrillary tangles. Aβ is produced by cleavage of the APP by β-site APP cleaving enzyme 1 (BACE1, the rate-limiting enzyme) and γ-secretase complex. AD also has deficiency of synaptic proteins, e.g., SNAP25. Vascular deterioration routinely co-occurs with AD, and impaired neural tissue perfusion contributes to neurodegeneration. Deficiencies in VEGF significantly contribute to impaired neural tissue perfusion. Diabetes also associates with cognitive decline and accelerated aging in all tissues. Diabetes induced tissue damage occurs via hyperglycemic

alteration of endothelial cell (EC) metabolism, which deranges gene expression to produce multiple dysfunctions, including protein *translational* misregulation. Such regulation is performed in part by microRNAs (miR/miRNA). miRNAs are ~20-25 nucleotide RNA molecules that serve as "recognition sockets" for RNA-induced silencing complex (RISC) modification of specific mRNA translation. A given miRNA provides target sequence specificity. APP and BACE1 are downregulated by specific miRNAs (Long et al., 2012; 2014). We show reduced expression normalized to U6 RNA, of specific miRNAs (miR146a and miR200b) in mouse hippocampus after streptozotocin induction of diabetes. We also examine hippocampal and brain cortical tissues from mice with endothelial specific overexpression of miR200b and miR146a (generated by us) with or without induced diabetes. Diabetes induction resulted in brain-region specific decrease of APP levels in hippocampus, decrease of VEGF levels in cortex, and increase of SNAP25 levels in cortex (and insignificant decrease in hippocampus), all in wild-type mice. Prior endothelial overexpression of miR200b prevented diabetes-induced changes for APP, VEGF and SNAP25. Endothelial overexpression of miR146a produced no preventative effects. Further, miR200b induced upregulation of BACE1 (insignificant change in Aβ) and downregulation of VEGF in hippocampus of diabetes-induced mice, while induction introduced no such protein level changes in wildtype mice. Hippocampal atrophy may be an early sign or precursor of AD. Our results suggest a potential prophylactic role for miR200b, as miRNA in our study were not delivered directly to the brain but were only expressed in ECs. Peripheral administration of miRNA mimics may be an effective early treatment for AD or pre-AD conditions. Supported by NIH grants to DKL.

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Nanosymposium

482. Alzheimer's Disease: Therapeutics in Animal Models

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Title: Dynamic assessment of tau antibodies in the brains of live animals by two-photon imaging

Authors: *Q. WU[1], Y. LIN[1], J. GU[1], E. SIGURDSSON[1,2];
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Abstract: Tau immunotherapies are a promising approach for Alzheimer's disease and related tauopathies. Our group published the first reports showing the effectiveness of active and passive tau immunizations in mouse models. These findings have now been confirmed and extended by several groups and a few clinical trials have already been initiated. However, the mechanisms of antibody-mediated clearance of tau aggregates are relatively unclear, although work by us and others has clarified to some extent in culture models various pathways that may be involved. Two monoclonal antibodies (mAbs) that we have generated against the P-Ser396, 404 tau region, 4E6 and 6B2, have markedly different properties. 4E6 is more effective in various culture, *ex vivo* and *in vivo* models in preventing/reducing tau pathology and associated cognitive impairments, whereas 6B2 or rather its smaller derivatives may be better suited as a diagnostic imaging marker. Here, using *in vivo* two-photon imaging, we investigated the dynamics of brain uptake and clearance of fluorescently tagged 4E6 and 6B2 in live transgenic tauopathy mice. Our preliminary results showed that both mAbs readily cross the blood brain barrier and co-localized with pathological tau marker 1-fluoro-2,5-bis (3-carboxy-4-hydroxystyryl) benzene (FSB) as assessed 4 days after femoral injection, with R=0.55 and R=0.64, respectively. Seven days post-injection, the signals from FSB, 4E6 and 6B2 had decreased to 69%, 60% and 62%, respectively, compared to 24 h after injection. Additional injections of FSB and/or antibodies are planned in the same animals to clarify if this decrease is due to degradation of probes and/or clearance of tau pathology. Viral expression of GFP in neurons at the imaging site allowed us to determine that the antibodies are primarily found within neurons (about 80%), which fits with our published ex vivo brain slice findings (Gu J et al JBC 288 2013). This type of approach provides valuable insight into the dynamics of uptake and clearance of mAbs in live animals, and may clarify their mechanism of action.

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Nanosymposium

482. Alzheimer's Disease: Therapeutics in Animal Models

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Title: Inhibition of astrocytic calcineurin/NFAT signaling in a mouse model of vascular cognitive impairment and dementia

Authors: *M. PLEISS, P. SOMPOL, I. ARTIUSHIN, S. KRANER, D. POWELL, V. BAKSHI, A.-L. LIN, P. NELSON, D. WILCOCK, C. NORRIS;
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Abstract: Astrocytes are one of the most abundant cell types in the brain, and play a vital role in maintaining healthy nervous tissue. Calcineurin (CN), a Ca^{2+}-sensitive protein phosphatase, appears at elevated levels in activated astrocytes associated with aging, injury, and disease. The signaling between CN and the transcription factor NFAT (Nuclear Factor of Activated T-cells) regulates several critical pathways in astrocytes, including those involved in excitotoxicity, inflammation, and neuronal death. Inhibition of the astrocytic CN/NFAT pathway in a mouse model of Alzheimer's disease was associated with reduced glial activation and improved synaptic and cognitive function. However, no studies that we know of have investigated the role of astrocytic CN/NFAT signaling in vascular cognitive impairment and dementia (VCID). Here, we used adeno-associated virus (AAV 2/5) vectors containing an astrocyte-specific promotor, Gfa2, and VIVIT, a potent NFAT inhibitor, to selectively inhibit astrocytic NFAT signaling in a diet-induced mouse model of VCID. AAV-treated mice were maintained on either control or methionine-enriched and folate-deficient diet for a minimum of 11 weeks to induce hyperhomocysteinemia (HHcy) associated with vascular pathology. HHcy diet was associated with a significant reduction in hippocampal synaptic strength and long-term potentiation (LTP). Both of these deficits were ameliorated by AAV-Gfa2-VIVIT, suggesting that astrocytic CN/NFAT signaling contributes to synaptic dysfunction during VCID. Other hallmarks of VCID, including cognitive decline, cerebral hypoperfusion, and metabolic disturbances are being assessed in a second cohort of HHcy mice using the radial-arm water maze (RAWM) task and MRI/MRS. Histochemical analyses are underway to characterize the relationship between the astrocytic CN/NFAT pathway and a variety of vascular abnormalities, including microhemorrhages and microinfarcts.

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Nanosymposium

482. Alzheimer's Disease: Therapeutics in Animal Models

Location: SDCC 33C

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Presentation Number: 482.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: A Grants-in-Aid for Scientific Research (C) (23500439) from the Ministry of Education, Culture, Sports, Science and Technology of Japan

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Title: Approach to identify anti-Alzheimer’s disease compounds that are delivered into the brain after oral administration of traditional medicine

Authors: *Z. YANG, T. KUBOYAMA, C. TOHDA;
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Abstract: Some traditional medicines and formulas could ameliorate memory deficits and pathological symptoms in Alzheimer’s disease (AD), but the active metabolite crossing BBB was rarely studied. Our recently study showed that Drynaria Rhizome (DR) water extract reversed Aβ (25-35)-induced axonal atrophy in cultured mouse cortical neurons, and restored the AD pathology and memory deficits in a mouse model of AD, 5XFAD. In this study, we aimed to identify active metabolites of DR in the brain, and clarify the mechanism for anti-AD effects of them.

After DR water extract was oral administered to 5XFAD mice for 5h, the brain cortex was dissected after perfusion with saline, then the cortex was homogenized and extracted with methanol, after centrifugation the supernatant was applied to LTQ-Orbitrap FT-MS/MS, three metabolites derived from DR in the mouse brain were detected and identified.

Since we consider axonal regeneration is critical for the fundamental therapies of AD, the metabolites were tested for evaluation of axonal regrowth activity. Two of them (naringenin and naringenin-7-*O*-glucuronide) significantly increased axonal density in cultured cortical neurons when administered for 4 days after Aβ (25-35) treatment. To explore effects of the metabolite *in vivo*, naringenin (5, 100 mg/kg) was orally administered for 19 days to 5XFAD mice (male, 8-12 months old). Object recognition memory (1-h interval time) was significantly improved by high dose of naringenin treatment. Immunohistochemistry of brain slices showed amyloid β plaques were significantly reduced in the perirhinal cortex and hippocampus. Abnormally swollen degenerated axonal terminals were also significantly decreased in the naringenin treated group. Combined with DARTS analysis, we investigated the starting point in the signaling axis of

naringenin. CRMP2 was identified as a candidate for direct target protein of naringenin. Naringenin reduced CRMP2 phosphorylation at Thr514, which was enhanced by Aβ treatment. Dephosphorylated CRMP2 is known to contribute to axonal growth.
Above results suggested that naringenin could penetrate BBB after oral administration of DR, and ameliorate memory deficits in 5XFAD mice, this might be related to modulation of CRMP2 state after binding with naringenin. Our study indicated that biochemical analysis coupled with pharmacological means could be a powerful method to search for lead compounds for AD and unravel their mechanism.

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Nanosymposium

482. Alzheimer's Disease: Therapeutics in Animal Models

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Title: PLGA nanoparticles loaded cerebrolysin attenuates CSF and brain levels of F-2 isoprostane and p-tau in Alzheimer's disease

Authors: ***G. TOSI**[1], A. SHARMA[2], B. RUOZI[3], D. BELLETTI[3], F. PEDERZOLI[3], M. A. VANDELLI[3], F. FORNI[3], A. NOZARI[4], J. V. LAFUENTE[5], D. F. MURESANU[6], H. MOESSLER[7], H. S. SHARMA[2];
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Sci., Univ. of Modena and Reggio Emilia, Modena, Italy; [4]Anesthesiol. & Critical Care Ctr., Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA; [5]Neurosciences, Univ. of Basque Country, Bilbao, Spain; [6]Clin. Neurosciences, Univ. of Med. & Pharm., Cluj-Napoca, Romania; [7]Drug Discovery & Develop., Ever Neuro Pharma, Oberburgau, Austria

Abstract: Alzheimer's Disease (AD) affecting millions of people every year Worldwide for which no suitable therapeutic measures are still available. Thus, exploration of novel therapeutic strategies to improve the functional parameters of the victims for a better healthcare and quality of life is urgently needed.

It appears that severe oxidative stress caused by a variety of internal factors and altered metabolism of enzymes could results in vascular, neuronal and glial damages in the brain leading to progression of AD brain pathology. Increased levels of lipid peroxidation and phosphorylation of tau (p-tau) protein occurs in the CSF and plasma of AD patients. Elevated levels of prostaglandin metabolite F-2 isoprostane (ISP) in CSF and plasma further confirm oxidative stress in AD. The levels of tau and ISP correlate well with the accumulation of amyloid beta protein (AβP) in AD brains. Thus, novel therapeutic strategies to reduce these elements may have potential neuroprotective effects in AD.

In AβP infusion model of AD in rats we showed that cerebrolysin- a multimodal drug when administered repeatedly (5 ml/kg, i.v.) 1 week after the onset of AβP infusion significantly reduced brain pathology, AβP deposition, breakdown of the blood-brain barrier and brain edema formation. In present investigation we examined the role of cerebrolysin on p-tau and ISP levels in CSF and brain of AD rats.

Intraventricularly administration of AβP (1-40) in the left cerebral lateral ventricle (250 ng/10 µl once daily) for 4 weeks resulted in AD like. In these rats, p-tau and ISP in the CSF collected from cisterna magna and brain tissues obtained from cerebral cortex, hippocampus and cerebellum was measured using commercial ELISA kit according to standard protocol.

Our results showed a 2-fold increase in p-tau in CSF and 3-to 4-fold in the cortex, hippocampus and cerebellum from control group (CSF 35±5 pg/ml, Brain 5±2 ng/g). Likewise, ISP was elevated 1.5 fold in CSF and 2-fold in the AD brain from control (CSF 45±6 pg/ml, Brain 0.56±0.03 pg/mg). Cerebrolysin (5 ml) administration significantly reduces the elevation of both p-tau and ISP in AD brain by 40 to 60 % and reduced brain pathology. Interestingly, PLGA nanoparticles labeled cerebrolysin resulted in reduction in p-tau and ISP levels by 80 to 90% and thwarted AD brain pathology. These results are the first to indicate that PLGA-labeled cerebrolysin has superior effects in reducing oxidative stress in AD and thereby inducing pronounced neuroprotection, not reported earlier.

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Nanosymposium

482. Alzheimer's Disease: Therapeutics in Animal Models

Location: SDCC 33C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Jazan University

Title: Cineole protects Alzheimer's type disease in rats

Authors: *F. ISLAM[1,2], M. M. SAFHI[2], A. KHAN[2];
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Abstract: Cineole, a terpenoid oxide present in many plant essential oils displays anti-inflammatory and strong anti-oxidative properties in animals. Increasing evidence demonstrates that oxidative stress and inflammation play an important role in pathogenesis of Alzheimer disease (AD). The present study was undertaken to evaluate the neuroprotective effect of cineole against sporadic dementia of Alzheimer type (SDAT)-induced neuronal damage. SDAT was induced by intracerebroventricular-streptozotocin (ICV-STZ) administration in the brain using double manipulator stereotaxic apparatus. Cineole was administered at (50, 100 and 200 mg/kg/day p.o.) for 7 days before surgery. The Morris Water maze test was performed from 16th to 20th day after the surgery to analyze the cognitive impairment in rats. Animals were sacrificed on 21st day from surgery and the brains were isolated to dissect the hippocampus and frontal cortex for the biochemical assays and whole brains were used for histopathological examination. Cineole protected the levels of TBARS and GSH as well as the activities of GPx, GR and catalase and cytokines in the lesion group pretreated with cineole. These results were supported by the immunohistochemical findings of NOS-2, COX-2, NF-κB and ChAT. Additionally, the histopathological observation of hippocampus revealed significant neural cell death in the hippocampus CA1 region after 21 days of post-surgery (49 % cell loss). Pretreatment with cineole attenuated the neuronal damage induced by ICV-STZ. The cineole significantly decreased the number of dead hippocampal neurons (30.06 % in cineole-treated). In conclusion, cineole is effective in protecting rats against ICV-STZ-induced damage in the rat hippocampus and frontal cortex. This spectacular protection makes cineole a promising agent in pathologies implicating neurodegeneration such as Alzheimer disease.

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Nanosymposium

482. Alzheimer's Disease: Therapeutics in Animal Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 EB003268

Title: Investigation of ultrasound mediated transient bbb opening in a natural canine model of ageing

Authors: *K. HYNYNEN[1], M. A. O'REILLY[2], R. JONES[2], E. BARRETT[3], E. HEAD[4]; [1]Med. Biophysics, Univ. of Toronto/ Sunnybrook Res. Inst., Toronto, ON, Canada; [2]Med. Biophysics, Univ. of Toronto, Toronto, ON, Canada; [3]Lovelace Resp. Res. Inst., Albuquerque, NM; [4]4Sanders-Brown Ctr. on Aging, Univ. of Kentucky, Lexington, KY

Abstract: Transient opening of the Blood-Brain Barrier (BBB) mediated by ultrasound and intravenously circulating microbubbles(MB), may have a role in the treatment of Alzheimer's disease (AD). Recent studies have shown that this approach can reduce Amyloid Beta plaque pathology and improve spatial memory in transgenic mouse models. Here we investigate these methods in a naturally occurring, large animal model of AD. Low frequency (0.28 MHz) ultrasound and MB were used to open the BBB unilaterally in aged beagle dogs (9-11 years, n=5) under MRI-guidance at 3T. The treatments were performed using an MRI-compatible 3-axis positioning system that mechanically moved the single-element, spherically focused transducer (focal spot size = 4.4 mm x 24.6 mm) around the treatment space. Sonications were performed interleaving exposures at 4 spots (2x2 grid with 4.5 mm spacing between targets). A wideband receiver captured the acoustic emissions during the treatments and a real-time control algorithm was used to analyze the frequency content of the detected signals and modulate the treatment pressures accordingly to stay within a safe yet effective exposure range. The sonications were repeated at new locations until full coverage of one hemisphere was achieved. MRI was used to assess the BBB integrity/tissue damage post treatment. Neurological testing was performed 24 hr post-treatment, and follow-up MR imaging was performed 1 week post treatment. The BBB was successfully opened in all animals. Neurological testing did not show any effects from the treatment. Follow-up MRI at 1 week post-treatment showed the BBB to be fully intact with no evidence of tissue damage. In the short term, whole-hemisphere opening of the BBB was well tolerated in the aged brain following a single treatment. Continuing studies will investigate the safety and impact of chronic treatments.

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Nanosymposium

483. Therapy of Parkinson's Disease: Alpha-Synuclein Target

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Presentation Number: 483.01

Topic: C.03. Parkinson's Disease

Support: Wellcome Trust: George K Tofaris 97479/Z/11/X

Title: Identification of Usp8 as a toxicity modifying deubiquitinase for alpha-synuclein

Authors: *Z. ALEXOPOULOU[1], J. LANG[1], R. PERRETT[1], H. T. KIM[3], A. GOLDBERG[3], O. ANSORGE[1], T. FULGA[2], G. TOFARIS[1];
[1]Nuffield Dept. of Clin. Neurosciences, [2]Weatherall Inst. of Mol. Med., Univ. of Oxford, Oxford, United Kingdom; [3]Harvard Univ., Cambridge, MA

Abstract: Objective: To study whether ubiquitination in Lewy bodies is directly relevant to α-synuclein turnover and Parkinson's pathogenesis.
Background: In Parkinson's disease, misfolded α-synuclein accumulates, often in a ubiquitinated form, in neuronal inclusions termed Lewy bodies. It is currently unknown whether ubiquitin conjugation in Lewy bodies is due to a defect in enzymes that regulate α-synuclein degradation. This is important as identification and pharmacological modulation of such enzymes could be targeted for therapies.
Methods: Comparative immunohistochemistry and immunoblotting between three brain regions was used to determine the type and abundance of ubiquitin conjugates as well as relevant interacting proteins in neurons with Lewy bodies (n=20 cases). The co-localisation of Usp8 and α-synuclein was investigated using bi-fluorescence complementation assays and in human iPSc-derived neurons. Transient overexpression and shRNA mediated knockdown of Usp8 was used in human cell lines to investigate the functional interaction between the two proteins. The deubiquitination of α-synuclein was investigated in cells and with purified proteins. Drosophila genetics were used to study the effect of Usp8 and other deubiquitinases against α-synuclein toxicity in vivo.
Results: By comparative analysis in human post-mortem brains, we found that ubiquitin immunoreactivity in Lewy bodies is largely due to K63-linked ubiquitin chains and markedly reduced in the substantia nigra compared to the neocortex. The ubiquitin staining in cells with Lewy bodies inversely correlated with the content and pathological localization of the deubiquitinase Usp8. Usp8 interacted and partly co-localized with α-synuclein in endosomal membranes and both in cells and after purification, it deubiquitinated K63-linked chains on α-synuclein. Knockdown of Usp8 in the Drosophila eye reduced α-synuclein levels and α-synuclein-induced eye toxicity. Accordingly, in human cells Usp8 knockdown decreased α-synuclein levels. In the dopaminergic neurons of the Drosophila model, unlike knockdown of

other deubiquitinases, Usp8 protected from α-synuclein-induced locomotor deficits and cell loss. **Conclusions:** These findings strongly suggest that removal of K63-linked ubiquitin chains on α-synuclein by Usp8 is a critical mechanism in slowing α-synuclein degradation in dopaminergic neurons that may contribute to α-synuclein accumulation in Lewy body disease.

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Nanosymposium

483. Therapy of Parkinson's Disease: Alpha-Synuclein Target

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Presentation Number: 483.02

Topic: C.03. Parkinson’s Disease

Support: DFG Center for Nanoscale Microscopy and Molecular Physiology of the Brain

Title: The mechanism of SIRT2-mediated protection against alpha-synuclein toxicity in models of Parkinson's disease

Authors: *T. F. OUTEIRO;
Univ. Med. Ctr. Goettingen, Goettingen, Germany

Abstract: Sirtuin genes have been known as as anti-aging genes, affecting multiple cellular pathways. Sirtuin 2 (SIRT2) was previously shown to modulate proteotoxicity associated with age-associated neurodegenerative disorders such as Alzheimer’s and Parkinson’s disease (PD). However, the precise molecular mechanisms involved were unclear. Here, we provide detailed mechanistic insight into the interplay between SIRT2 and alpha-synuclein (aSyn), the major component Lewy bodies that are pathognomonic protein inclusions in PD and other synucleinopathies. We found that aSyn is acetylated on lysine residues, and that these residues are deacetylated by SIRT2. Genetic manipulation of SIRT2 levels, in vitro and in vivo, alter the levels of acetylated aSyn, and modulate its aggregation in cell models of synucleinopathy. Strikingly, mutants mimicking or blocking acetylation modulate aSyn toxicity in rat primary cortical neurons and in vivo, in the substantia nigra of rats. Altogether, our study identifies aSyn acetylation as a key regulatory mechanism governing aSyn aggregation and toxicity, and provides mechanistic insight into the protective role of SIRT2 in cell and animal models of PD, demonstrating its therapeutic value.

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Nanosymposium

483. Therapy of Parkinson's Disease: Alpha-Synuclein Target

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Presentation Number: 483.03

Topic: C.03. Parkinson's Disease

Support: Amercian Parkinson's Disease Association

Michael J Fox Foundation LRRK2 LEAPS Award

National Institutes of Health Grants R01 NS064934

Title: G2019S-LRRK2 facilitates formation of alpha-synuclein inclusions

Authors: *L. A. VOLPICELLI-DALEY[1], H. ABDELMOTILIB[2], Z. LIU[2], L. STOYKA[2], H. ZHAO[3], W. HIRST[4], A. WEST[2];
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Abstract: Pathologic inclusions are a defining characteristic of α-synucleinopathies that include Parkinson disease (PD). The G2019S *LRRK2* mutation is the most common genetic cause of PD. This mutation increases LRRK2 kinase activity. We show that G2019S-LRRK2 expression increases the formation of fibril-induced recruitment of endogenous α-synuclein into inclusions in both cultured neurons and dopaminergic neurons in the rat substantia nigra. In contrast, expression of WT-LRRK2 in neurons reduces the formation of α-synuclein inclusions. Potent and selective LRRK2 kinase inhibitors and anti-sense oligonucleotides to α-synuclein or LRRK2 likewise reduce α-synuclein inclusion formation. G2019S-LRRK2 expression increases total levels of α-synuclein in neurons, while WT-LRRK2 expression decreases total levels of α-synuclein in neurons. Fluorescence recovery after photobleaching experiments reveal that G2019S-LRRK2 expression increases pools of mobile α-synuclein that persist after the formation of α-synuclein inclusions. We hypothesize that LRRK2 influences α-synuclein inclusion formation by altering mobile pool concentrations of α -synuclein. Furthermore, we find that expression of G2019S-LRRK2 alters association of both LRRK2 and α-synuclein to synaptic vesicle and endosome enriched fractions. These findings support the hypothesis that G2019S-LRRK2 may increase the progression of pathological α-synuclein inclusions after the initial formation of α-synuclein pathology.

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Nanosymposium

483. Therapy of Parkinson's Disease: Alpha-Synuclein Target

Location: SDCC 24A

Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 483.04

Topic: C.03. Parkinson's Disease

Title: Antisense oligonucleotides to lrrk2 ameliorate alpha-synuclein pathology and behavioral deficit induced by pre-formed alpha-synuclein fibrils

Authors: *H. T. TRAN[1], A. WEIHOFEN[2], K. M. IKEDA-LEE[1], E. SWAYZE[1], H. B. KORDASIEWICZ[1];
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Abstract: LRRK2 mutations are the major cause of familial late-onset PD. However, the interplay between LRRK2 and alpha-synuclein in PD pathophysiology is still of debate and undergoing extensive research. To determine whether LRRK2 expression modifies alpha-synuclein pathology, we lowered endogenous LRRK2 by antisense oligonucleotide (ASO) in mice injected with pre-formed fibrils (PFF) made of recombinant alpha-synuclein protein. Wildtype mice were injected intracerebroventrically (ICV) with LRRK2 ASOs 2 weeks before intra-striatal inoculation of alpha-synuclein PFF. At 8 weeks post ICV injection of LRRK2 ASOs, mice were assessed on the wirehang task for motor coordination and grip strength. LRRK2 mRNA, protein, and phosphorylated alpha-synuclein pathology were assessed by RT-QPCR, western blots, and immunohistochemical methods, respectively. We demonstrated that preventive ASO-mediated suppression of endogenous LRRK2 reduced pathological spread of alpha-synuclein pathology and protected mice against pathology-induced wirehang deficit in the PFF inoculation mouse model. Thus, LRRK2 may play an important role in alpha-synuclein pathology formation and progression. Therefore, ASO targeting LRRK2 is of potential therapeutic use for PD and other synucleinopathies.

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Employment/Salary (full or part-time): Biogen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biogen. **K.M. Ikeda-Lee:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals, Inc. **E. Swayze:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals, Inc. **H.B. Kordasiewicz:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals, Inc.

Nanosymposium

483. Therapy of Parkinson's Disease: Alpha-Synuclein Target

Location: SDCC 24A

Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 483.05

Topic: C.03. Parkinson’s Disease

Title: Snca targeted antisense oligonucleotides modulate progression of pathological deposition in alpha synuclein rodent transmission models of Parkinson’s disease

Authors: *T. COLE[1], K. PAUMIER[2], T. COLLIER[2], A. BOOMS[2], S. CELANO[2], E. SCHULZ[2], A. STRAUSS[2], B. DALEY[2], H. ZHAO[1], A. WEIHOFEN[3], H. KORDASIEWICZ[1], E. SWAYZE[1];
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Abstract: The objective was to investigate the effect of alpha synuclein antisense oligonucleotides on the spread and toxicity of a pathological form of alpha synuclein. Parkinson’s disease is a prevalent neurodegenerative disease for which there are no approved disease-modifying therapies. Alpha synuclein accumulation is a pathological hallmark of PD. Multiplication of the alpha synuclein gene, *SNCA*, alone can result in PD. Antisense oligonucleotides designed to target *Snca* RNA result in reduced production of alpha synuclein and thus have the potential to slow spread of pathology and modulate disease progression. Endogenous mouse and rat alpha synuclein transmission pre-formed fibril (PFF) inoculation models were evaluated. These models are based on intrastriatal injection of alpha synuclein fibrillar fibrils (PFFs) that lead to the deposition and aggregation of alpha synuclein, which accumulates and spreads through neural networks over time. Following characterization of

timing of fibril deposition in the models both pre (preventive) and post (therapeutic) fibril deposition paradigms were evaluated with central delivery (intracerebroventricular) of *Snca* targeting antisense oligonucleotides. Following study completion *Snca* RNA was evaluated by RT-PCR and phosphorylated alpha synuclein pathology was evaluated histologically. *Snca* targeting antisense oligonucleotides in both species led to *Snca* RNA target reduction and slowed deposition and spread of a phospho-specific form alpha synuclein. Antisense oligonucleotides designed against *SNCA* have the potential to be a disease modifying therapeutic for Parkinson's disease patients.

Disclosures: **T. Cole:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals. **K. Paumier:** A. Employment/Salary (full or part-time): Michigan State University. **T. Collier:** A. Employment/Salary (full or part-time): Michigan State University. **A. Booms:** A. Employment/Salary (full or part-time): Michigan State University. **S. Celano:** A. Employment/Salary (full or part-time): Michigan State University. **E. Schulz:** A. Employment/Salary (full or part-time): Michigan State University. **A. Strauss:** A. Employment/Salary (full or part-time): Michigan State University. **B. Daley:** A. Employment/Salary (full or part-time): Michigan State University. **H. Zhao:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals. **A. Weihofen:** A. Employment/Salary (full or part-time): Biogen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biogen. **H. Kordasiewicz:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals. **E. Swayze:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals.

Nanosymposium

483. Therapy of Parkinson's Disease: Alpha-Synuclein Target

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Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

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Topic: C.03. Parkinson's Disease

Support: Region Östergötland LIO-567661

Title: The effect of alpha-synuclein oligomer/protofibril selective antibodies on uptake and transfer of alpha-synuclein species in neuronal primary cells

Authors: *L. CIVITELLI[1], E. SEVERINSSON[2], J. BERGSTRÖM[3], E. NORDSTRÖM[4], F. ERIKSSON[4], L. LANNFELT[3], M. INGELSSON[3], M. HALLBECK[2];
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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the depletion of dopamine-producing cells within the substantia nigra pars compacta and the accumulation of amyloid aggregates composed mainly of the α-synuclein (α-syn) protein. Aggregation of α-syn promotes its pathological prion-like transfer, a process that involves a self-seeding mechanism, which may begin by the uptake of misfolded α-syn aggregates. Passive immunotherapy targeting aggregated species of amyloid proteins effectively decreased protein aggregation and neurodegeneration. Passive immunization with the monoclonal tau antibody, PHF1, lessened the levels of insoluble tau in tangle mice, and monoclonal antibody against α-syn reduced behavioral deficits in mice overexpressing α-syn. Previously, antibodies that selectively target soluble oligomer/protofibril α-syn species (oα-syn) were produced and characterized. Extracellular administration of such an antibody, mAb49, on a cell culture model successfully prevented the formation of oα-syn, whereas another antibody, mAb47, reduced the levels of soluble toxic oα-syn in the spinal cord of transgenic mice. In this study, three oα-syn selective antibodies were investigated for their ability to prevent the uptake and cell-to-cell transfer of exogenous oα-syn in rat primary cortical neurons. All three antibodies were able to significantly reduce cellular uptake of oα-syn. Interestingly, preliminary results from blocking the Fcγ II/III receptors suggested that the uptake of oα-syn may be blocked by the formation of α-syn complexes with mAbs before their internalization into the cells. Furthermore, addition of these mAbs to our donor-acceptor 3-D co-culture model system successfully reduced the cell-to-cell propagation of oα-syn among neuronal cells. Overall, these findings demonstrate that these antibodies, highly selective for oα-syn, play an important role in blocking the uptake of exogenous oα-syn as well as cell-to-cell transfer of soluble oα-syn oligomer/protofibril species, thus highlighting their potential use for immunotherapy in PD and other disorders with α-syn brain pathology.

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Nanosymposium

483. Therapy of Parkinson's Disease: Alpha-Synuclein Target

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Presentation Number: 483.07

Topic: C.03. Parkinson's Disease

Support: MJF Therapeutic Pipeline Program/ID 11742

NINDS/R37NS033123

Title: Accumulation of α-synuclein in SCA2 models

Authors: *D. P. HUYNH, W. DANSITHONG, S. PAUL, D. R. SCOLES, S. M. PULST; Dept Neurol, Univ. Utah, Salt Lake Cty, UT

Abstract: Accumulations of misfolded α-synuclein (α-syn) are neurotoxic and HADC inhibitors reversed the cytotoxic effects of increased α-syn levels in Parkinson disease (PD) models. This suggests that reduction of α-syn expression likely protects DA neurons in PD patients. Parkinsonism phenotypes were observed in a sub-population of expanded polyglutamine (polyQ) diseases including SCA2, but there was no association of polyQ expansion in the ATXN2 gene mutated in SCA2 with a large cohort of idiopathic PD. We investigated α-syn processing in SCA2 cell and animal models to determine if PD pathogenesis mediated by α-syn is modified by polyQ expanded ATXN2. **Methods:** Western blots, RT-PCR, and RNA sequencing were employed to examine the effect of ATXN2 polyQ expansion in SH-SY5Y cells, human SCA2 lymphoblastoid cells, and SCA2 mice. An α-syn-luciferase (luc) reporter cell line (Luc6B) was created by direct knock-in of a luc cDNA in-frame with the last codon of the SNCA gene by ZFN methods. **Results:** RNA sequencing of SCA2[Q127] transgenic mouse models showed moderate elevation of α-syn mRNA levels. SH-SY5Y transfected with flag-tagged ATXN2 elevated α-syn levels causing aggregation of α-synuclein. Co-transfection of α-syn-luc and ATXN2 expression plasmids in SH-SY5Y cells increased α-syn-luc expression, as did transfection of ATXN2 plasmids to the stably expressed α-syn-luc Luc6B cell line. Overexpression of polyQ expanded ATXN2 caused susceptibility of cells to rotenone cytotoxicity. Western blotting and quantitative RT-PCR used to investigate BAC-ATXN2[Q72] and Pcp2-ATXN2[Q127] transgenic mouse models showed high levels of α-synuclein in the cerebellum. SCA2 lymphoblastoid cell lines which endogenously expressed expanded polyQ ATXN2 showed slight increase of α-synuclein levels. Analyses of the Luc6B cell line showed that Luc6B cells produced full-length α-syn-luc fusion protein. Valproate and bafilomycin A1 treatments of Luc6B cells increased the levels of α-syn-luc mRNA, endogenous α-synuclein, and α-syn-luc fusion proteins. A high Z-score of 0.75 suggested that the Luc6B cell line is highly suitable for high-throughput screening (HTS) for compounds lowering α-syn expression. **Conclusions:** Our observations that α-synuclein levels and aggregations in SCA2 models were

similar to previous observations in HD and SCA3 models. We conclude that expanded polyQ proteins alter cellular posttranslational processing and that expanded polyQ proteins may act as agents of α-synuclein aggregation. Alternatively, α-synuclein accumulations may catalyze the aggregation of expanded polyQ protein causing cytotoxicity in polyQ protein models and SCA2 patients.

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Nanosymposium

483. Therapy of Parkinson's Disease: Alpha-Synuclein Target

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Title: PARIS (ZNF746) is an important pathologic mediator of α-synuclein-induced neurodegeneration.

Authors: *P. GE[1], S. BRAHMACHARI[2], C. YUAN[2], S. S. KARUPPAGOUNDER[2], H. JIANG[2], Y. LEE[3], H. KO[2], V. L. DAWSON[2], T. M. DAWSON[2];
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Abstract: The heterogeneity of Parkinson's disease (PD) pathogenesis has made the development of effective neuroprotective therapies challenging. As such, identifying pathologic mediators that play a crucial role in both the familial and sporadic disease is essential for developing effective neuroprotective therapies. Recent work has demonstrated that accumulation of parkin substrate PARIS (ZNF746) is necessary for neurodegeneration following parkin inactivation. Here, we demonstrate that accumulation of PARIS (ZNF746) is also critical for neurodegeneration in multiple mouse models of PD and α-synucleinopathies. We show that overexpression of human (h)A53T α-synuclein (α-syn) leads to parkin inactivation, and a corresponding accumulation of parkin substrates AIMP2 and PARIS. PGC-1α, a target of PARIS

transcriptional repression and promoter of mitochondrial biogenesis, is likewise downregulated. We further find that PARIS-KO substantially reduces accumulation of aggregated and phosphorylated α-syn, ameliorates motor deficits, and restores average lifespan by 50 percent in hA53T α-syn overexpressing mice. Finally, we demonstrate PARIS-KO protects against dopaminergic cell death in the SNpc caused by virally-induced overexpression of hA53T α-syn or by striatal injections of exogenous α-syn preformed fibrils. Because α-syn pathology characterizes the vast majority of sporadic and a substantial portion of familial PD, these data indicate that PARIS accumulation could be central disease process in a majority of PD cases and could prove to be an attractive therapeutic target.

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Nanosymposium

483. Therapy of Parkinson's Disease: Alpha-Synuclein Target

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Presentation Number: 483.09

Topic: C.03. Parkinson's Disease

Title: A characterization of the onset, progression, and reversibility of morphological changes in mouse lung following pharmacological inhibition of LRRK2 kinase activity

Authors: *D. K. BRYCE[1], C. M. WARE[1], J. D. WOODHOUSE[2], L. G. HEGDE[2], S. KURUVILLA[2], M. L. MADDESS[2], C. G. MARKGRAF[2], K. M. OTTE[2], A. PATEL[2], F. M. POULET[2], J. M. ELLIS[2], M. E. KENNEDY[1], M. J. FELL[1];
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Abstract: Reducing LRRK2 kinase activity via pharmacological inhibition has been postulated as a treatment for Parkinson's Disease (PD); however, morphological changes in lungs of non-human primates following treatment with LRRK2 kinase inhibitors have brought the safety of this mechanism into question. Chronic treatment with the highly potent and selective LRRK2 kinase inhibitor MLi-2, dosed to mice via formulated chow, induces similar pulmonary changes and provides a rodent model by which we can study the long-term effects of LRRK2 kinase inhibition. Here we demonstrate that MLi-2 induces morphological changes in mouse lung within one week following dosing in-diet or PO. Pulmonary changes develop only when systemic MLi-2 exposure is maintained at ≥10x the in vivo IC_{50} for LRRK2 kinase inhibition, as measured by pSer935 dephosphorylation. Immunohistochemical and histological analysis of lung sections from affected mice reveal a significant increase in surfactant protein C staining and morphology consistent with enlarged type II pneumocytes. With MLi-2 treatment continued up

to 6 months, these pulmonary changes progress only modestly and are fully and rapidly reversed upon removal of MLi-2 from diet. Surfactant levels in bronchoalveolar lavage fluid could serve as a translatable biomarker for LRRK2 inhibitor effects in lung. Surfactant protein D levels were unchanged with MLi-2 treatment. These data describe a model for the study LRRK2 inhibitor-induced changes in lung, allowing for elucidation of safety concerns surrounding LRRK2 kinase inhibitors in PD.

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Nanosymposium

483. Therapy of Parkinson's Disease: Alpha-Synuclein Target

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Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 483.10

Topic: C.03. Parkinson’s Disease

Title: Pathogenic hlrrk2 r1441g mutation abolishes the maturation of d2-autoreceptor response in substantia nigra dopamine neurons

Authors: Q. QIN[1,2], L. ZHI[1], L. CHEN[3], G. LI[2], *H. ZHANG[1];
[1]Neurosci., Dept. of Neurosci., Philadelphia, PA; [2]The First Affiliated Hosp. of Harbin Med. Univ., Harbin, China; [3]Fudan Univ., Shanghai, China

Abstract: Substantia nigra pars compacta (SNc) dopamine (DA) neurons are particularly prone to degenerate in Parkinson disease (PD). Emerging evidence suggests that synaptic dysfunction is an early event in the pathogenesis of PD occurring prior to the onset of symptoms. In order to develop more effective therapeutic strategies, we need a better understanding of the underlying mechanisms of synaptic dysfunction of PD. Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene are the most prevalent causes of familial and sporadic PD, demonstrating an unprecedented significant role in PD pathogenesis. Recently a transgenic (TG) mouse model with over-expression of human LRRK2-R1441G has been shown to recapitulate the robust motor behavioral, neurochemical and pathological features of PD (*Li et al., 2009*). In this study, we used patch-clamp and pharmacological tools to examine the SNc DA neuron intrinsic firing properties and somatodendritic D2 autoreceptor response (D2-AR) in acute midbrain slices from postnatal day 14 (P14), 1, 3, 5 and 10 month old R1441G-LRRK2 TG mice and their wild type (WT) littermates. We found that whereas D2-AR-mediated inhibition of SNc DA neuron pacemaker activity is pronounced during maturation in WT mice, the maturation of the D2-AR response is absent in the LRRK2-R1441G TG mice. SNc DA neurons from adult (= > 3 months)

WT mice displayed pronounced sensitized D2-AR responses depending on Ca^{2+} and neuronal calcium sensor NCS-1/D2-AR interactions. NCS-1 is crucial for the D2-AR sensitization in both WT and LRRK2 R1441G TG mice. Using laser microdissection and RT-qPCR, we found that NCS-1 is decreased in SNc DA neurons from adult R1441G TG mice compared to WT littermates. Therefore, the pronounced desensitization of D2-AR in R1441G TG mice may lead to excitatory toxicity, which may result in the neurite degeneration in TG mice during aging. Our results provide novel insights into the impact of pathogenic LRRK2 mutations on DA neurons and reveal novel mechanisms underlying the development of PD.

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Nanosymposium

483. Therapy of Parkinson's Disease: Alpha-Synuclein Target

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Focused Ultrasound Foundation

Kavli Institute

Kinetics Foundation

Title: Neurorestoration of the nigrostriatal pathway through focused ultrasound facilitated drug delivery

Authors: *M. KARAKATSANI, G. SAMIOTAKI, S. WANG, T. KUGELMAN, C. ACOSTA, E. KONOFAGOU;
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Abstract: Current Central Nervous System (CNS) drug delivery techniques are confined to either targeted but invasive or to non-targeted and non-invasive methods. Focused Ultrasound (FUS) coupled with the systemic administration of microbubbles has been proven to open the Blood Brain Barrier (BBB) locally, transiently and non-invasively. IV delivery of the neurotrophic factor Neurturin following BBB opening has been demonstrated to activate the downstream signaling pathway. The aim of the current study is to investigate and compare the

neurorestorative effect of single and triple delivery sessions of Neurturin in a Parkinsonian mouse model. For this study, wild type mice were infused with sub-acute dosages of MPTP causing apoptotic degeneration in the nigrostriatal pathway. The treatment groups were sonicated on the left hemisphere targeting twice the Caudate Putamen region (CPu) and once the Sabstantia Nigra region (SN) while one received an IV injection of 0.5mg Neurturin. The survival period after the last treatment, lasted for 28 days allowing the neurotrophic factor to develop its restorative effects while coronal sectioning for tissue processing followed. Brain sections of both the SN and the CPu were stained for tyrosine hydroxylase positive cells (TH^+) with a custom protocol. The stained slices were imaged to count the TH^+ nerve cell bodies on the SN while the dendritic and terminal areas were quantified by a custom MATLAB algorithm by computing the percentage of the relative difference (RD) between the two hemispheres. There was no significant difference in the number of neurons between the two hemispheres. This result was in accordance with our knowledge of Neurturin restoring impaired neuronal cells and not regenerating them. The RD was found to be significantly higher for both single and triple treatment-treated group at the SN but only for the triple treatment-treated group at the CPu region. These findings indicate the potential of multiple treatments on the reversal of the Parkinsonian phenotype with significance reported in both the dendritic (SN) and terminal (CPu) site.

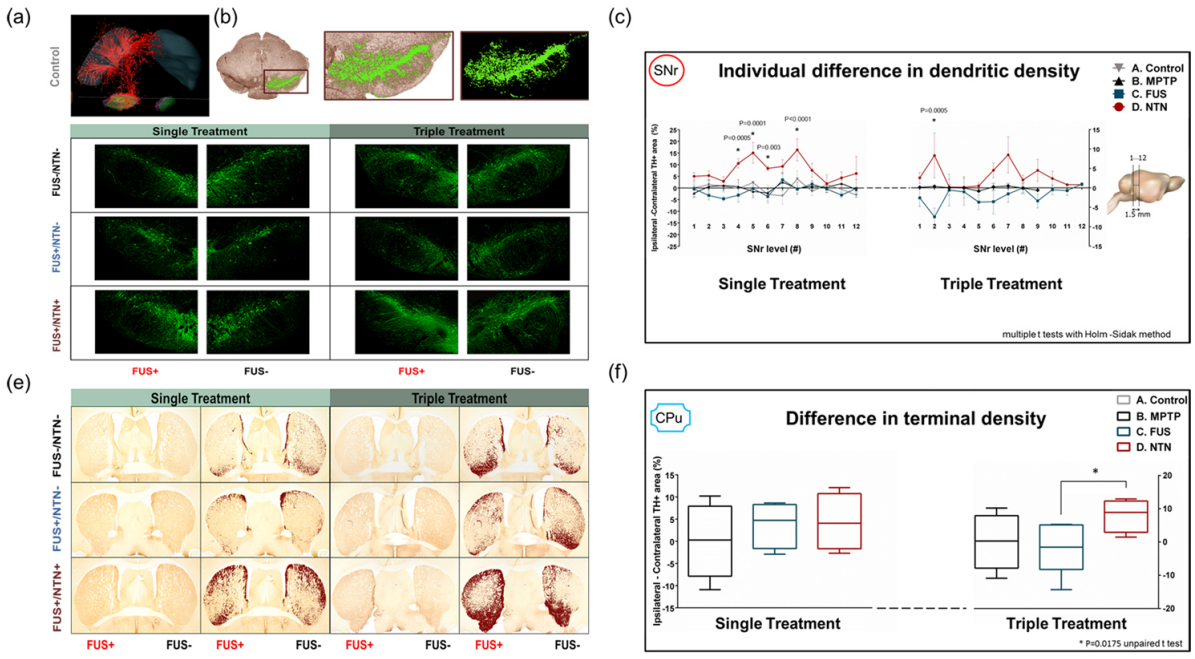

Figure 1: Substantia Nigra: a) Atlas 3D representation of the nigrostriatal pathway and the involved structures, Substantia Nigra (SN) and Caudoputamen (CPu). (b) Coronal section at a cross-section of the SN in increasing magnification. (c) Fluorescent images of both hemispheres at the SN region for every group and each treatment regime, single and triple. (d) Quantification of the dendritic density for both treatments regimes. Caudoputamen: (e) Coronal sections of the Cpu region for each group and treatment regime. The first column shows the raw data while the second the pixels that surpass the threshold in TH+ staining. (f) Quantification of the terminal density of each group.

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Nanosymposium

483. Therapy of Parkinson's Disease: Alpha-Synuclein Target

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Title: Common variant near SNCA gene predicts motor phenotype, rate of disease progression, and SNCA brain expression in Parkinson's disease

Authors: *N. JAIN[1], C. A. COOPER[1], M. D. GALLAGHER[1], D. WEINTRAUB[2], S. X. XIE[3], Y. BERLYAND[5], A. J. ESPAY[6], J. QUINN[7], K. L. EDWARDS[8], T. MONTINE[9], V. M. VAN DEERLIN[4], J. TROJANOWSKI[4], C. P. ZABETIAN[10], A. S. CHEN-PLOTKIN[1]; [1]Neurol., [2]Psychiatry, [3]Biostatistics and Epidemiology, [4]Pathology and Lab. Med., Univ. of Pennsylvania, Philadelphia, PA; [5]Harvard Med. Sch., Boston, MA; [6]Dept. of Neurol., Univ. of Cincinnati, Cincinnati, OH; [7]Dept. of Neurol., Oregon Hlth. and Sci. Univ., Portland, OR; [8]Dept. of Epidemiology, Univ. of California Irvine, Irvine, CA; [9]Dept. of Pathology, [10]VA Puget Sound Hlth. Care Syst. and Dept. of Neurol., Univ. of Washington, Seattle, WA

Abstract: Parkinson's disease (PD) presents clinically with several motor subtypes that exhibit a variable treatment response and prognosis. The neurobiological underpinnings of these PD subtypes remain undetermined. In the present study, we investigated 10 SNPs, previously associated with PD risk, for association with tremor-dominant (TD) vs. postural-instability gait disorder (PIGD) motor subtypes. SNPs that correlated with the TD/PIGD ratio in a discovery cohort of 251 PD patients were then evaluated in a multi-site replication cohort of 559 PD patients. SNPs associated with motor phenotype in both cross-sectional cohorts were next evaluated for association with (1) rates of motor progression in 230 PD patients followed longitudinally for up to 7 years and (2) brain (cerebellum, substantia nigra, caudate, and frontal cortex) alpha-synuclein (SNCA) expression in the GTEx (Genotype-Tissue Expression project)

consortium database. We found that genotype at rs356182, near SNCA, correlated with the TD/PIGD ratio in both the discovery (Bonferroni-corrected p=0.04) and replication cohorts (p=0.02). The rs356182 GG genotype was associated with a more tremor-predominant phenotype and predicted a slower rate of motor progression (1-point difference in annual rate of UPDRS-III motor score change, p=0.01). Finally, the rs356182 genotype was associated with SNCA expression in the cerebellum (p=0.005). Our study thus demonstrates that the GG genotype at the rs356182 SNP 3' to the SNCA gene provides molecular definition for a clinically-important endophenotype associated with (1) more tremor-predominant motor phenomenology (2) slower rates of motor progression, and (3) decreased brain expression of SNCA. Such molecularly-defined endophenotyping in PD may benefit both clinical trial design and tailoring of clinical care as we enter the era of precision medicine.

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Nanosymposium

483. Therapy of Parkinson's Disease: Alpha-Synuclein Target

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Title: TRIM28 regulates the nuclear accumulation and toxicity of both alpha-synuclein and tau

Authors: *M. W. ROUSSEAUX[1], M. DE HARO[1], C. A. LASAGNA-REEVES[1], A. DE MAIO[1], J. PARK[2], P. JAFAR-NEJAD[1], I. AL-RAMAHI[1], A. SHARMA[1], L. SEE[1], N. LU[1], L. VILANOVA VELEZ[1], T. F. WESTBROOK[1], R. KAYED[3], J. TRONCOSO[4], J. BOTAS[1], H. Y. ZOGHBI[1,5];
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Abstract: A number of neurodegenerative diseases are driven by the toxic gain-of-function of specific proteins within the brain. Elevated levels of alpha-synuclein (α-Syn, *SNCA*) appear to drive neurotoxicity in Parkinson's Disease (PD) and Parkinson's Disease Dementia (PDD); cases of SNCA multiplication show a dose-response relationship between wild-type α-Syn levels and phenotype. Similarly, neuronal accumulation of the microtubule-associated protein tau (*MAPT*) is a hallmark of Alzheimer's disease (AD), and tau overexpression causes neurodegeneration in model organisms. Despite the clinical differences between AD and PD, several lines of evidence suggest that α-Syn and tau overlap in pathogenic states. These multiple connections between α-Syn and tau led us to ask whether the two proteins might be regulated through a shared pathway whose dysfunction leads to pathogenesis. We therefore screened for genes that affect post-translational levels of both α-Syn and tau. We found that TRIM28 regulates α-Syn and tau levels in cells and *in vivo* and that reduction of TRIM28 rescues toxicity in *Drosophila* and mouse models of tau- and α-Syn-mediated degeneration. Conversely, overexpression of TRIM28 exacerbates α-Syn and tau pathology. TRIM28 increases the overall bioavailability of both proteins and promotes their nuclear localization, accumulation, and toxicity through its E3 ligase function; the nuclear localization of α-Syn and tau contributes to their pathogenicity. Lastly, TRIM28 is found to be aberrantly localized in human cases of synucleinopathy and tauopathy and this correlates with nuclear localization of α-Syn and tau in human brains. Taken together, intersecting screens across comorbid proteinopathies can reveal shared mechanisms, exemplified here with the discovery of TRIM28-driven toxicity of nuclear α-Syn and tau. Similar strategies across additional proteinopathies may point the way toward therapeutic entry points.

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Nanosymposium

484. Traumatic Brain Injury: Models, Mechanisms, and Deficits

Location: SDCC 32B

Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 484.01

Topic: C.09. Brain Injury and Trauma

Title: Exploring gender differences in female vs male rodents following exposure to a single mild blast

Authors: *L. KAWA, M. ANGERIA, U. ARBORELIUS, T. HÖKFELT, M. RISLING; Karolinska Institutet, Stockholm, Sweden

Abstract: Traumatic brain injury (TBI) is a heterogeneous disease, with the global burden shifting from death to years lost as a result of living with a disability; tens of millions of people battle with physical and /or mental disabilities as a result of suffering a TBI. Mood/anxiety-disorders is one the most prevalent complaints post-TBI, particularly post-exposure to a blast wave, which results in a unique form of TBI termed blast TBI. There is substantial literature that indicates there may be gender differences in the systems involved in emotional regulation. But pre-clinical research has largely focused on using either male or female rodents to address this active area of research, and there has been little comparison of data from both sexes. Previous studies in our laboratory using male rodents have revealed compelling changes in the noradrenaline, serotonin, and the neuropeptide galanin systems following exposure to a single blast. In this study we aimed to replicate these findings in female rodents. Similar to the male studies, we do not observe evidence of white matter injury, cell death, or blood brain barrier breakdown post-exposure to blast-induced TBI. Brain sections from the regions of interest processed for in situ hybridisation to investigate the transcript levels of the rate limiting enzymes tyrosine hydroxylase (TH) and tryptophan hydroxylase two (TPH2), reveal similar increased levels in the acute time points post-exposure (1 d). But in the case of TPH2, it is more pronounced, extensive, and remains elevated even at 7 d post-exposure in the females, but not the males. We see similar levels of Progesterone and Estradiol across the female animals, possibly eliminating a hormone induced effect on differences across sham and exposed groups. We are still processing data on the galanin system, the enzyme cyclooxygenase-2 (COX-2), and several biomarkers. Our preliminary data suggests post-exposure to a blast-induced TBI, in the absence of visible structural damage to the brain, the same salient players in emotional regulation are activated, but this may be more prolonged and more extensive in the females vs males. These studies demonstrate the value and need in considering gender, when studying the underlying mechanisms of TBI.

Disclosures: L. Kawa: None. **M. Angeria:** None. **U. Arborelius:** None. **T. Hökfelt:** None. **M. Risling:** None.

Nanosymposium

484. Traumatic Brain Injury: Models, Mechanisms, and Deficits

Location: SDCC 32B

Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 484.02

Topic: C.09. Brain Injury and Trauma

Support: NIH/NIA “Institute on Aging" (IOA) Training Grant (T32) #5T32AG00213

Veterans Administration Hospital MERIT Review AWARD # 1I21RX001371

Title: hCG treatment reverses hypogonadism induced by a penetrating controlled cortical impact injury in adult male rats

Authors: *R. I. GEDDES[1], K. HAYASHI[1], A. KAPOOR[2], Q. BONGERS[1], M. M. WEHBER[1], I. M. ANDERSON[1], A. D. JANSEN[1], C. NIER[1], G. FARQUHAR[1], E. FARES[1], A. ABDULSALAM[1], S. VADDAKADATH-MEETHAL[1], T. E. ZIEGLER[2], C. S. ATWOOD[1,3,4]; [1]Med., [2]Assay Services Unit and Inst. for Clin. and Translational Res. Core Laboratory, Natl., Univ. of Wisconsin at Madison, Madison, WI; [3]Geriatric Research, Educ. and Clin. Ctr., Veterans Admin. Hosp., Madison, WI; [4]Sch. of Exercise, Biomed. and Hlth. Sci., Edith Cowan Univ., Joondalup, Australia

Abstract: Traumatic brain injury (TBI) has been demonstrated to induce hypogonadotropic hypogonadism in a significant percentage (36-87%) of patients. Aging-related endocrine dyscrasia, like that in TBI, is associated with decreased neurogenesis and cognitive function in humans. To test if TBI induces hypogonadism in rats, 5-month old adult male Sprague Dawley rats were subjected to a bilateral moderate-to-severe controlled cortical impact (CCI) injury (Coordinates A/P = +2.5 mm from Bregma (b); M/L = 0.0 mm from midline; D/V = -3.0 to -3.5 mm from brain surface; impactor size = 5 mm; velocity = 2.25 m/s; dwell = 100 ms) to the medial prefrontal cortex. The concentration of circulating testosterone (T) and progesterone (P_4) concentrations in CCI animals (CCI-vehicle: 1.7 ± 0.6 ng/mL and 3.4 ± 1.0 ng/mL, respectively) was markedly decreased compared with pre-injury circulating concentrations (4.3 ± 1.2 ng/mL and 10.1 ± 5.2 ng/mL, $p < 0.01$; respectively). Since human chorionic gonadotropin (hCG) and its adult homolog, luteinizing hormone, can reverse hypogonadism and have neurogenic properties, we treated CCI animals with hCG (400 IU/kg/2 days) over 28 d. hCG treatment reversed the hypogonadism in these animals (10.0 ± 2.8 ng/mL and 12.0 ± 4.4 ng/mL, respectively for T and P_4), indicating that CCI-rats have the capacity to produce sex steroids at normal reproductive levels. hCG-treated CCI animals also displayed significantly smaller cystic infarcts and improvements in vestibulomotor performance and learning and memory as compared with saline-treated CCI controls. Taken together, our data demonstrate the presence of post-traumatic (hypogonadotropic) hypogonadism in our CCI model that is reversible with hCG

treatment. We are continuing to explore whether the restoration of the reproductive hormone axis is responsible for the cognitive and motor benefits of this treatment.

Disclosures: **R.I. Geddes:** None. **K. Hayashi:** None. **A. Kapoor:** None. **Q. Bongers:** None. **M.M. Wehber:** None. **I.M. Anderson:** None. **A.D. Jansen:** None. **C. Nier:** None. **G. Farquhar:** None. **E. Fares:** None. **A. Abdulsalam:** None. **S. Vaddakadath-Meethal:** None. **T.E. Ziegler:** None. **C.S. Atwood:** None.

Nanosymposium

484. Traumatic Brain Injury: Models, Mechanisms, and Deficits

Location: SDCC 32B

Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 484.03

Topic: C.09. Brain Injury and Trauma

Support: CDMRP W81XWH-13-2-0019

CDRMRP W81XWH-13-2-0018

Title: Identification of abnormalities in the ferret brain following mild brain injury using voxelwise diffusion MRI microimaging

Authors: ***E. B. HUTCHINSON**[1], S. C. SCHWERIN[2], M. E. KOMLOSH[1], K. L. RADOMSKI[2], M. O. IRFANOGLU[1], S. L. JULIANO[2], C. M. PIERPAOLI[1];
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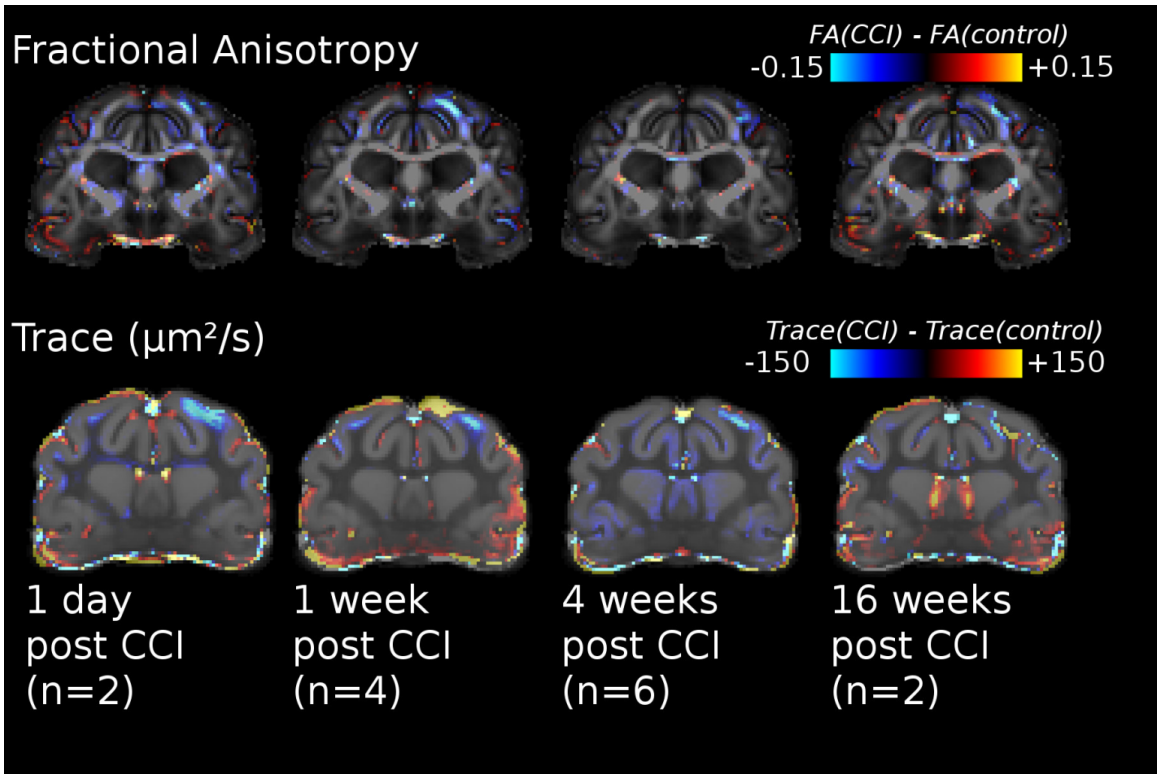
Abstract: Introduction. The combination of high-resolution, quantitative MRI methods with a human-similar experimental model of brain injury is a promising approach to identify and spatially map abnormalities that accompany traumatic brain injury (TBI). We have employed a mild TBI model in the ferret, which has a gyrencephalic cortex and relatively high white matter volume, to examine the ability of diffusion tensor imaging (DTI) to non-invasively detect abnormalities at various times following injury. Voxelwise statistical comparisons of high-resolution ex-vivo DTI maps showed several compelling patterns of brain abnormalities according to time after injury.
Methods. Perfusion fixed brain specimens were taken from 8 uninjured ferrets and 14 ferrets following mild controlled cortical impact for the following times after injury: 1d(n=2), 1w(n=4), 4w(n=6) and 16w(n=2). For each brain, 297 high-resolution (250 μm^2 voxels) DWIs were acquired with b=100-10,000 s/mm^2. The TORTOISE pipeline was used for image corrections and DTI model fitting. Next, diffusion tensor images were warped into a common space using the DRTAMAS registration algorithm. Voxelwise difference maps were generated for Trace and

FA to compare groups and individual brains with different time after injury to the control group. Results. Voxelwise group difference maps for TR and FA examining the effect of time after injury are shown in the included figure. FA was reduced in the white matter near the injury with the greatest change at 1 week after CCI and persistence of this finding out to 16 weeks. TR was decreased in cortex and white matter regions most robustly at one day post-CCI. At 16 weeks TR was increased in the white matter.
Conclusions. The imaging approach shown here provides a high-resolution, whole brain, quantitative and bias-free analysis method for determining anatomical and microstructural abnormalities following TBI. In this study, this approach has enabled the identification of spatial and temporal features of post-traumatic brain changes in a ferret TBI model.



Disclosures: **E.B. Hutchinson:** None. **S.C. Schwerin:** None. **M.E. Komlosh:** None. **K.L. Radomski:** None. **M.O. Irfanoglu:** None. **S.L. Juliano:** None. **C.M. Pierpaoli:** None.

Nanosymposium

484. Traumatic Brain Injury: Models, Mechanisms, and Deficits

Location: SDCC 32B

Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 484.04

Topic: C.09. Brain Injury and Trauma

Support: Children's Hospital Foundation Research Grant

Title: Acute pathological progression of axonal injury and neuroinflammation following diffuse moderate brain injury in the pediatric rat.

Authors: *N. MILLER FERGUSON[1], A. LAFRENAYE[2];
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Abstract: Pediatric traumatic brain injury (TBI) is a leading cause of mortality and morbidity in children under 17 years of age with a significant impact on children 2 years of age and under compared to older children. While elegant studies have explored pathology following contusive brain injury, very little is known regarding the progression of pathologies following diffuse TBI during early development and the majority of clinical interventions are based on studies done in adults. To begin addressing this gap in knowledge we developed a model of moderate (2.05±0.15 atm) diffuse traumatic brain injury using the central fluid percussion injury model in postnatal day (PND) 17 (± 1 day) male and female rats. This model induced diffuse brain injury via a fluid pressure pulse transduced through a hub affixed to a 3.0mm craniotomy located between bregma and lambda along the sagittal suture. To evaluate the extent of axonal injury following pediatric diffuse TBI tissue was labeled with amyloid precursor protein (APP), which accumulates at sites of axonal transport disruption. Cellular morphological alterations as well as increased Iba-1 expression were used to evaluate acute microglial activation over the first 24h following diffuse TBI. Pediatric rats sustaining moderate diffuse TBI exhibited pronounced post-injury apnea lasting for minutes following injury, requiring CPR, however, following regained breathing animals recovered well with normal systemic physiology within 15min post-injury. While substantial sub-arachnoid hemorrhage was apparent, very little gross pathology was observed. Microscopic assessment of axonal injury showed diffuse axonal injury (DAI) within the subcortical white matter, thalamus and, to a lesser extent, the lateral cortex. This DAI peaked at 6h post-injury and was drastically reduced by 12h post-TBI. Neuroinflammation was also readily apparent within the above domains, with striking activation observed at 1d post diffuse-TBI in both regions with DAI as well as areas within discernable DAI throughout the acute temporal window. This work represents the initial characterization of a novel model of diffuse TBI in a pediatric rat correlating to a 2 year old, and could prove invaluable for the development of therapeutics for young children with TBI.

Disclosures: **N. Miller Ferguson:** None. **A. Lafrenaye:** None.

Nanosymposium

484. Traumatic Brain Injury: Models, Mechanisms, and Deficits

Location: SDCC 32B

Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 484.05

Topic: C.09. Brain Injury and Trauma

Title: Sulfonylurea receptor 1-Transient receptor potential melastatin 4 (Sur1-Trpm4) expression is not associated with cell death following diffuse moderate traumatic brain injury in the rat

Authors: *A. D. LAFRENAYE;
VCU, Richmond, VA

Abstract: Traumatic brain injury (TBI) is a highly prevalent disease that has significant personal, societal and financial costs. Although progress has been made in understanding the complex pathobiological consequences of contusive TBI, questions still remain regarding the diffuse responses to injury. Recently expression of the sulfonylurea receptor 1- transient receptor potential melastatin 4 (Sur1-Trpm4) channel has been linked to cell death during hemorrhagic contusion expansion, however, nothing is known about the expression patterns of the Sur1-Trpm4 channel following diffuse TBI, in which hemorrhagic contusions do not form. To explore the expression pattern of Sur1-Trpm4 following diffuse TBI, adult male Sprauge Dawley rats were subjected to a moderate (2.05±0.05atm) central fluid percussion injury and survived for time points ranging from 3h to 8w. Double immunolabeling of the Trpm4 and Sur1 components of the Sur1-Trpm4 channel verified coexpression of the two components throughout the temporal window. For subsequent assessments the Trpm4 component of the Sur1-Trpm4 channel alone was used. At 3h post-injury, Trpm4 labeling within stellate cells was seen within the white matter of the hippocampal fissure. By 1w following diffuse TBI cells expressing Trpm4 encompassed the entire hippocampus, fimbra and aspects of the subcortical white mater and lateral neocortex, however, was limited in other brain regions. Diffuse Trpm4 expression within the hippocampus was observed as chronically as 8w following moderate diffuse TBI. To investigate the cell type expressing Trpm4 following diffuse TBI immunohistochemistry against the common astrocyte marker, GFAP, and the common microglial marker, Iba-1, was done. Trpm4 was found to be exclusively express by astrocytes following diffuse TBI. Correlative assessment of cell damage/death via Nissl stain demonstrated little hippocampal or subcortical white matter cell loss suggesting that expression of Sur1-Trpm4 by hippocampal astrocytes does not precipitate cell death. This indicates that astrocyte expression of Sur1-Trpm4 following diffuse brain injury might be involved in a different and potentially adaptive response, not seen in models producing expanding hemorrhagic contusion.

Disclosures: A.D. Lafrenaye: None.

Nanosymposium

484. Traumatic Brain Injury: Models, Mechanisms, and Deficits

Location: SDCC 32B

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Presentation Number: 484.06

Topic: C.09. Brain Injury and Trauma

Support: Academy of Finland

COST Action ECMNET [BM1001]

ERA-NET Neuron

Doctoral Program in Molecular Medicine

Epilepsy Foundation

Finnish Epilepsy Research Foundation

Title: Pdgfr-beta expression in blood-brain barrier-related cells after traumatic brain injury

Authors: *J. KYYRIÄINEN, X. E. NDODE-EKANE, A. PITKÄNEN;
Neurobio., A. I. Virtanen Inst., Kuopio, Finland

Abstract: Platelet-derived growth factor receptor β (PDGFRβ) is a tyrosine-kinase receptor expressed in brain by parenchymal cells and vascular cells such as pericytes and endothelial cells. Reports suggest that PDGFRβ is involved in post-injury tissue remodeling by regulating cell proliferation and migration. Recent data demonstrate that PDGFRβ deficiency results in leaky blood-brain barrier (BBB) and compromised scar formation. Our objective was to (1) elucidate the spatio-temporal evolution of PDGFRβ expression in the cortex and thalamus, and (2) to determine whether PDGFRβ-expressing cells are associated with areas of scar formation in the cortex after traumatic brain injury (TBI). TBI was induced in rats using the lateral fluid-percussion injury in adult rats which were sacrificed at 2 d, 7 d and 3 months after injury by transcardiac perfusion of 4% paraformaldehyde. The brains were cut into 30 μm coronal sections, and immunohistochemically stained using antibodies raised against PDGFRβ. Some sections were double or triple immuno-labeled for PDGFRβ and GFAP (astrocyte marker) or NG2 or RECA-1 (endothelial cell marker). In controls, PDGFRβ+ parenchymal cells were distributed homogenously throughout the cortex and thalamus. Double-labeling showed that PDGFRβ was expressed in all NG2 cells and in 40-60% of astrocytes. PDGFRβ expression was highest at 2 d post-TBI, and then reduced progressively over the next 3 months. PDGFRβ+ cells clustered in the perilesional cortex and thalamus, particularly during the first recovery week. Like in controls, PDGFRβ expression was detected in parenchymal NG2 cells, and 40-60% of astrocytes. Additionally, PDGFRβ expression was observed in perivascular pericytes, detached

fusiform pericytes and endothelial cells. In particular, PDGFRβ-expressing blood vessels were detected around the cortical glial scar for up to 3 months after injury. Our data indicate an acute accumulation of different types of PDGFRβ+ cells in injured tissue, suggesting the role of PDGFRβ in post-injury blood vessel and tissue recovery.

Disclosures: J. Kyyriäinen: None. **X.E. Ndode-Ekane:** None. **A. Pitkänen:** None.

Nanosymposium

484. Traumatic Brain Injury: Models, Mechanisms, and Deficits

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Presentation Number: 484.07

Topic: C.09. Brain Injury and Trauma

Support: CIHR MOP 60788

Alzheimer Society Research Program

UBC Four Year Fellowship

Comissao Technica de Atribuicao de Bolsas para Estudos Pos-Graduados Macao

Title: CHIMERA (Closed-head impact model of engineered rotational acceleration) traumatic brain injury exacerbates neuropathology in APP/PS1 mice

Authors: *W. CHENG[1], K. MARTENS[1], N. DHANANJAY[1], K. MCINNES[2], A. WILKINSON[1], C. HUANG[1], T. WARD-ABLE[1], P. CRIPTON[2], C. WELLINGTON[1]; [1]Dept. of Pathology and Lab. Med., [2]Departments of Mechanical Engin. and Orthopaedics, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Background/objectives:
The annual incidence of traumatic brain injury (TBI) is over 3.5 million in North America. Severe and moderate TBI can increase risk of Alzheimer's disease (AD), and repetitive exposure to mild TBI, which comprises over 75% of all TBI cases, can lead to long-term neurodegenerative diseases with AD-like neuropathologies. This study aims to study how repetitive mild TBI (rmTBI) induced by the CHIMERA (Closed-Head Impact Model of Engineered Rotational Acceleration) model exacerbates neuropathology in an APP/PS1 model of amyloidosis. It further investigates how age at injury affects the injury outcomes.
Methods:
CHIMERA is an experimental rodent TBI model recently developed by our laboratory. It is an impact-acceleration model that induces reproducible closed-head TBI without constraint of head

movement. Male APP/PS1 mice at either 5-mo or 13-mo were subjected to 2 mild TBI spaced 24hr apart. Age-matched wildtype (WT) littermates served as genotype controls. Mice that experienced isoflurane and body restraint but no head impact served as sham-injury control. Mice were sacrificed at 6 hr to 14d after sham or TBI injury. Behavioral analysis includes loss of righting reflex (LRR), neurological severity score (NSS), accelerating rotarod (RR), and Barnes maze. Histological and biochemical assays included amyloid-beta (6E10), fibrillary amyloid (thioflavin S), axonal (silver and neurofilament SMI312), and microglial (Iba1) changes.
Results:
Compared to sham and independent of APP/PS1 or WT genotype, rmTBI prolongs loss of consciousness (LRR), increases neurological deficits (NSS) and impairs motor performance (RR). Ageing and injury synergistically exacerbate motor deficits. Current data show that after rmTBI, at Day2 post-injury, there is a transient increase of 6E10+ve amyloid-beta deposits in 5-mo APP/PS1 mice but not in 13-mo APP/PS1 mice. Interestingly, in 13-mo APP/PS1 mice, there is a decrease of ThioS+ve fibrillary amyloid at Day2 and Day7, suggesting a structural remodelling of fibrillary amyloid. Iba-1 staining indicates microglial activation at white matter areas including the optic tract, brachium of superior colliculus, and olfactory bulb, from 6h to Day7, in both APP/PS1 and WT mice. Argyrophilic fibres and SMI312+ve neurofilament axonal bulbs are observed at the same white matter areas, indicating diffuse axonal injury. Blood brain barrier integrity is being assessed by serum protein extravasation.
Conclusions:
Clinically around 40% of CTE brains show amyloid deposits, and the mechanisms by which TBI is involved not yet clear. Our results suggest that age may be a factor in amyloid-beta neuropathology after rmTBI.

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Nanosymposium

484. Traumatic Brain Injury: Models, Mechanisms, and Deficits

Location: SDCC 32B

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Presentation Number: 484.08

Topic: C.09. Brain Injury and Trauma

Support: OU College of Pharmacy Seed Grant to HOA

Partial funding provided by National Institutes of Health, National Institute of General Medical Sciences (Grant 1U54GM104938), an IDeA-CTR to the University of Oklahoma Health Sciences Center

Title: Earlier recovery from vestibulomotor deficits compared to neurological deficits and increased pain sensitivity in rats with a mild traumatic brain injury

Authors: *H. O. AWWAD[1,5], A. C. EDWARDS[2], M. P. JOHNSTON[6], M. R. LERNER[3], S. H. ABDELKADER[4], S. CHEN[4];
[1]Pharmaceut. Sciences, Col. of Pharm., Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK; [2]Pharmaceut. Sciences, Col. of Pharm., [3]Surgery, [4]Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma city, OK; [5]Oklahoma Ctr. for Neurosci., University of Oklahoma Health Sciences Center, OK; [6]Oklahoma Sch. of Sci. and Mathematics, Oklahoma city, OK

Abstract: Traumatic Brain Injury (TBI) is the leading cause of disability and death in young Americans (< 40 yrs old). Mild TBI (mTBI) patients experience transient neurological and behavioral deficits and increased pain sensitivity following injury, some symptoms persist up to 1 year. mTBI consists of more than 80-90 % of TBI cases, yet its underlying mechanisms are still not completely understood. Our specific aim was to characterize the temporal profile for neurological and behavioral deficits as well as increased pain sensitivity induced by mTBI in Sprague Dawley male rats compared to sham and naïve rats. mTBI rats underwent stereotaxic surgery involving a craniotomy and a controlled impact to the left cortex at fixed coordinates. Sham rats received a craniotomy only and naïve rats received neither. Modified neurological severity score tests indicated that mTBI rats received a mild injury score of 3.56 ± 0.59 (n=8) on day 1 and 2.40 ± 0.62 (n=5) on day 8 post-injury. mTBI rats showed a significant reduction in vestibulomotor function on the rotarod during the first 4 days post-injury and returned to levels similar to naïve and sham rats by day 7 post-injury. mTBI rats displayed ~25% reduction in sensorimotor function on days 1 and 8 post-injury as assessed by the modified sticky tape test. Tactile allodynia and thermal hyperalgesia were demonstrated by reduced paw withdrawal pressures and latencies respectively. mTBI-induced pain sensitivity appeared as early as day 3 post-injury and was maintained through day 7 post-injury. Brain tissue histology at day 1 and day 8 post-injury indicate injury in the sensorimotor cortex, hippocampus and thalamus. In conclusion, mTBI-induced vestibulomotor deficits were transient and rats showed complete recovery within 7 days of injury, whereas the same animals did not recover from mTBI-induced neurological deficits, tactile allodynia and thermal hyperalgesia during that same post-injury period.

Disclosures: H.O. Awwad: None. **A.C. Edwards:** None. **M.P. Johnston:** None. **M.R. Lerner:** None. **S.H. Abdelkader:** None. **S. Chen:** None.

Nanosymposium

484. Traumatic Brain Injury: Models, Mechanisms, and Deficits

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Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 484.09

Topic: C.09. Brain Injury and Trauma

Support: Ruth D & Ken M Davee Pediatric Neurocritical Care Program

Title: Thrombin induces down-regulation of glast and glt-1 and contributes to depression after traumatic brain injury Via par-1

Authors: *C. PIAO[1], A. HOLLOWAY[2], S. HONG[2], M. S. WAINWRIGHT[2];
[1]Ann & Robert H. Lurie Children's Hosp. of Chica, Chicago, IL; [2]Ann & Robert H. Lurie Children's Hosp., Chicago, IL

Abstract: Background Depression is a major chronic complication after traumatic brain injury (TBI) but the mechanism by which TBI causes depression is not known. Human autopsy and preclinical studies implicate loss of astrocyte glutamate transporters and dysregulation of glutamate signaling in the mechanisms of depression. We have previously shown in primary astrocytes that thrombin causes a decrease in glutamate transporter via activation of Rho-kinase. Here we tested the hypothesis that TBI activates thrombin, resulting in down-regulation of glutamate transporters which contributes to the depressive-like behaviors after TBI. **Methods** We used a mouse closed skull TBI model, and measured the activation of thrombin, expression of glutamate transporters and the depressive-like behaviors at 1 month after TBI. To determine whether prevention of GLT-1 downregulation can ameliorate the depression after TBI, we treated mice with ceftriaxone after TBI. To elucidate whether thrombin/PAR-1 activation results in down-regulation of glutamate transporter , we administered SCH 79797, a selective PAR-1 antagonist, after TBI, and evaluated the expression of GLAST and GLT-1. **Results** Following TBI there was a significant decrease in cortex and hippocampal GLAST and GLT-1 levels at 1-month recovery. Depressive behavior measured by tail suspension, forced swim, and sucrose preference tests were also increased compared to sham controls. Thrombin was activated after TBI, and inhibition of PAR-1 by SCH79797 ameliorated the down-regulation of GLAST and GLT-1 in TBI mice compared to controls. Treatment with ceftriaxone attenuated the depressive behaviors in mice subjected to TBI. **Conclusions** These data suggest that thrombin contributes to the down-regulation of hippocampal and cortical astrocyte transporters caused by TBI. This response is mediated by the PAR-1 receptor. These results also provide indirect evidence linking decrease in glutamate transporter expression to depression, a major cause of morbidity following moderate and severe TBI.

Disclosures: C. Piao: None. **A. Holloway:** None. **S. Hong:** None. **M.S. Wainwright:** None.

Nanosymposium

484. Traumatic Brain Injury: Models, Mechanisms, and Deficits

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Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 484.10

Topic: C.09. Brain Injury and Trauma

Support: CBIR15IRG022

Title: Electrophysiological patterns of the cerebellum injury with subdural recordings

Authors: *G. ORDEK[1,2], M. SKOTAK[3], N. CHANDRA[3], M. SAHIN[2];
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Abstract: Oscillatory activity in certain rhythms that can describe the behavioral state of an animal can be a potential marker of brain injury in a well-defined neural morphology, such as that of the cerebellum. Brain injuries that result from trauma have long been investigated, where the imminent deficits in the brain structure or the individual's behavior were detected by conventional clinical measures such as magnetic resonance imaging (MRI) or computed tomography (CT). Recent reports argued that initial effects of most brain injuries, particularly the mild cases, can be too subtle to detect during diagnosis. In this preliminary work, we developed a highly sensitive electrophysiological measure to monitor progression of mild injuries in rats that were exposed to a single shock wave. Our approach utilized electrocorticography (ECoG) in the rats implanted with multi-electrode arrays (MEAs), which allowed the cerebellar signals to be monitored during an on-going state of injury. The cerebellum, a major brain site in motor related functions, has been viewed as a secondary brain region affected in the TBI context. However, recent reports from both human and animal studies showed that the cerebellum is highly susceptible to brain insults. In fact, prolonged cell deaths and micro lesions in the blood-brain barrier (BBB) were found even at mild levels of injury. We assessed the cerebellar injury progress by characterizing the cerebellar local field potentials (LFPs) containing sensory evoked potentials (EPs) and the cerebellar oscillations in the anesthetized as well as in awake animals. The EPs that contained the mossy and climbing fiber responses showed almost 3 fold decline in amplitude at the end of the survival period. Interestingly, we found critical changes on days 1 and 3 post-injury where signals were deteriorated by 62% and 38%, respectively, compared to the pre-injury measurements. We also performed the spectral analysis of LFP oscillations for commonly defined frequency bands; theta (5-8 Hz), beta (12-20), low gamma (30-80 Hz) and high gamma (80-150 Hz). While oscillations in the theta band showed an increased mean power (about +5dB) and inter-channel correlation, high gamma oscillations indicated significant drops in the mean power (about -8dB) as well as in correlation between the ECoG channels. These

results demonstrate the potential utility of continuously monitoring the electrophysiological response as a tool to pinpoint acute and delayed phases of the post-injury period.

Disclosures: **G. Ordek:** None. **M. Skotak:** None. **N. Chandra:** None. **M. Sahin:** None.

Nanosymposium

484. Traumatic Brain Injury: Models, Mechanisms, and Deficits

Location: SDCC 32B

Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 484.11

Topic: C.09. Brain Injury and Trauma

Title: Intranasal administration of low-dose paclitaxel is neuroprotective for cognitive deficits after repeat concussive brain injury in mice

Authors: ***M. M. CLINE**[1], B. NEEL[1], C. CROSS[2], G. G. GARWIN[3], J. YUMUL[1], D. MURRA[1], L. HYSA[1], E. BRIM[1], S. MINOSHIMA[3], D. J. CROSS[3];
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Abstract: Introduction: Therapeutics for the clinical management of traumatic brain injury (TBI) are currently under investigation. We have previously shown both direct application and IP administration of the microtubule-stabilizing drug *paclitaxel* following open-skull controlled cortical impact (CCI) improves spatial cognition, sensorimotor function, and reduces injury volume and edema on brain MR imaging in mice. The aim of the current study was to extend these findings to a more clinically relevant model of concussive injury (repetitive closed skull CCI). Additionally, as *paclitaxel* does not readily cross an intact BBB, the drug was administered via the intranasal route.
Methods: Mice (C57BL6 male, 10 wks) received a mild closed-skull CCI (CS-CCI) injury (n=15) or sham surgery (n=8, anesthesia, but no impact)) once per day for five consecutive days (5mm impact tip, 1mm depth, 5 m/s velocity, 100 msec dwell time; Impact One, Leica Biosystems). Low-dose intranasal *paclitaxel* (0.6 mg/kg; n=6) or saline (n=9) was administered two hours following the first CS-CCI, but not on subsequent days. Sensorimotor function was evaluated using Gridwalk testing at 6-7 days post-CCI, and again at 28 days. Radial water tread (RWT) maze was used to test short- and long-term spatial cognition at 10 days post-CCI. Finally, whole brain glucose metabolism was assed via SUV corrected FDG-PET, with 60 min uptake of 250 μCi FDG delivered ip, 32-41 days post-CCI.
Results: Saline treated CS-CCI mice exhibited significant short-term memory deficits compared to both *paclitaxel* and sham mice (79.03±58.2 vs 17.22±17.0s and 14.28±16.4s: $p<0.05$), and significant long-term memory deficits compared to shams (96.26±54.6s vs 30.79±30.1, $p<0.05$).

Paclitaxel treated mice did not differ significantly from shams on any of the RWT maze testing days. FDG-PET revealed a 42% increase in whole glucose brain metabolism in mice treated with *paclitaxel* compared to saline (130.6±23.3 vs. 91.8±18.8, $p<0.01$).
Conclusions: Clinical use of systemically delivered *paclitaxel* for TBI is limited, because reduced BBB permeability requires higher doses that may have adverse side effects. Our results suggest that intranasal administration of *paclitaxel* following a single closed skull CCI can be neuroprotective for subsequent concussive impacts that impair cognitive function in mice. These findings were supported by in vivo imaging of increased metabolic function. The results from this study may significantly impact the development of new therapeutic options in the treatment of TBI.

Disclosures: **M.M. Cline:** None. **B. Neel:** None. **C. Cross:** None. **G.G. Garwin:** None. **J. Yumul:** None. **D. Murra:** None. **L. Hysa:** None. **E. Brim:** None. **S. Minoshima:** None. **D.J. Cross:** None.

Nanosymposium

484. Traumatic Brain Injury: Models, Mechanisms, and Deficits

Location: SDCC 32B

Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 484.12

Topic: C.09. Brain Injury and Trauma

Support: BranchOut Neurological Foundation

Alberta Children's Hospital Research Institute

Alberta Children's Hospital

Title: Chronic alterations in mitochondrial dynamics in a rat model of pediatric mild traumatic brain injury.

Authors: *E. FRAUNBERGER[1], T. SHUTT[2], M. J. ESSER[3];
[2]Med. Genet., [3]Pediatrics, [1]Univ. of Calgary, Calgary, AB, Canada

Abstract: Mild traumatic brain injury (mTBI) and concussion have become major health concerns with extensive public awareness, particularly with respect to long term consequences in children. While most children recover completely after a mTBI, 10-15% will have symptoms beyond 3 months and 5-10% may have symptoms beyond one year. Currently, there are no effective treatments for pediatric mTBI, nor are there any reliable prognostic biomarkers to indicate susceptibility to long-term impairment. This may be related to an incomplete understanding of the pathophysiological mechanisms underlying individual differences in

recovery from mTBI. In particular, there is substantial evidence to suggest that mitochondrial dysfunction contributes significantly to the prolonged disability after mTBI, yet the specifics of this hypothesis are poorly understood. Further, while acute cerebral metabolic changes and mitochondrial fragmentation have been demonstrated in an adult TBI model, little is known about the mitochondrial network in pediatric mTBI. Therefore, our project sought to characterize the relationship between post-mTBI behavioural impairments and chronic alterations in mitochondrial dynamics and function. Based on our preliminary studies, we hypothesize that impaired outcomes in an animal model of mTBI will coincide with measures of altered mitochondrial form and function.
In the present study, we used a pediatric modified weight drop rat model with a mTBI at postnatal day 30 (P30). Behavioural assessments including beam walk, open field, elevated plus maze, novel context mismatch, and forced swim were conducted between P31 and P50 followed by sacrifice for mitochondrial preparation. Corroborating previous results, rats that experienced a mTBI had changes in measures of affect, motor function, and short-term memory. On a molecular level, mitochondrial respiration, measured using a Seahorse XF24 analyzer, was impaired in mTBI rats three-weeks following the injury. In addition, the expression of key regulators of mitochondrial dynamics (Mfn2, Drp1, OPA1) was depressed in mTBI rats at three weeks post-injury. Collectively, our preliminary studies suggest chronic impairments in behaviour, as well as mitochondrial dynamics and function, persist following a mTBI in the developing brain. Further molecular and behavioural analyses are underway to better elucidate this relationship and to determine the presence of sex differences in behavioural and molecular outcomes.

Disclosures: **E. Fraunberger:** None. **T. Shutt:** None. **M.J. Esser:** None.

Nanosymposium

484. Traumatic Brain Injury: Models, Mechanisms, and Deficits

Location: SDCC 32B

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Presentation Number: 484.13

Topic: C.09. Brain Injury and Trauma

Support: Science Foundation Arizona

American Sleep Medicine Foundation

NIH F31NS090921

Title: Post-traumatic sleep as a personalized physiological biomarker of vulnerability to repetitive traumatic brain injury.

Authors: *R. ROWE[1,2,3], J. L. HARRISON[4], J. LIFSHITZ[2];
[1]Child Hlth., Univ. of Arizona Col. of Med. Phoenix, Phoenix, AZ; [2]Barrow Neurolog. Inst. at Phoenix Children's Hosp., Phoenix, AZ; [3]Veteran Affairs Healthcare Syst., Phoenix, AZ; [4]Interdisciplinary Grad. Program in Neurosci., Arizona State Univ., Tempe, AZ

Abstract: Chronic neurological impairments can manifest from repetitive traumatic brain injury (rTBI), particularly when subsequent injuries occur before the previous one has completely healed. For sport-related TBI, return-to-play guidelines are trending towards a personalized approach, rather than a time-dependent prescription, based on symptomology. The opportunity exists to define quantitative metrics - biomarkers - that represent risk for worse outcomes in the event of rTBI. We have pioneered research on post-traumatic sleep identifying injury-induced sleep lasting 6hrs in brain-injured mice as a bio-indicator of unregulated inflammation. Here, we apply post-traumatic sleep as a physiological biomarker of vulnerability to hypothesize that a second TBI occurring during the period of post-traumatic sleep worsens functional and histological outcome compared to mice receiving one TBI or a second TBI after post-traumatic sleep resolves. Mice were subjected to sham or midline fluid percussion injury (FPI). Brain-injured mice received one FPI, or two FPIs (3hr or 9hr interval). As expected, FPI groups showed significantly more post-traumatic sleep (non-invasive monitoring cages) over 24hrs. For mice with rTBIs within 3hrs, functional assessments showed significantly lower latencies on rotarod and increased Neurological Severity Scores. Anxiety-like behaviors in the open field task were significantly increased for mice with rTBIs at 3hrs. Neuropathology in mice from both rTBI groups was significantly greater at 28 days post-injury (DPI) compared to sham and single-FPI, measured by pixel density of silver accumulation. Neuroinflammation in mice receiving rTBI at 3hrs was significantly increased at 7, 14, and 28DPI compared to sham, as measured by Iba-1 positive, activated microglia morphology distributions. Orexin-positive neurons regulate wakefulness and contribute to chronic sleep disturbances after TBI. Here, orexin-A positive cells in the lateral hypothalamus did not differ across groups, indicating loss of wake-promoting neurons did not contribute to post-traumatic sleep. Thus, post-traumatic sleep served as a physiological biomarker defining a period of vulnerability to a second TBI. Monitoring individual post-traumatic sleep is a potential clinical tool for personalized TBI management, where regular sleep patterns may inform rehabilitative strategies and return-to-play guidelines.
Support: Science-Foundation-Arizona, Halle Foundation

Disclosures: **R. Rowe:** None. **J.L. Harrison:** None. **J. Lifshitz:** None.

Nanosymposium

485. Visual Cognition: Decision Making

Location: SDCC 4

Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 485.01

Topic: D.06. Vision

Support: NEI Intramural Research Program

Title: Context-dependent changes of visual detection induced by optogenetic stimulation in the mouse striatum

Authors: *L. WANG, R. J. KRAUZLIS;
NIH, Natl. Eye Inst., Bethesda, MD

Abstract: The circuits for visual decision-making include not only sensory cortex, but also action selection mechanisms through the basal ganglia. We hypothesize that the striatum, the input nucleus to the basal ganglia, plays a crucial role in identifying the sensory and behavioral context associated with valued actions. This hypothesis predicts that artificial activation of striatal neurons will bias visual detection, and that this effect will depend on the particular behavioral context.

We optogenetically activated specific neuronal populations in the striatum of mice trained to report changes in the orientation of drifting visual gratings embedded against noise background. Head-fixed mice ran on a styrofoam wheel and the drift rate of two bilateral vertically oriented Gabor gratings depended on running speed. On each trial, after mice traveled a randomized distance, one of the two gratings changed its orientation. The task of the mice was to lick the spout if an orientation change happened and to otherwise withhold from licking. We used *Drd1a-Cre* and *A2a-Cre* mice and virus (*AAV2-DIO-ChR2*) to target direct- and indirect-pathway medium spiny neurons (MSNs) in the striatum. Brief optogenetic stimulation (150ms) was delivered unilaterally at the onset of the orientation change in a subset of trials. We calculated detection sensitivity and response criterion with and without optogenetic stimulation. Our data show that stimulating *direct* MSNs caused an equal reduction (~1.0) in sensitivity for detecting visual changes either ipsilateral or contralateral to the site of stimulation, but a much larger reduction in response criterion for the contralateral (~1.0) than the ipsilateral side (~0.5). In contrast, stimulating *indirect* MSNs caused a similar reduction (~1.0) of sensitivity for both sides, and a smaller but equal reduction in criterion (~0.5) for both sides. In addition, these effects of optogenetic stimulation were specific to the visual task. In a randomized subset of stimulation trials, we turned off the Gabor patches and noise background, while keeping the timing and overall illumination the same. In both genotypes, the probability of licking evoked by optogenetic stimulation in these “no visual stimuli” control trials was much lower than that during the catch trials with visual stimuli present ($p<0.001$, Chi-square test).

In summary, we found that: 1) activation of the striatal direct but not the indirect pathway causes a spatially biased shift in decision criterion during a visual detection task, and 2) these changes in decision criterion depend on the behavioral context. Our results provide insights on the function of the striatum in visual decision-making.

Disclosures: L. Wang: None. **R.J. Krauzlis:** None.

Nanosymposium

485. Visual Cognition: Decision Making

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Topic: D.06. Vision

Support: Wellcome Trust PhD Studentship Grant 527813

Wellcome Trust Grant 095668

Wellcome Trust Grant 095669

Title: Widefield imaging of sensory cortices during visual and audio-visual behaviour in mice

Authors: *E. A. JACOBS, M. OKUN, N. A. STEINMETZ, C. P. BURGESS, D. SHIMAOKA, M. CARANDINI, K. D. HARRIS;
UCL, London, United Kingdom

Abstract: Different behavioral states are associated with different brain states, and there is substantial interest in knowing how these brain states vary during sensory tasks and affect sensory processing.
To examine how brain states might correlate with performance of a sensory task, we performed widefield imaging of genetically encoded calcium indicators in behaving mice. We expressed GCamp6f selectively in cortical excitatory neurons using a triple-transgenic strategy (Madisen et al, *Neuron*, 2015), and imaged their activity unilaterally across visual, auditory and posterior somatosensory cortices. We trained mice to perform head-fixed two-alternative forced choice tasks (Burgess et al, *bioRxiv*, 2016) using visual stimuli that were either presented alone or together with task-irrelevant auditory pips. Trials were separated by >4s intervals, during which we collected baseline measurements. After stimulus onset mice had a 2-3 s window to turn a steering wheel to provide a response and obtained a water reward for a correct choice.
We assessed brain state from the patterns of spontaneous cortical activity during the baseline period prior to each trial. Brain state differed between trials when the mouse subsequently made a left or right choice (here referred to as Go-trials) and trials when the animal missed responding before the time-out (No-Go trials). Go trials were preceded by a decrease in low frequency power across visual, auditory, and somatosensory cortices in both visual and audio-visual tasks, indicating a globally desynchronized state. Low frequency power was also negatively correlated with the amount of pre-stimulus movement, as assessed by deflections in the steering wheel. Stimulus responses differed during Go and No-Go trials, but also showed more variability than could be explained by subsequent Go or No-Go responses alone.
These results suggest that changes in brain state can be assessed through widefield imaging of calcium indicators. Furthermore, task-related brain states appear to manifest globally and be

related to factors such as movement and task performance. Behavioral states modulate stimulus responses, and further analysis will examine how additional measurements, such as pupil diameter, relate to stimulus responses and brain states.

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Nanosymposium

485. Visual Cognition: Decision Making

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Presentation Number: 485.03

Topic: D.06. Vision

Support: Wellcome Trust 095669

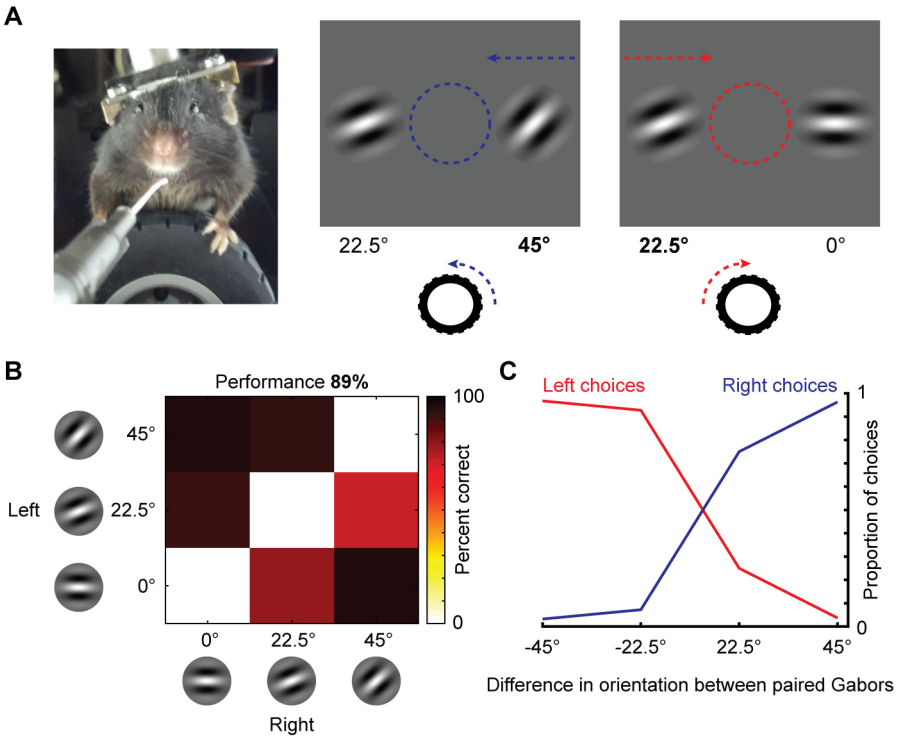
Wellcome Trust 095668

Title: An orientation comparison task for head-fixed mice

Authors: ***S. W. FAILOR**, M. CARANDINI, K. HARRIS;
Visual Neurosci., Univ. Col. London, London, United Kingdom

Abstract: Many choices faced by an animal involve not only recognizing an ideal stimulus feature, but also comparing that ideal to other presently available alternatives.
To elucidate neural processes underlying visual feature comparisons, we developed a two-alternative forced choice orientation comparison task. The task is an adaptation of a similar task that we developed for contrast discrimination (Burgess et al, *bioRxiv* 2016). On each trial, two Gabor patches of equal contrast and spatial frequency were presented simultaneously in opposite visual hemifields [Figure A]. They could each take one of three possible orientations with equal probability (45°, 22.5°, and 0°). Mice were rewarded for correctly identifying the Gabor whose orientation was at or closest to the ideal 45°. Thus, the intermediate orientation (22.5°) would be either correct or incorrect, depending on its pairing.
While this task was more difficult for mice to learn than simple contrast detection or discrimination, 67% of mice were able to perform the task at >80% accuracy within 3 months of training [Figure B]. Trial accuracy depended on the difference in orientation between presented stimuli [Figure C].
This result demonstrates that mice can be trained to perform an orientation comparison task and leads to two questions: (1) Are responses in visual cortex to the same stimulus (22.5°) different when it is perceived as the correct choice? (2) Are there modifications in cortical circuitry that

allow the mouse to perform this task? To answer these questions we implanted a head-plate and an imaging window over the visual cortex of mice expressing GcAMP6f exclusively in excitatory cells of cortex (Madisen et al., 2015). We are currently performing longitudinal 2-photon imaging of visual cortex to determine how the presence of a competing alternative stimulus affects sensory responses, and whether learning the task is accompanied by alterations in these sensory responses. The stimulus comparison task provides a method to address these and other questions about the neural mechanisms of visual perception.



Disclosures: **S.W. Failor:** None. **M. Carandini:** None. **K. Harris:** None.

Nanosymposium

485. Visual Cognition: Decision Making

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Presentation Number: 485.04

Topic: D.06. Vision

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R01NS088628

Title: Decoding perceptual confidence signals in the superior colliculus

Authors: *B. ODEGAARD[1], P. GRIMALDI[2], S. H. CHO[3], M. A. K. PETERS[1], H. LAU[1], M. A. BASSO[4];
[1]Psychology, [2]Dept. of Psychiatry, [3]Integrative Biol. and Physiol. department, [4]Departments of Psychiatry and Biobehavioral Sci. and Neurobio., UCLA, Los Angeles, CA

Abstract: When we view the visual world, our subjective perceptual experience is often accompanied by an assessment of how confident we are in what we see. It has been reported that this ability is due in part to activity in the lateral intraparietal area (Kiani & Shadlen, 2009) and the frontal eye fields (Middlebrooks & Sommer, 2012), but recent data from our laboratory indicates that the superior colliculus (SC) may also play a role in encoding perceptual confidence (Grimaldi et al., 2016). Specifically, we showed that whereas some neurons in the SC have significantly higher discharge rates for responses indicating higher confidence in a visual percept, other neurons have higher discharge rates for responses indicating reduced confidence. This heterogeneity in how perceptual confidence may be encoded in the SC introduces an interesting question: can this varied neural representation of confidence be decoded to predict perceptual choices? To answer this we collected single- and multi-neuron recordings from the SC of two monkeys during a perceptual decision-making task (n=410). Monkeys viewed random dot motion stimuli with varying coherence, and reported the perceived direction of motion with eye movements. Critically, on half of the trials, monkeys received an opt-out target option. If monkeys chose correctly, they received a reward, and if they chose incorrectly, they received no reward. On trials in which an opt-out target appeared, monkeys could choose this third target and receive a smaller but guaranteed reward. We implemented a support vector machine (SVM) classifier using spike counts within a sliding window beginning 100ms before cue onset and extending for 800ms. Spike counts from each electrode were used as features to predict choices on a trial-by-trial basis. Our SVM analyses yielded three main findings: (1) Decoding motion direction choices on trials when the opt-out choice was available (high-confidence choices) resulted in significantly larger SVM decision values compared to trials without the opt-out choice (mix of high- and low-confidence choices), providing evidence that a signature of perceptual confidence can be decoded from population activity in the SC. (2) The capacity of the decoder to distinguish between confident and opt-out responses emerged approximately 250ms after stimulus onset. (3) A decoder trained on correct responses from trials without the opt-out option generalized to classify trials with the opt-out option, indicating a similar population code for choice across conditions. These results provide evidence that choice and confidence are encoded in the population activity of superior colliculus neurons.

Disclosures: **B. Odegaard:** None. **P. Grimaldi:** None. **S.H. Cho:** None. **M.A.K. Peters:** None. **H. Lau:** None. **M.A. Basso:** None.

Nanosymposium

485. Visual Cognition: Decision Making

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BRAIN Initiative Grant

Title: How populations of neurons in V1 encode information for perceptual detection.

Authors: *A. R. ANDREI, S. POJOGA, R. JANZ, V. DRAGOI;
Neurobio. & Anat., Univ. of Texas Hlth. Sci. Ctr. At Houston, Houston, TX

Abstract: It is currently unknown how information across large populations of neurons is integrated during the formation of sensory percepts. In this study, we used optogenetic methods to investigate how the population activity of glutamatergic neurons in primary visual cortex (V1) impacts an animal's ability to detect near-threshold visual stimuli. Studying the system at the limits of sensory detection allows for us to observe how additional, artificially-induced, spiking information is incorporated with the endogenously visually-evoked activity to alter visual detection performance. We delivered the channelrhodopsin (ChR2) gene to multiple sites in V1 using a lentivirus vector with a CaMKII promoter in two monkeys (*macaca mulatta*). Starting 4 weeks after the injection, single and multi-unit activity were recorded using laminar electrodes and a laser-connected fiber optic cable positioned 0.6mm from the nearest recording site. Monkeys performed a visual detection task - while maintaining fixation, oriented gratings with differing luminance contrast levels were presented parafoveally over the receptive fields of the neurons of interest. Monkeys signaled the presence or absence of a stimulus by releasing or holding a response bar. Half of the trials were paired with simultaneous optical stimulation (20-50Hz, for ~300ms). We recorded a total of 48 sessions, and analyzed a total of 473 light-responsive single and multi-units. 22/48 sessions activated neuronal populations optimally tuned to the stimulus orientation, while 14/48 sessions activated non-optimally-tuned populations and 12 session included both types orientations. We found that optical stimulation of populations of excitatory neurons tuned to the visual stimulus resulted in an 8.0% ± 2.2 SEM improvement in behavioral detection of near-threshold stimuli (P=0.0022, Wilcoxon signed rank test). In contrast, optical stimulation of neurons unresponsive to the visual stimuli resulted in no change in task performance. Since orientation, but not luminance contrast is represented in V1 in a topographical manner, the orientation dependency of the performance increase with the optical stimulation suggests that spiking activity used for detection is integrated in local, spatially defined pools. At the neuronal level, while all sessions showed robust firing rate augmentation and increases in signal to noise ratio, pairs of neurons from optimally-tuned sessions also showed

a significant decrease in noise correlations (P<0.0001, Wilcoxon signed rank test, n=2436 pairs) following optical stimulation in conditions when detection was augmented.

Disclosures: **A.R. Andrei:** None. **S. Pojoga:** None. **R. Janz:** None. **V. Dragoi:** None.

Nanosymposium

485. Visual Cognition: Decision Making

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Topic: D.06. Vision

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Title: Activity propagation along the visual cortical hierarchy during the emergence of awareness

Authors: *P. R. ROELFSEMA[1], B. DAGNINO[1], D. VARTAK[1], H. SAFAAI[2], S. PANZERI[2], B. VAN VUGT[1];
[1]Netherlands Inst. for Neurosci., Amsterdam, Netherlands; [2]Inst. Italiano di Tecnologia, Rovereto, Italy

Abstract: How the brain gives rise to conscious experience is one of the central unsolved problems in neuroscience. Along the cortical hierarchy a progressively larger proportion of cells modulate their spiking activity according to the subject's perceptual state. We hypothesized that the state of the cortex might determine the efficiency of the propagation of activity from lower to higher cortical areas and thereby the emergence of awareness.
We therefore compared activity propagation from lower to higher cortical areas between perceived and identical non-perceived stimuli in V1, V4 and the dorsolateral prefrontal cortex of macaque monkeys. The monkeys carried out a task in which they reported the presence of a low-contrast stimulus with an eye movement. We found that low contrast stimuli that reached awareness elicited a stronger initial feedforward response at all these levels of the cortical hierarchy than stimuli that remained subliminal.
The difference between the amount of neuronal activity elicited by perceived and non-perceived stimuli increased at higher levels of the cortical hierarchy. Thus, the feedforward propagation of visual information from lower to higher areas is less efficient when perception fails.
Interestingly, the stronger stimuli only got lost in frontal cortex, implying a failure of propagation toward these higher cortical levels. As a direct test of propagation failures within

cortex, we induced phosphenes with weak microstimulation pulses in V1, close to the perceptual threshold. Perceived V1 phosphenes elicited stronger V4 activity than phosphenes that were missed, suggesting that an inefficiency of the cortico-cortical interactions is at least partly responsible for the failures of propagation.
The efficiency of signal propagation toward higher visual areas depended on the prestimulus brain state, as indexed by alpha and gamma power in the EEG as well as the spontaneous firing rate of neurons in visual and frontal cortex. Our results provide new insights into the fate of subliminal stimuli and elucidate how stimuli of constant strength can yield different perceptual outcomes.

Disclosures: **P.R. Roelfsema:** None. **B. Dagnino:** None. **D. Vartak:** None. **H. Safaai:** None. **S. Panzeri:** None. **B. van Vugt:** None.

Nanosymposium

485. Visual Cognition: Decision Making

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Fundacao para a Ciencia e Tecnologia

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Simons Collaboration on the Global Brain

NIH Pioneer 1DP1OD006409

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Title: Real-time decoding of a decision variable during a perceptual discrimination task

Authors: ***D. PEIXOTO**[1,2], R. KIANI[3], J. C. KAO[4], P. NUYUJUKIAN[4,5], C. CHANDRASEKARAN[4], J. R. BROWN[8], S. FONG[8], K. SHENOY[4,6,7,8], W. T. NEWSOME[6,8]; [1]Stanford Univ. Sch. of Med., Stanford, CA; [2]Champalimaud Neurosci. Programme, Lisbon, Portugal; [3]Ctr. for Neural Science, NYU, New York, NY; [4]Electrical Engin., [5]Neurosurg., [6]Neurobio., [7]Bioengineering, Stanford Univ., Stanford, CA; [8]Stanford Univ. /HHMI, Stanford, CA

Abstract: In dynamic environments, subjects often integrate multiple samples of a signal and combine them to reach a categorical judgment. This evidence integration process can be described by a time-varying decision variable (DV) reflecting the current judgment of the subject. We previously showed (Peixoto et al. SFN ’14) that during a motion discrimination task, population firing rates in dorsal premotor cortex (PMd) and motor cortex (M1) carry choice-predictive signals that (i) are consistent with a DV representation and (ii) allow for high prediction accuracy during the evidence integration epoch. However, calculating single trial DVs and assessing the meaning of their fluctuations has remained extremely challenging. In this study we tackled these exact challenges, by (i) estimating the current DV in real-time and (ii) testing its relationship with decision commitment. To do so, we assembled a real-time setup that uses a logistic classifier to estimate an instantaneous DV every 10 ms using the last 50 ms of spiking data on 96-192 channels from 1 or 2 Utah arrays in PMd/M1. We obtained excellent online choice prediction accuracy: 93%/95%during the second half (600-1200 ms) of stimulus presentation for Monkey F/H. Leveraging our accurate real-time readout we performed 2 closed loop experiments in which the termination of the stimulus was contingent on the value and/or history of the DV. First, we established threshold values for DV that, if reached, stopped the stimulus and cued the monkey to report its decision. Strikingly, the choice probability observed for DVs that triggered termination closely followed the predictions from the logistic function (average difference 1.8%/1.5% for Monkey F/H) suggesting that, fluctuations in DV have a lawful relationship to choice behavior. Second, we triggered the stimulus termination on robust changes in DV sign, interpreted as potential “Changes of Mind” (CoMs), that met specific parameters within our control. The statistical regularities for our putative CoMs were the same as (i) those reported behaviorally in humans (Resulaj et al.2009) and (ii) estimated post-hoc from neural activity during the delay period of an identical task (Kiani et al 2014). We went beyond these previous studies by capturing covert CoMs online during evidence integration and directly validating the final choice of the subject. In summary, our study shows that real time fluctuations in DV reflect meaningful fluctuations in the cognitive state, both in terms of choice probability and CoMs. These results open the possibility of using real-time closed loop experiments to shed light on other covert cognitive processes.

Disclosures: **D. Peixoto:** None. **R. Kiani:** None. **J.C. Kao:** None. **P. Nuyujukian:** None. **C. Chandrasekaran:** None. **J.R. Brown:** None. **S. Fong:** None. **K. Shenoy:** None. **W.T. Newsome:** None.

Nanosymposium

485. Visual Cognition: Decision Making

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Howard Hughes Medical Institute

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Fundacao para a Ciencia e Tecnologia

Title: Do decision-related firing rates of dorsal premotor cortex neurons "ramp" or "step" on single trials?

Authors: *C. CHANDRASEKARAN[1], J. SOLDADO-MAGRANER[4], D. PEIXOTO[5,2], M. SAHANI[4], K. V. SHENOY[1,2,3,6];
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Abstract: Dorsal premotor cortex (PMd) is a brain region thought to be important for somatomotor decisions. We recently showed that trial-averaged firing rates (FRs) of a subpopulation of PMd units that increased their FR after visual stimulus onset ("Increased units") had an organized relationship to stimulus difficulty, choice, and RT (Chandrasekaran et al., SFN '13, '14, '15). These are consistent with the properties of a candidate decision-variable (DV) predicted by a drift-diffusion model (DDM). Here, as a first step, we examined if a recently developed Bayesian model comparison technique (Latimer et al., 2015) could shed light on whether this DV arises from averaging gradual DDM-like "ramps" or instantaneous jumps (a "step") in FR that occur at different times on different trials.
Our database consisted of 131 Increased units recorded from PMd of two monkeys (T & O) performing a visual reaction time (RT) discrimination task with arm movements as the behavioral report (Decision times: up to 220 ms for O; up to 500 ms for monkey T). We used the method from Latimer et al. (2015) to fit the FRs for the preferred ("PREF") and null ("NULL") directions and then computed the penalized model comparison metric, Deviance Information Criterion score (DIC). Our analysis initially focused on the PREF direction because of the robust FR modulations observed. DIC scores for the majority of the PMd units argued against the step model as a descriptor of the single-trial FR dynamics in the PREF direction (75/131 had DIC < 0, 57%). Moreover, computing DIC only for long RT trials (>500 ms) suggested that PREF direction single-trial FR dynamics in an even greater proportion of PMd units were inconsistent with stepping (85/131 had DIC < 0, 65%, Shadlen et al., 2016). When we pooled over both PREF and NULL directions to compute the DIC score, we found the opposite result. Many units classified as rampers based on the PREF direction DIC score were classified as steppers based on the pooled DIC score. Now, when using the pooled DIC score, a majority (67%, 87/131 units had DIC > 0) of the population was better described by the step model.
Thus, using this method from Latimer et al. (2015) to conclude whether PMd FRs step or ramp on single trials appears to depend on properties of the trials used (i.e., tuning and RT). This sensitivity identified here might apply more broadly. More generally, the sensitivity of this

model comparison method to these factors and temporal complexity in decision-related FRs in PMd (Chandrasekaran et al., SFN '13) which perhaps make both "steps" and "ramps" poor models suggests that population-level techniques might be needed to understand how decision variables arise in PMd during decision-making.

Disclosures: C. Chandrasekaran: None. **J. Soldado-Magraner:** None. **D. Peixoto:** None. **M. Sahani:** None. **K.V. Shenoy:** None.

Nanosymposium

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NIH Grant EY007024

Title: Synchronous fluctuations in visual cortex impact neural coding and behavioral performance

Authors: *C. BEAMAN, S. EAGLEMAN, V. DRAGOI;
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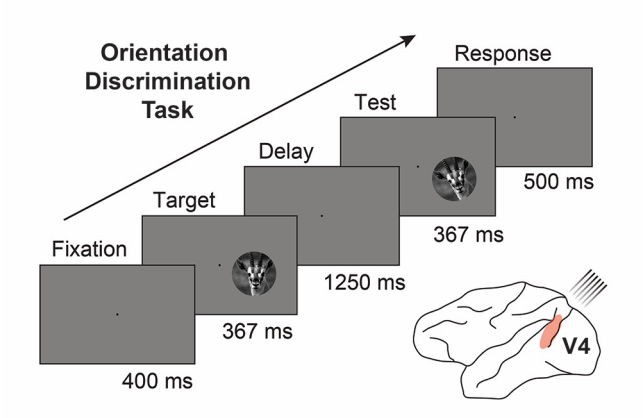
Abstract: The mammalian cortex fluctuates through continuous states of synchrony during the day. Thus, neural population activity varies between the 'synchronized' state during sleep and the 'desynchronized' state during waking. During wakefulness, little is known about the association between fluctuations in population synchrony and neural coding in behaving animals. To investigate this relationship, we conducted recordings in area V4 of monkeys participating in a match-to-sample orientation discrimination task. We examined the synchrony of neural populations on a trial-by-trial basis and found that in desynchronized trials, correlated variability was decreased, while network and behavioral performance were increased. These findings demonstrate that the structure of variability in neuronal population response is not noise but rather controls how sensory information is optimally integrated with ongoing processes to guide network coding and behavior.



Disclosures: **C. Beaman:** None. **S. Eagleman:** None. **V. Dragoi:** None.

Nanosymposium

485. Visual Cognition: Decision Making

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Topic: D.06. Vision

Support: Nottingham Research Fellowship

Title: Brain rhythms shape Bayesian integration of prior predictions with precision weighted sensory information for perceptual decisions

Authors: ***M. BAUER**[1], M. WISLOWSKA[4], T. VEALE[1], P. G. MORRIS[2], P. F. LIDDLE[3], H. R. HEEKEREN[5], M. J. BROOKES[2];
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Abstract: Perception is ambiguous due to the complexity of the world and the limited information provided by sensory signals. The state of the world has to be inferred from these signals, without the existence of a unique solution. Research predominantly into multisensory cue combination has provided strong evidence that brains compute Bayesian statistics to limit this ambiguity, but the universality of such a mechanism has been questioned and the underlying neuronal processes remain hypothetical. We show in an ambiguous visual object recognition task that humans integrate prior probabilities of visual object category (face/house) with stimulus

information in a Bayesian sense, weighted by stimulus precision. The implicitly learned prior probabilities affect perception through modulation of anticipatory occipital alpha-/beta-oscillations, even in the absence of any cue and without participants explicit awareness of this probability manipulation. By contrast, the precision of face/house representations is encoded in visually induced gamma-oscillations and these are further enhanced by prior predictions, potentially reflecting a posterior belief signal. The causal relevance of these mechanisms was established through transcranial alternating current stimulation (tACS, sinusoidal at 1mA), applied in different sessions at participants' individual alpha- and gamma-frequencies whilst they performed the perceptual task. Crucially, acute alpha-tACS enhanced the influence of prior probabilities on perceptual decisions, whereas acute gamma-tACS enhanced the impact of sensory stimuli, hence fully congruent with our electrophysiological results. Concerning direct measures of the efficacy of tACS in modulating brain activity, we confirmed previous findings that *several minutes after the end of tACS*, the EEG showed sustained enhancement of power compared to before tACS (predominantly in alpha-/beta-band following alpha-tACS, vs a more broadband effect including high frequencies following gamma-tACS), hence clearly showing the efficacy of tACS in modulating brain activity, even at weak amplitudes. Thus, our results provide fully congruent evidence for a causal role of alpha-oscillations in relaying predictive feedback signals and gamma-oscillations forwarding sensory signals and we show this whilst participants successfully integrate precision weighted sensory evidence with prior probabilities for Bayesian inference of visual object identity.

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Nanosymposium

485. Visual Cognition: Decision Making

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Title: Prospective optimization and visual representation

Authors: ***J. SNIDER**[1], S. GEPSHTEIN[2];
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Abstract: We developed a new task to identify the strategies employed by humans to plan risky actions for multiple future steps using incomplete and rapidly varying information. A triangular lattice of disks of different value scrolls down on a touch screen at different speeds. By touching disks in a rapid sequence, subjects choose an upward path through the lattice to maximize the cumulative reward. A path is a sequence of binary choices made by selecting between the two disks directly above the current disk.
We parameterize human strategies by depth of computation (d) and recalculation period (r). Depth d is the number of rows subjects use to evaluate potential paths. Period r is how often subjects reassess their planned behavior. For example, a subject may look ahead for two rows (d=2) and develop a plan of action. She may then reassess the plan after 1 step (r=1) or play out the whole plan (r=2). The lattices were optimized so different strategies led to paths in different directions, allowing to estimate (r, d) strategies from individual choices. We also tested whether subjects used simple heuristics, such as 'seek out the large' or 'avoid the small.'
In the initial study (Snider et al, 2015, PLoS Comp Biol, e1004501) subjects exchanged deeper computation for less frequent reassessment. Surprisingly, there was no evidence that subjects relied on heuristics or pruning. They behaved as if they exhaustively performed all the sums along an exponential number of paths. When time pressure increased, subjects chose to decrease d, but kept the exhaustive computations.
How can such computations be performed? We hypothesize that the parallel organization of the visual system enables the comprehensive evaluation of potential paths, taking advantage of the analogue representation of value by disk size. We can interfere with the visual computation by representing value by numerals that engage memory-based serial cortical mechanisms. Indeed, the numerical task decreased depth d by about one row and forced subjects to rely on heuristics. In the fast numerical condition, they only aimed for the largest disks. In a more rigorous approach, choice behavior is parameterized using such machine learning algorithms as Partially Observable Markov Decision Process. The data requirements for estimating parameters in such models are staggering. We are partnering with a game company to create a mobile version of the task that entertains subjects and increases user pools from dozens to perhaps millions, to satisfy the data hungry models. We present simulations of how we store data and analyze subjects' choice behavior at the scale of Big Data.

Disclosures: J. Snider: None. **S. Gepshtein:** None.

Nanosymposium

485. Visual Cognition: Decision Making

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NIH 1R44NS073417

NIH 5R44NS063537

Title: Pushing the brain into overdrive: the role of the metabolism in overcoming the speed-accuracy tradeoff

Authors: *A. T. VU[1,2], D. A. FEINBERG[1,2];
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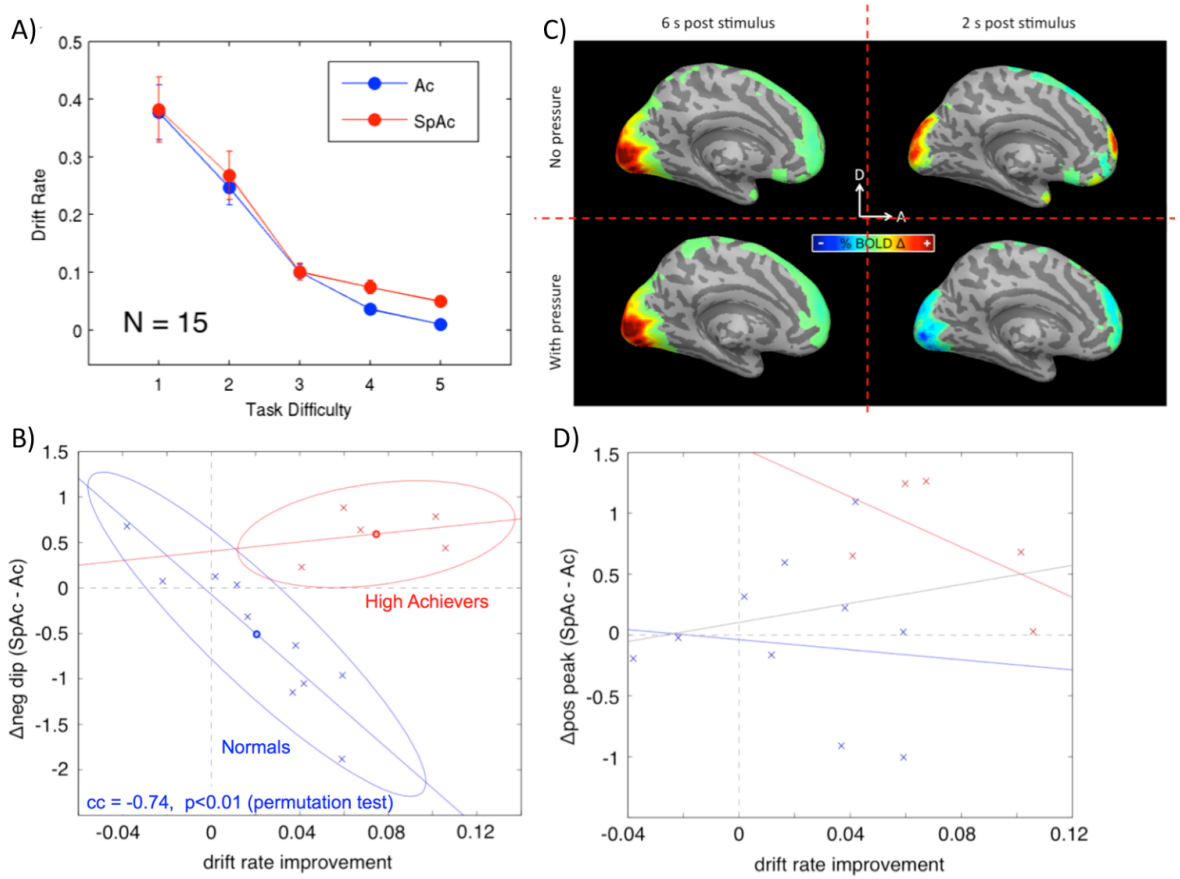
Abstract: Speed (Sp) - accuracy (Ac) tradeoff (SAT) theory dictates that decisions can be made more quickly by sacrificing accuracy. This tradeoff has been attributed to changes in the amount of sensory information accumulated prior to making a decision (response threshold) with the assumption that the rate of sensory information accumulation (drift rate) remains constant. We find that under difficult, time pressured situations, subjects can overcome SAT to improve both speed and accuracy relative to non-time pressured situations ($p<0.01$; Fig A). Using highly accelerated MB fMRI (TR=333ms) to scan 15 subjects performing a motion discrimination task, we found that the increase in drift rate is correlated with a brief metabolic overdrive (initial dip) in visual cortex proportional to the degree of improvement in information processing efficiency ($p<0.01$; Fig B). Interestingly, the positive response traditionally analyzed in fMRI experiments did not change with time-pressure or degree of drift rate improvement (Fig CD), suggesting that the initial dip can be more sensitive and/or specific to underlying neuronal activity. A minority group of "high achiever" subjects excelled under time-pressure with above average improvements in drift rates under SpAc emphasis (Fig B). Interestingly, for these subjects, they already exhibited the initial dip in visual cortex under the Ac condition. Under SpAc, their negative dip reduced and positive response increased suggesting that these subjects are better able to predict when increases in metabolism will arise and/or their vasculature is able respond faster and stronger to the increase in neural metabolism. The variations in neurovascular responses across subjects could explain in part why the initial dip has remained controversial to this day. Future studies controlling for these variations, in conjunction with multiband accelerated, high spatial and temporal resolution fMRI at ultra high field, will be important for elucidating the source and taking advantage of the initial dip as a biological marker.



Disclosures: **A.T. Vu:** A. Employment/Salary (full or part-time): Advanced MRI Technologies. **D.A. Feinberg:** A. Employment/Salary (full or part-time): Advanced MRI Technologies.

Nanosymposium

485. Visual Cognition: Decision Making

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SC2-GM-099626

Title: Supramodal decision-related activation in simultaneously recorded human EEG and fMRI

Authors: *N. A. STEINEMANN[1], J. H. BALSTERS[2], C. KELLY[3,4], R. G. O'CONNELL[5], S. P. KELLY[6,7];
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Abstract: Single unit studies in animals have examined perceptual decisions communicated through response-mode dependent neural populations e.g. in the intraparietal sulcus (Shadlen and Newsome, 2001). Recently, human electrophysiology studies have identified a decision-related centro-parietal positivity (CPP) that traces decision formation independent of the sensory modality of the evidence or response requirements, indicating a response-mode independent computation (O'Connell et al., 2012). Several functional imaging (fMRI) studies have sought to localize such an abstract decision mechanism in the human brain, but due to lack of consensus in assumed evidence-dependent changes, and the difficulty in distinguishing neural sensory evidence signals from evidence accumulation signals due to lack of temporal resolution (Kelly and O'Connell, 2014), a convergent set of regions has failed to emerge across studies (e.g. Heekeren et al., 2004; Ho et al., 2009).
Here we attempted to localize the neural site of supramodal decision formation by applying similar logic to that applied in the identification of the CPP (O'Connell et al., 2012): participants performed several versions of a continuous monitoring task for intensity changes, with the requirement to respond (counting versus button press), sensory modality (visual contrast vs auditory volume) and direction of the change (up vs down) varied across blocks, while EEG and fMRI were acquired simultaneously.
Preliminary analyses show that, as previously, measures of perceived sensory evidence (instantaneous steady-state visually and auditory evoked potentials), decision-related activity (CPP), and motor preparation (lateralized μ and β power) were traceable in parallel in EEG. In the fMRI data, contrasts of the target intensity changes (up vs down) localized early auditory and visual areas implicated in sensory evidence representation, as expected. Areas involved in decision formation were identified by target-evoked positive activation for all five conditions, including the non-response condition. This revealed a set of areas including the bilateral intraparietal sulcus to be involved in perceptual decision formation independent of evidence-type and response-mode. Future analysis will employ a combination of EEG source analysis, and EEG-informed fMRI analysis to further characterize the dynamics of activation profiles in regions of interest extracted from the fMRI analysis.

Disclosures: N.A. Steinemann: None. **J.H. Balsters:** None. **C. Kelly:** None. **R.G. O'Connell:** None. **S.P. Kelly:** None.

Nanosymposium

486. Learning to Reach

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Presentation Number: 486.01

Topic: E.04. Voluntary Movements

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Title: Monetary incentives differentially modulate fast and slow motor learning

Authors: *L. J. VOLZ[1], A. ASTURIAS[1], M. CIESLAK[1], T. G. LEE[2], S. GRAFTON[1]; [1]Univ. of California, Santa Barbara, Santa Barbara, CA; [2]Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Introduction
Recent models of trial-by-trial sensorimotor learning propose at least two simultaneous processes operating on different time scales: a fast learner involved in immediate improvement and a slow learner accounting for later and more gradual, but persisting gains in performance [1]. While it is clear that incentives (reward or punishment) impact sensorimotor learning [2], it is not known how incentive interacts with learning on these different time scales. We address this question in the framework of a sequential pointing task where subjects must adapt to a visuo-motor rotation (on a fast scale), and simultaneously form sequence knowledge (on a slow scale).
Methods
40 healthy subjects performed a discrete sequence production joystick task, with 8 consecutive elements, consisting of an out-and-back movements (Fig. 1). Two sequences (A, B) were learned at distinct rotations (rotation A: -25 degrees, rotation B: 20 degrees) over 200 trials divided into 10 blocks at two levels of incentives. For each trial, subjects earned or lost points depending on successful performance. Two-factorial ANOVAs (BLOCK, INCENTIVES) were calculated to assess the change in fast-rate (average MT of the first 5 trials of the first and last block), and slow-rate (average MT of the last 15 trials of the first and last block).
Results
For the change in slow-rate improvement, we observed a significant main effect of BLOCK ($F_{(1,39)}$=186.87, p<0.001) and a BLOCK x INCENTIVE interaction ($F_{(1,39)}$=9.17, p=0.004). Post-hoc testing showed singificantly improvement for the high compared to the low incentive condition.
For fast-rate improvement, we also found a significant BLOCK x INCENTIVE interaction ($F_{(1,39)}$=4.96, p=0.032). Here, significantly higher improvement in the low incentive compared to high incentive trials was observed. Thus, low stake incentives led to pronounced improvements in fast-rate motor adaptation compared to high incentives.

Conclusions
Slow-rate learning of a motor sequence is sensitive to high reward training whereas adaptation is not. The results may have functional relevance for skill learning in various contexts like sports or neurorehabilitation, where a task- and situation-dependent accentuation of fast or slow learning processes may be profitable.

Refrences
[1] Wolpert, D. M., & Flanagan, J. R. (2016). Computations underlying sensorimotor learning. Current Opinion in Neurobiology, 37, 7-11.
[2] Galea, J. M., Mallia, E., Rothwell, J., & Diedrichsen, J. (2015). The dissociable effects of punishment and reward on motor learning. Nat Neurosci, 18(4), 597-602.

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Nanosymposium

486. Learning to Reach

Location: SDCC 23A

Time: Tuesday, November 15, 2016, 8:00 AM - 11:15 AM

Presentation Number: 486.02

Topic: E.04. Voluntary Movements

Support: NSF SBE 0542013

Title: A conservation law for self-paced movements

Authors: *D. HUH, T. J. SEJNOWSKI;
Salk Inst., San Diego, CA

Abstract: Optimality principles for modeling regularities in biological movements introduce external task factors to specify the pace of movements. Here, we present the dual to the principle of optimality based on a conserved quantity, called “drive”, that represents the influence of internal motivation level on movement pace. Optimal control models and drive conservation provide equivalent descriptions for the regularities observed within individual movements. For regularities across movements, drive conservation predicts a novel scaling law between the overall size and speed of various self-paced hand movements in the absence of any external tasks, which we confirmed with psychophysical experiments. Drive can be interpreted as a high-level control variable that sets the overall pace of movements and may be represented in the brain as the tonic levels of neuromodulators that control the level of motivation, thus providing insights into how internal states affect biological motor control.

Disclosures: **D. Huh:** None. **T.J. Sejnowski:** None.

Nanosymposium

486. Learning to Reach

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Topic: E.04. Voluntary Movements

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Rhode Island Foundation 20144132

NSF CAREER 1555006

Title: Paradoxical benefits of attentional distraction for visuomotor memory without explicit awareness

Authors: ***J.-H. SONG**[1], H. IM[1,2];
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Abstract: A pilot learning to operate an aircraft and a stroke victim recovering the ability to walk share the task of acquiring or reacquiring pertinent motor skills. Importantly, these motor skills are often learned and retrieved in complex environments where attention is often distracted by other events. Traditionally, attention has been viewed as a necessary resource that facilitates many cognitive functions, including learning, and thus previous studies have focused exclusively on the immediate detriment effects of divided attention on motor performance. However, the question of how divided attention affects memory formation or retrieval remains unanswered. Using a novel dual task paradigm in which visuomotor learning was concurrently paired with an attention demanding secondary task, we recently demonstrated that motor skill learned under distraction was correctly retrieved, during a separate recall phase, only under similar distraction. A possible explanation is that the presence of attentional distraction acts as a context for visuomotor memory encoding and retrieval (Song & Bédard, 2015; Im et al., 2015). In the current study, we investigated whether this paradoxical benefit of attentional distraction manifests in the absence of awareness and explicit cognitive strategies. Unlike the previous study, participants in the current study experienced a gradually increasing visuomotor distortion (0.3°/trial) instead of an abrupt visuomotor distortion (45°). Participants are generally unaware of learning during gradual visuomotor distortion and thus this procedure minimizes the potential use of cognitive strategies to solve the visuomotor discordance. Consistent with abrupt

adaptation, we continue to observe that the benefit from consistent attentional-context-dependent memory. This result suggests that explicit visual feedback during initial visuomotor learning is unnecessary for binding attentional states and visuomotor memory. Furthermore, altering the attentional distraction task between learning and recall (e.g., from RSVP task to visual search task or vice versa) did not affect this finding. It appears that participants do not simply associate the specific distraction task with visuomotor. Rather, the presence of a distraction task activates a specific attentional state that is bound to the visuomotor memory; all of which is happening without explicit awareness of cognitive strategy. Our new discovery of attentional-context-dependent memory highlights the necessity of updating current models of attention, motor learning, and memory to fully understand visuomotor learning processes.

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Nanosymposium

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Topic: E.04. Voluntary Movements

Support: NIH Grant 1P20GM103645

Rhode Island Foundation 20144132

NSF CAREER 1555006

Title: Attentional context-dependent generalization of visuomotor memory

Authors: *T. WANG, J.-H. SONG;
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Abstract: In daily life, people are constantly presented with situations to learn and acquire new motor skills, often in complex environments where attention is often distracted by other events. We have recently discovered that adding a concurrent, attentionally distracting task (e.g., difficult RSVP task) did not impair the original learning of a simple visuomotor rotational adaptation task. Paradoxically, successful recall of the visuomotor skill only occurred when a similar level of attentional distraction was present. This finding suggests that in contrast to a traditional view of attention as a cognitive resource, performing a distractor task acts as an internal 'task context' for encoding and retrieving of motor memory (Song & Bédard, 2015). Since the acquired motor representation must be performed in different environments, a crucial part of visuomotor learning is generalization. How does attentional distraction affect the

generalization of visuomotor adaptation? Our previous study (Bédard & Song, 2013) showed that visuomotor training with the dual-task reduces the gain, and narrows the tuning of the generalization function. Importantly, however, this effect of divided attention on generalization was only studied under inconsistent task-context between the training (dual task) and the generalization phase (single task). The critical question of whether inconsistency between task-contexts reduces generalization remains unknown. Here, we examined how attention task-contexts affect visuomotor generalization. Using a similar dual-task paradigm, we replicated that switching the attentional context from training (dual task) to generalization (single task) narrowed the tuning of the generalization function. However, in accord with the notion of attentional context-dependent memory, maintaining the presence the RSVP task throughout both training and generalization increased the tuning of the generalization function. Together, these findings show that the subjects' attentional state is encoded with the visuomotor memory and that retrieval is dependent on consistency of the attentional state between training and generalization. The findings highlight how attention may interact with motor learning and the need for current models of motor learning to incorporate concepts of attention and memory.

Disclosures: T. Wang: None. **J. Song:** None.

Nanosymposium

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Support: VA Advanced Fellowship Neurosciences/Psychiatric Research

Title: Reorganization of low-frequency oscillatory dynamics during skilled motor learning.

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Abstract: Introduction: Prior studies have demonstrated that low-frequency oscillations organize motor cortical circuits involved in well-learned sub-movements. However, little is known about how these low-frequency neural dynamics are modulated with training. This study aimed to investigate changes in low-frequency oscillations in motor cortex of rodents following

skilled motor learning.
Method: Long-Evans rats were implanted with 16-channel micro-wire arrays spanning M1. Animals next underwent training on a skilled-forelimb reach task while we recorded changes in single unit activity and local field potentials (LFP). We analyzed oscillatory power, inter-trial phase coherence and spike-field coherence in association with skilled motor learning. In addition, we investigated changes in low-frequency principle-component trajectories, a previously described way of understanding large-scale oscillatory dynamics during movement.
Results: Skilled motor training was associated with a reorganization of motor-related slow-oscillations. We found increased low-frequency oscillatory power and increased phase-locking in association with skilled motor learning. Low-frequency oscillations strongly modulated spiking activity, with single-units showing a clear task-related change in spike-field coherence at these slow oscillatory frequencies. Finally, we found that skilled motor learning strongly reorganized low-frequency PC trajectories, indicating meso-scale reorganization of these oscillatory dynamics as a result of skilled motor training.
Conclusion: Skilled motor learning is associated with increased binding of low-frequency oscillations external task-demands. This is evidenced by changes in power, phase-locking and PC trajectories.

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Nanosymposium

486. Learning to Reach

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Topic: E.04. Voluntary Movements

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BWF

Title: Population-level changes in neural activity during learning

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Abstract: -----Learning requires changes in how we generate neural activity. We and others have found that patterns of activity across many neurons tend to reside within a low-dimensional subspace, which we refer to as the intrinsic manifold. We have previously shown that rhesus monkeys can learn new behaviors by adapting activity within this intrinsic manifold, but the structure and neural strategies underlying these adaptations are not yet known.
-----To characterize these learning-related population-level changes, we leveraged multi-electrode recordings in monkeys and a brain-computer interface (BCI) paradigm. By perturbing the BCI mapping, we could systematically induce behavioral learning and study the underlying changes in population activity. Because the animals showed substantial learning, and because behavior is directly determined by the recorded neural activity under BCI, there must be learning-related changes in the recorded neural activity.
-----First, we asked whether learning-related changes were restricted to the high-covariance modes of population activity (i.e., the dimensions which capture the most shared variability across the population). Interestingly, we found that the low-covariance modes also play a substantial role during learning. The monkeys learned to activate the correct modes of activity at the correct times during the task, even if those modes explained only a small fraction of the population covariance prior to learning.
-----Next, we asked how the distribution of neural activity patterns changed throughout learning. At perturbation onset, behavioral performance drops substantially. For performance to improve for a particular intended movement, the neural activity patterns generated for that intended movement must change. One might hypothesize that the joint set of activity patterns, taken across all intended movements, reorients to better align with the perturbed BCI mapping. We did not find this to be the case. Alternatively, because perturbations altered the magnitude of influence that each mode of activity had on behavior, another reasonable hypothesis is that each mode of activity rescales its dynamic range to restore the influence it had on behavior prior to the perturbation. We also did not find this to be the case. Although we did detect changes in the across-movement distribution of activity patterns, these findings suggest that there may be limitations to how much these distributions of activity can change, even within the intrinsic manifold. Taken together, these findings begin to elucidate the neural strategies subserving the brain's flexibility in learning novel and abstract behaviors.

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Nanosymposium

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Title: Functional relation between primary motor cortex and dorsal premotor cortex is altered during curl field learning

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Abstract: The motor system has an impressive ability to learn new skills and adapt to new environments. This motor learning has been studied using a "curl field" (CF), a velocity-dependent force applied orthogonally to reaching movements. We have shown that the dynamics between primary motor cortical (M1) activity and movement are fixed during learning and thus cannot account for the altered behavior. Instead, learning must be mediated by altered recruitment of M1 neurons by upstream brain areas. Since dorsal premotor cortex (PMd) plays a role in movement planning, we hypothesized that behavioral adaptation may be driven by changes in these inputs to M1. We recorded spiking activity simultaneously from M1 and PMd neurons using chronically implanted electrode arrays while a monkey made center-out reaching movements. After a Baseline period, the monkey adapted to a CF (Force period). We then removed the CF and the monkey re-adapted to normal reaching dynamics (Washout). We analyzed seven sessions with an average of 22 M1 and 32 PMd cells per session.
We tested the hypothesis that CF learning is driven by a change in the functional relationship between populations of PMd and M1 neurons. We used a Poisson Generalized Linear Model to predict the spiking of each M1 or PMd neuron from the spikes of all other M1 or PMd neurons recorded in the session, the self-history of the predicted neuron, and limb kinematics. We built models relating M1 to M1, PMd to M1, and PMd to PMd. We evaluated model performance using relative pseudo-R^2 (RPR^2), which compares the full model against a basic model with only kinematics and self-history. This represents the information that can be inferred from the population of neurons alone. The Baseline models significantly predicted spiking for the majority of cells (146 of 155 for M1-M1, 178 of 228 for PMd-PMd, and 126 of 155 for PMd-M1; 95% C.I. of bootstrapped $RPR^2 > 0$)

We then applied the models calculated from Baseline data to data collected during Force and Washout. Decreased prediction performance over the course of learning would be indicative of a change in the functional relation between the neurons. We found no significant change in normalized RPR^2 for either M1-M1 or PMd-PMd as the monkey adapted (t-test, beginning vs end of Force; p=0.54 and 0.24, respectively). However, there was a decrease in PMd-M1 performance during learning (p=0.04), and a subsequent increase during Washout (p=0.01). The final Washout prediction accuracy was not significantly different from Baseline (p=0.40). The altered relation from PMd to M1 that was concurrent with the behavioral changes supports the hypothesis that CF adaptation involves changes in the recruitment of M1 neurons by PMd.

Disclosures: M. Perich: None. **P.N. Lawlor:** None. **L.E. Miller:** None.

Nanosymposium

486. Learning to Reach

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NIH NINDS K02

Title: Interactions between motor cortex and dorsolateral striatum during skilled motor learning

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Abstract: Introduction: The functional connectivity between motor cortex and dorsolateral striatum is known to evolve during the acquisition of new motor skills. However, the details of when these changes occur during motor learning is not fully understood. Additionally, how different behavioral states, such as offline sleep periods, relate to learning-related changes in corticostriatal interactions is unknown. We chronically monitored neural activity from both primary motor cortex (M1) and dorsolateral striatum (DLS) throughout motor skill learning to determine the temporal dynamics of these changes.
Methods: We chronically recorded single units, multiunit activity, and local field potentials (LFP) from microelectrode arrays simultaneously placed in both M1 and DLS while rats learned a single pellet reach-to-grasp task. Motor skill acquisition was assessed using task accuracy, skill

performance speed, and movement consistency. Electromyography (EMG) activity, LFP, and video-based tracking were used to classify behaviorally relevant states. We further explored how changes in corticostriatal connectivity evolved in relation to the acquisition of the motor skill, as well as how these interactions evolved during different behavioral states.
Results: Coherence between the low frequency components of LFP across both M1 and DLS emerged, time-locked to significant improvements in behavioral performance. Interestingly, changes in coherence during offline periods tracked the process of skill acquisition.
Conclusion: The temporal dynamics of the evolving M1 and DLS coherence suggests that it plays an important role in long-term skill acquisition.

Disclosures: S.M. Lemke: None. **D.S. Ramanathan:** None. **K. Ganguly:** None.

Nanosymposium

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Les Fonds Québécois de la Recherche sur la Nature et les Technologies, Québec (FQRNT)

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Title: Make no mistake, acquisition of novel sensorimotor maps without error correction: beyond model-based learning

Authors: *F. T. VAN VUGT[1], D. J. OSTRY[1,2];
[1]Psychology, McGill Univ., Montreal, QC, Canada; [2]Haskins Labs., New Haven, CT

Abstract: One of the puzzles of learning to talk or play a musical instrument is how we learn which movement produces a particular sound: an audiomotor map. Existing research typically uses mappings that are well-learned at the outset such as using a computer mouse to control the movement of a cursor on a screen. Previously, we documented acquisition of novel sensorimotor maps by having participants learn center-out arm movements to auditory targets. However, virtually nothing is known about what process enables the formation of a sensorimotor map. One dominant view is that motor learning is governed by error correction: subjects compare their movements to a target (model), compute an error vector, and then produce motor commands to correct for a fraction of this error. We hypothesise that sensorimotor maps can be learned without

error correction and test this idea by having participants learn a mapping without having been presented targets, and thus without errors. We first tested one group of participants who learned an audiomotor map through active movements to auditory targets. Then, participants in a passive group experienced the same target, the same kinematic trajectory produced by a robot arm, and the same auditory feedback as a yoked active participant. An additional passive group experienced the same movements and the same auditory feedback but were never presented with the auditory targets. Passive movement replay thus allowed us to control for the distribution of performed movements while exclusively removing target information. If learning is driven by error correction, the groups who are deprived of targets (and therefore of error) should show impaired learning. We found instead that passive presentation of movements without auditory targets yielded improvements in performance following training that were comparable to those found in the active group. Furthermore, the passive group with auditory targets showed less learning, not more, than the group without targets, directly contradicting a predicted improvement in performance if learning was driven by error correction. In sum, learning sensorimotor maps is not based on error correction. Instead, the process governing sensorimotor map learning appears to be the formation of an association table of movements and their sensory feedback.

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486. Learning to Reach

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Topic: E.04. Voluntary Movements

Support: US Department of Defense Grant N66001-15-C-4015

Title: Extending a Bayesian estimation approach to model human movements

Authors: ***D. BLUSTEIN**, J. SENSINGER;
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Abstract: Bayesian estimation is one approach used to describe the way humans plan and execute movements. This approach to describing nervous system operation is necessarily an abstraction because of our incomplete picture of the complicated neurophysiology of the human brain. Nevertheless, computational modeling represents a powerful tool to help leverage neural principles in applications of biomedical engineering such as brain-machine interfaces and prosthetic control. Here we describe a new computational motor control model better aligned

with our understanding of the human nervous system and containing fewer free parameters than a previous model developed by Berniker and Kording (*Nature Neuroscience*, 2008, 11(12):1454-1461) and extended by Johnson (*Northwestern University Dissertation*, 2015). The Bayesian model we describe has two phases. During the state estimation phase, system states (e.g. position and velocity) are predicted and observed to iteratively generate a movement. The predicted result of an intended movement is compared with the feedback from the actual movement to drive a Kalman filter that weights the ongoing state estimates based on the relative source of the error (prediction and feedback). During the parameter estimation phase, system parameters (e.g. object mass and stiffness) are estimated and the uncertainty of the estimate is updated. In the previous implementations the parameter estimate uncertainty and sensory feedback uncertainty (in the parameter estimation phase) were set as fixed values. Now these values update continuously and are iteratively influenced by the ratio of predicted to estimated performance error, resulting in fewer parameters to be set arbitrarily. With parameter estimate uncertainty tied to movement error, if error persists so will uncertainty. Previously the uncertainty could reduce to zero even with movement errors. The sensory feedback uncertainty during the parameter estimation phase is now set equal to the state estimation uncertainty at the end of the state estimation phase. This value was previously static and determined by initial model parameters. Now the level of uncertainty from the state estimation phase influences the uncertainty in the parameter estimation phase. This simple model to describe human movements generates data that match what we see in humans. We show that the model can accurately predict human performance during a Schmidt-style movement task as measured by trial-by-trial adaptation to errors and perturbation detection during a two-alternative forced choice test.

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Nanosymposium

486. Learning to Reach

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Title: Reinforcement but not error-based learning contributes to motor transfer

Authors: B. YACOUBI, A. CASAMENTO-MORAN, Y. CHEN, M. H. KWON, *E. A. CHRISTOU;
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Abstract: Task acquisition is essential for motor learning and comprises of two phases. The initial phase, termed error-based learning, refers to the reduction of error relative to the task goal. The following phase, termed reinforcement learning, refers to the reduction of variability around the task goal. It is currently unclear how each of these two phases contribute to transfer of a motor task (motor learning). The purpose of this study, therefore, was to determine the individual contribution of the error-based learning and reinforcement learning phase during task acquisition to the ability to transfer the practiced motor task.Twenty healthy adults (10 young: 25.1 ± 3.9 yrs, 5 men; 10 older: 71.5 ± 4.8 yrs, 5 men) participated in this study. The participants practiced 100 trials of a rapid goal-directed task with ankle dorsiflexion and were tested one day later with elbow flexion (ipsilateral transfer). The targeted position was 9^o for ankle dorsiflexion and 18^o for elbow flexion. The targeted time was 180 ms for both tasks. Error-based learning was quantified as the slope of the error reduction in the initial 20-30 trials. Reinforcement learning was quantified as the endpoint variability of the last 50 trials. Motor learning (ipsilateral transfer) was quantified with the endpoint error during elbow flexion. Error-based learning was not associated with motor performance during the transfer task (R^2=0.001; P>0.3). In contrast, reinforcement learning was associated with motor transfer. Specifically, greater variability during the last 50 trials of task acquisition was associated with greater error during the ipsilateral transfer task (R^2=0.35; P<0.01). Similarly, greater variability during the initial 20 trials also was associated with greater error during the transfer task (R^2=0.30; P<0.01). These findings provide novel evidence that greater variability during task acquisition impairs motor transfer. Greater variability during acquisition likely impairs the extraction of task relevant information and inhibits the formation of memories that can be generalized.

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Nanosymposium

486. Learning to Reach

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Title: Behavioral encoding of reaching when eye and hand are dissociated in depth and direction.

Authors: ***A. BOSCO**, V. PISERCHIA, P. FATTORI;
Univ. of Bologna, Bologna, Italy

Abstract: The encoding of reaching movements in physiological situations that involve also the depth dimension has been the focus of few studies. Here, we analysed the reaching behavior in terms of kinematics, coordinate systems and the type of action control when reaching to targets on a horizontal surface. 12 healthy participants were tested in 2 experimental paradigms where reaching targets were presented at different depths and directions in foveal and peripheral viewing conditions. Each participant executed a memory guided task in which he/she had to reach the memorized position of the target and a visually guided task in which target was always visible. The peripheral and foveal viewing conditions consisted in three eye/hand configurations: in the constant-gaze configuration, the eye fixated a central fixation target and the hand reached one of the peripheral reaching targets, in the constant reach configuration, eyes fixated one of the peripheral targets and the hand reached always the central target, and in the foveal reach configuration, the fixation and reaching targets were coincident. We found that peak velocity and movement time were statistically influenced by depth and direction in the majority of our conditions, whereas reaction time was affected by the type of task (visually guided or memory guided). The analysis of on-line movement corrections (feedback control) showed that the execution of a reaching movement towards peripheral positions required more control and hence corrections, either when the reaching was foveated (foveal reach configuration) or when it was not foveated (constant gaze configuration). We did not find any significant modulation of feedforward control among the two tasks in the three eye/hand configurations. The analysis of reach endpoint errors showed that in both tasks (visually guided and memory guided), a mixed eye-centered/spatial encoding was used, and this occurred both in direction as well as in depth. Present results suggest that the modulation of multiple behavioral parameters of reaching actions executed in complex geometries is based on the combination of flexible coordinate systems and feedback control.

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Nanosymposium

486. Learning to Reach

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Title: Role of post-trial performance feedback after visual occlusion in multi-finger force production tasks

Authors: *V. SKM[1], S. S. EMBRANDIRI[1], A. GUPTA[2], S. CHITUPROLU[1];
[1]Indian Inst. of Technol. Madras, Chennai, India; [2]The Hebrew Univ. - Hadassah Med. Sch., Jerusalem, Israel

Abstract: Planning and execution of any movement involves the complex interplay of sensory and motor modalities, which regulate movement through feed-forward and feed-back control. Recently, the role of visual feedback on constant force production tasks has been studied well. The absence of visual feedback leads to a characteristic drift in the force values depending on the magnitude of the original force. This phenomenon has been explained in the framework of the referent configuration hypothesis, which posits that the drift in force production can be attributed to the drift in a hypothetical referent point to the actual fingertip coordinates in the Euclidean space. In this work, we investigate if this effect can be controlled by cognitive learning through post-session visual feedback. Data recorded from 14 subjects (8 males) performing two distinct set of tasks on the ATI Nano-17 (ATI Industrial Automation, USA) has been considered for this work. The primary task consists of constant force production using four fingers (Index, Middle, Ring, Little) with visual feedback of individual finger forces for 8 seconds followed by 8 seconds of force maintenance without the visual feedback. The next task consists of the same visual on-off paradigm of 8-8 seconds followed by a post-trial feedback of the entire performance (the subject is shown their performance in both vision-on and vision-off state). Both sets of the task are repeated 30 times each. By comparing the variation in drift characteristics of the secondary task relative to the primary task, we attempt to explore the role of the memory derived cognitive corrective modality in force production tasks. The extent of this correction that can be achieved and the nature of the corrective cognitive effect have been studied. Our results indicate the nature of the drift to significantly change upon inclusion of the post-trial feedback, with the original decay rate and deviation from target dropping significantly. A large degree of inter-subject variability was observed in the decay and learning characteristics. The error values, measured as the deviation from projected profile, show an oscillatory characteristic with time that correlates with the cognitive learning associated with the task. Taken together, we suggest that the learning characterized by the error values is not optimally regulated but tends to fluctuate across trials, eventually converging to an optimum. The findings substantiate modeling of the cognitive learning modality as an under-damped oscillatory feedback control module.

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Nanosymposium

487. Enduring Consequences of Early Stress I

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Topic: F.04. Stress and the Brain

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Battaglia Endowed Chair, CHOP

Title: Neonatal pain experienced in the presence of the caregiver has short and long-term consequences for pain and emotion

Authors: *G. A. BARR[1], R. E. PERRY[2], M. OPENDAK[2], K. KAYSER[3], R. M. SULLIVAN[2]; [1]Anesthesiol. and CCM, Children's Hosp. of Philadelphia, Philadelphia, PA; [2]NYU Sackler Inst. at NYU Sch. of Med., New York, NY; [3]Nathan S. Kline Inst. for Psychiatric Res., Orangeburg, NY

Abstract: Modern medical care permits survival of premature infants born months too early, who spend much of their early lives in a neonatal intensive care unit where they have ~10 medically essential but painful procedures each day. Repeated painful experiences in the NICU are associated with long-term negative consequences, as is the long-term use of pharmaceuticals for pain reduction. Therefore, non-pharmacologic interventions are increasingly used to reduce the pain of these procedures. One popular environmental manipulation is for the caregiver to hold or swaddle the infant during painful procedures, which we model here. This reduces the infant's behavioral and physiological responses to pain. Although effective, there are no assessments of the enduring effects of repeatedly pairing pain with the caregiver at a time when mother-infant attachments are being formed. The existing animal literature suggests that such pairings may have enduring effects; pairings have the immediate effect of reducing the behavioral response to pain, consistent with the clinical findings. However, repeated pairing of pain with the mother's presence during infancy produces precocious amygdala development and has long-term consequences on affect. Infant rats were given mild tail shock (0.5mA, 1s every 4-min for 32 min) either with the mother present or absent for 5 consecutive days. Two age ranges were chosen to represent the sensitive period for pain programming (PN5-9) and the age at which maternal presence has major neurobehavioral effects, including suppression of amygdala activity (PN10-14). Activity and ultrasonic vocalizations at both ages were reduced by the mother's presence during exposure to painful shock. Following treatment at PN10-14 pups only, Fos expression in the periaqueductal gray was elevated by the shock alone and reduced by the mother. In adulthood, pain thresholds, social behavior, and unlearned fear behavior were assessed as a function of infant pain experience. Adults treated at PN5-9 had reduced carrageenan-induced hyperalgesia, reduced social behavior, but no changes in fear behavior if

they had had pain-mother pairings. In contrast, when treated at PN10-14, the adult had no change in hyperalgesia, and showed disruption of social behavior. Shock with or without the mother decreased fear responding only if treated at PN10-15. The social behavior deficits were normalized by two weeks of environmentally-enriched rearing after weaning. The results of these studies aid in our understanding of the impact of nursery procedures used to attenuate pain on later outcomes focused on affective behaviors, and potentially provide a strategy to reduce those effects.

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Nanosymposium

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Topic: F.04. Stress and the Brain

Support: HD083217

MH091451

Title: Amygdala PKM zeta increases with functional emergence of amygdala-dependent fear learning in rat pups

Authors: *M. OPENDAK[1], R. M. SULLIVAN[1], P. A. SERRANO[2];
[1]Child and Adolescent Psychiatry, New York Univ., New York, NY; [2]Dept of Psychology, Hunter College, CUNY, New York, NY

Abstract: During infancy rapid learning associated with attachment and orientation to a caregiver is essential to survival. This developmental period also prevents the acquisition of avoidance learning. In rodent models this developmental time window occurs prior to post-natal day 10 (PND 10), during which pups display heightened preference learning accompanied by decreased aversion learning. PND 10 rats presented with odor-shock pairings fail to avoid the odor associated with shock, and show a preference for the paired odor. Older pups (PND-12) given odor-shock pairings develop an aversion to the odor at subsequent test. Even though pups at any age find the shock itself aversive, as measured by vocalizations and overall activity. One key developmental mechanism that appears to direct the change from a preference for the odor associated with shock to an aversion, involves the activation of the amygdala by corticosterone. Corticosterone is low in pups during the sensitive period and increases at PND 10. We

investigate synaptic markers which may be important for establishing the avoidance memory and that are likely activated by corticosterone. Recently, protein kinase M zeta (PKMζ), which is important for late-phase LTP and long-term memory, is also upregulated during stress (Sebastian et al 2013, PLoS One, vol 8, e79077). Therefore, we investigated the role of PKMζ in avoidance vs preference learning in rat pups using the paired odor-0.5mA shock fear-conditioning paradigm. PND 8 and PND 12 pups were given either paired (simultaneous odor and shock) or unpaired (shock 2 min after odor) training and tested 24hr later on a Y maze with one arm containing the conditioned stimulus (CS) odor and the other a familiar odor. Immediately after Y maze test, pups were sacrificed and amygdalae were harvested. The tissues were separated into cytosolic and synaptic cellular fractions. Each fraction was analyzed by Western blots. Pups in the unpaired condition showed no preference for either arm. PND 8 paired pups in the paired condition preferred the CS odor and PND 12 pups avoided the CS odor ($p<0.01$). PND 12 in the paired condition had higher cytosolic PKMζ in the amygdala compared to unpaired pups ($p<0.05$) with no change in synaptic PKMζ. PN8 paired did not show any changes in cytosolic or synaptic PKMζ. Thus, increased PKMζ expression following the sensitive period plays a role in the activation process of the amygdala and the formation of aversive memories.

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487. Enduring Consequences of Early Stress I

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Topic: F.04. Stress and the Brain

Title: Neonatal pain and reduced maternal care interact to impact brain development

Authors: ***S. M. MOONEY-LEBER**, S. BRUMMELTE;
Wayne State Univ., Detroit, MI

Abstract: Preterm infants are exposed to a multitude of painful procedures while in the neonatal intensive care unit (NICU), most of which are mandatory for survival. Recently, it has been suggested that exposure to painful procedures during the neonatal period results in impaired brain development and cognitive functioning. However, less is known about the underlying mechanisms producing these negative outcomes. In conjunction with pain exposure, preterm infants experience reduced maternal care due to the constraints of the NICU. Animal models have demonstrated that reduced maternal care has a profound negative impact on biobehavioral development, but there is a dearth of knowledge surrounding the impact of reduced maternal care in combination with neonatal pain. The current study sought to investigate the biological

consequences of both stressors by employing a standard repetitive needle poke pain regimen and a novel reduced maternal care paradigm to mimic standard NICU care. First, rat pups within a litter were assigned to one of 5 groups: unhandled control, tactile control, pain group, reduced maternal care group, and pain and reduced maternal care. Painful procedures consisted of needle insertion into alternating paws several times a day for the first 4 days of life. Rat pups in the reduced maternal care groups were placed in a tea-ball infuser (which provide olfactory cues while restricting direct maternal care) for 30 minutes immediately following administration of painful procedures or tactile stimulation and returned to their home cage. We observed maternal care during and after tea-ball encapsulation and on postnatal day 8 rat pups underwent a cardiac perfusion and brains were collected for histological analysis. Preliminary results revealed that the presence of a tea-ball infuser did not influence maternal care and that group assignment did not alter pup body weight, suggesting that tea-ball pups received less maternal care (as intended) but still received sufficient nursing. Further, we hypothesize that reduced maternal care and neonatal pain individually will produce impairments in brain development and that this deleterious effect will be exacerbated in neonatal pups that experienced both stressors.

Disclosures: S.M. Mooney-Leber: None. **S. Brummelte:** None.

Nanosymposium

487. Enduring Consequences of Early Stress I

Location: SDCC 5B

Time: Tuesday, November 15, 2016, 8:00 AM - 11:30 AM

Presentation Number: 487.04

Topic: F.04. Stress and the Brain

Support: NIMH Grant 5R21MH097182

Title: Early life stress leads to sex-dependent precocial limbic innervation into the PFC: a putative mechanism of stress-related pathological vulnerability

Authors: *J. A. HONEYCUTT, H. C. BRENHOUSE;
Psychology, Northeastern Univ., Boston, MA

Abstract: Early life experiences significantly shape the behavioral and neural trajectory of an organism across development. Therefore, disruptions during early developmental periods may set the course for aberrant brain maturation. In fact, children who have experienced early life adversity (i.e. abandonment, maltreatment) often exhibit deleterious effects which can manifest as maladaptive behaviors, cognitive impairments, and an increased susceptibility to mental illness (e.g. schizophrenia, anxiety). Increasing evidence in human populations who have experienced childhood adversity points to a role of atypical corticolimbic circuit development,

leading to changes in functional connectivity between the basolateral amygdala (BLA) and prefrontal cortex (PFC). In rodent models of early life adversity via maternal separation (MS) during the postnatal period (P2-21) comparable behavioral and neural phenotypes are observed, including loss of PFC inhibitory tone and asynchrony in addition to increased anxiety-like behaviors. The distinct mechanistic underpinnings leading to these findings following MS remain unknown, however it is likely that this dysfunction is driven, in part, by precocial BLA innervation of the PFC. In order to determine the impact of MS and sex on the developmental time course of BLA-PFC connectivity, we performed targeted microinjections of the anterograde tracer biotinylated dextran amines (BDA) into the BLA at key developmental milestones spanning between juvenility and adulthood: P25, P35, P45, and P90. BDA-labeled fibers from targeted BLA PFC-projecting neurons were quantified in the pre- and infra-limbic PFC. Here we present novel and exciting data indicating that MS drives BLA afferents to precocially innervate the PFC, thereby conferring increased vulnerability in a sex- and age-dependent manner, such that MS female innervation as early as P25 resembles that of their P45 control counterparts. Our findings suggest an important role for early life experiences on corticolimbic development and provide putative mechanistic insight into the underlying etiology of adversity-induced vulnerability and resilience.

Disclosures: J.A. Honeycutt: None. **H.C. Brenhouse:** None.

Nanosymposium

487. Enduring Consequences of Early Stress I

Location: SDCC 5B

Time: Tuesday, November 15, 2016, 8:00 AM - 11:30 AM

Presentation Number: 487.05

Topic: F.04. Stress and the Brain

Support: U01 AA014834

F32 AA022561

Title: Associations between socioeconomic status and cortical thickness are altered in adolescents with prenatal alcohol exposure

Authors: *K. A. UBAN[1], E. C. KAN[3], B. HERNANDEZ[2], M. M. HERTING[3], J. WOZNIAK[4], S. MATTSON[5], C. COLES[6], E. R. SOWELL[3];
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Abstract: Background
Fetal alcohol spectrum disorder (FASD) is completely preventable, yet remains among the top 3 known causes of intellectual disability. Socioeconomic status (SES) for youth is characterized by a combination of factors, including family income, parental educational attainment and occupational status, and is known to be an influential factor for cognitive function. Differences in brain volumes exist between youth from low and high SES samples. SES was associated with FASD-related cognitive and behavioral problems among human adolescents in South Africa. The present study examined associations between SES and cortical thickness among adolescents with prenatal alcohol exposure (PAE) compared to non-exposed Controls.
Methods
T1-weighted MRI data were obtained in adolescents with PAE and age- sex- matched Controls (n=206, 49% with PAE, 44% female, 6.5-19.9 years old), collected across 4 imaging sites as part of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD). All data were processed through FreeSurfer v5.1. General linear modeling was utilized to examine the impact of SES on whole-brain cortical thickness for adolescents with PAE and Controls, while controlling for sex, age and imaging site. Parental educational level and income were utilized as measures of SES. Monte Carlo simulation was utilized to correct for multiple comparisons.
Results
Significant positive associations between SES measures and cortical thickness were observed within the frontal, temporal and occipital lobes among Controls (corrected $p \leq 0.05$), but not youth with PAE.
Discussion
These findings demonstrate that associations between SES and cortical thickness are altered among human children and adolescents with PAE. Animal studies have demonstrated that neuroplasticity in response to environmental factors is often attenuated by PAE. The current results are consistent with a PAE-related reduction in plasticity to environmental factors (SES) in the human developing brain. The present results increase our understanding of the impact of PAE on the developing human brain within varying environmental contexts, and may inform experience-related FASD-interventions.
(Research supported by CIFASD U01 AA014834; F32 AA022561)

Disclosures: **K.A. Uban:** None. **E.C. Kan:** None. **B. Hernandez:** None. **M.M. Herting:** None. **J. Wozniak:** None. **S. Mattson:** None. **C. Coles:** None. **E.R. Sowell:** None.

Nanosymposium

487. Enduring Consequences of Early Stress I

Location: SDCC 5B

Time: Tuesday, November 15, 2016, 8:00 AM - 11:30 AM

Presentation Number: 487.06

Topic: F.04. Stress and the Brain

Support: ARC grant DP150104835

NHMRC grant APP1031688

Australian Postgraduate Award

Title: Impaired social buffering following direct & indirect maternal separation stress

Authors: *J. M. KAN, R. RICHARDSON;
Univ. of New South Wales, UNSW Sydney, Australia

Abstract: Social buffering, particularly early in life, has been shown to be a powerful regulator of stress and pain reactivity, and is a protective factor against later psychiatric illness. In this research, we show that exposure to early life stress attenuates social buffering in infant rodents. In addition, we show a generational effect of stress on social buffering, such that reduced buffering is observed when infants are indirectly exposed to stress through their mother. Females were mated and either standard-reared or maternally separated from their infant offspring for 3 hr/daily from postnatal day (PD) 2-14. The first set of buffering tests was conducted on these directly separated offspring at PD15. Infant offspring of standard-reared (SR) or maternally-separated (MS) mothers were given unpredictable shocks either alone or in the presence of an anaesthetised mother. Both the intensity of the animal's behavioural reactions as well as the number of ultrasonic vocalisations (USVs) emitted were measured. The same mothers were mated a subsequent time, but no further maternal separation occurred. A second set of maternal buffering tests was conducted on these subsequent offspring (MS-SUB) at PD15 and involved the same measures described above.

All groups exhibited strong behavioural responses when tested alone. Importantly, while offspring of SR mothers decreased their responding in the presence of a mother (i.e., they exhibited social buffering), both MS and MS-SUB offspring continued to show high levels of responding. In other words, these infants were impaired in social buffering. Similarly, all groups exhibited high levels of USVs when tested alone. When tested with a mother, SR infants emitted fewer USVs, and MS-SUB infants continued to emit high USVs - a pattern consistent with the behavioural responses exhibited by these two groups. In contrast to the behavioural response test, directly exposed MS infants emitted decreased USVs to the shocks when tested with a mother, indicating a buffering effect.

Together, this research adds to the body of evidence demonstrating the maladaptive consequences of stress exposure. The inconsistency between behavioral reactions and USV emissions in directly exposed MS infants will be discussed. Finally, findings in the subsequent offspring illustrate that past maternal experiences can have a potent and lasting generational effect, and highlights the need for stress research to extend beyond the individual to account for caregiver influences.

Disclosures: **J.M. Kan:** None. **R. Richardson:** None.

Nanosymposium

487. Enduring Consequences of Early Stress I

Location: SDCC 5B

Time: Tuesday, November 15, 2016, 8:00 AM - 11:30 AM

Presentation Number: 487.07

Topic: F.04. Stress and the Brain

Title: Behavioral effects of *In utero* sertraline exposure (a selective serotonin reuptake inhibitor) on male and female rats

Authors: *J. M. KOTT[1], S. M. MOONEY-LEBER[1], S. A. PERRINE[2], S. BRUMMELTE[1]; [1]Psychology, [2]Psychiatry and Behavioral Neurosciences, Wayne State Univ., Detroit, MI

Abstract: Sertraline, a commonly prescribed selective serotonin reuptake inhibitor (SSRI), has the ability to cross the placental barrier in pregnant women and may consequently impact the development of a fetus. Anti-depressant-induced changes in the serotonergic system may have drastic effects on brain development and thus the behavioral outcome of the offspring. This study seeks to investigate the long-term consequences of sertraline exposure during pregnancy in a translational animal model of preconceptional stress/depression. In this model, female Sprague-Dawley rats were first treated with a vehicle (sesame oil) or the stress-hormone corticosterone (CORT, 40 mg/kg, s.c.) for 21 days to induce a depressive-like phenotype in the animals. After 16 days of CORT or oil administration, these "depressed' or healthy (control) rats were further divided to either receive sertraline (20 mg/kg, p.o.) or a vehicle (water) daily and then mated with healthy males one day after the cessation of CORT treatment. This approach was chosen to mimic a situation in which a woman experiencing depression requires treatment with an SSRI, but then becomes pregnant, and is faced with the choice whether or not to continue or discontinue the antidepressant medication given the potential consequences for the developing fetus. Therefore, half of the animals receiving sertraline discontinued the treatment at gestational day (GD) 16, and the remaining half continued treatment through parturition to better understand the implications of continued medication vs. withdrawal during pregnancy for the offspring. Results revealed altered neurotransmitter levels in the brains of neonatal animals (postnatal day (PD) 1) exposed to sertraline during gestation, but surprisingly no group differences in anxiety behavior on the Elevated Plus Maze in adulthood. Further, preliminary data suggests, that the discontinuation of sertraline during pregnancy does not result in a 'better' behavioral outcome compared to the continued exposure. There is a critical need for more research on the effects of exposure to antidepressant medication compared to depression per se during pregnancy, and how each impacts neurochemical profiles during development and later behavioral outcome.

Disclosures: J.M. Kott: None. **S.M. Mooney-Leber:** None. **S.A. Perrine:** None. **S. Brummelte:** None.

Nanosymposium

487. Enduring Consequences of Early Stress I

Location: SDCC 5B

Time: Tuesday, November 15, 2016, 8:00 AM - 11:30 AM

Presentation Number: 487.08

Topic: F.04. Stress and the Brain

Support: NIH R01 MH091864

NSF GRFP DGE-1144087

Title: Parental deprivation induced alterations in amygdala-cortical functional connectivity across human development as risk and resilience factors for concurrent and long-term internalizing symptomatology

Authors: *L. GABARD-DURNAM[1], D. FARERI[3], B. GOFF[4], J. FLANNERY[7], D. GEE[8], E. TELZER[9], K. L. HUMPHREYS[10], C. CALDERA[5], M. SHAPIRO[6], N. TOTTENHAM[2]; [1]Columbia Univ., New York, NY; [2]Columbia Univ., New York City, NY; [3]Adelphi, New York City, NY; [4]UCLA, Los Angeles, NY; [5]UCLA, los angeles, CA; [6]UCLA, Los Angeles, CA; [7]U. Oregon, Eugene, OR; [8]Weill Cornell, New York City, NY; [9]U. Illinois Urbana-Champagne, Champagne, IL; [10]Stanford, Paolo Alto, CA

Abstract: A rich non-human animal literature has demonstrated that early life stress in the form of parental deprivation in the early postnatal period has potent effects on the structure and function of developing amygdala-cortical circuitry. In humans, a similar parental deprivation is experienced by previously institutionalized (PI) youths adopted out of orphanages. These PI youth have been shown to manifest early emergence of mature-like negative amygdala-medial prefrontal cortex (PFC) functional connectivity in response to emotional face stimuli that is associated with lower internalizing symptomatology, suggesting this circuitry alteration serves as a short-term adaptation. The present study aimed to examine how parental deprivation affects the subsequent development of amygdala-cortical resting-state functional architecture relative to typically-raised, typically-developing youth, and how altered network connections may serve as either adaptations or risk-factors relating to emotion regulation difficulties in both the short term (concurrent measurements) and long term (2 and 4 years later). Fifty PI youths aged 6 to 18 years old and fifty typically-developing comparison youths matched for age, gender, puberty, motion, and usable data length contributed usable resting-state data. Internalizing symptomatology was measured using parent-reported Revised Child Anxiety and Depression Scales (RCADS-P) scores. PI youth showed altered amygdala-cortical connectivity with regions of the PFC and sensory cortices relative to the typically developing group in a whole-brain analysis ($p < 0.05$, $FWE < 0.05$). A significant group x age interaction revealed that PI youth recruit additional lateral and medial PFC regions to their amygdala network across development that are not recruited in typical development. Moreover, PI youth did not show age-related change in

connectivity between the amygdala and a medial PFC region observed in a prior typically developing sample. These amygdala-PFC connectivity alterations differentially related to the RCADS-P internalizing scores as well. Together, these results suggest that robust alterations occur in amygdala-cortical, and especially amygdala-PFC networks as a result of early parental deprivation. Moreover, these alterations may serve as both resilience and risk factors for internalizing symptomatology across childhood and adolescence.

Disclosures: **L. Gabard-Durnam:** None. **D. Fareri:** None. **B. Goff:** None. **J. Flannery:** None. **D. Gee:** None. **E. Telzer:** None. **K.L. Humphreys:** None. **C. Caldera:** None. **M. Shapiro:** None. **N. Tottenham:** None.

Nanosymposium

487. Enduring Consequences of Early Stress I

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Presentation Number: 487.09

Topic: F.04. Stress and the Brain

Support: NIGMS 1P20GM103653

Title: Altering the epigenetic landscape: Counteracting the effects of early stress with epigenome modifiers

Authors: ***T. S. DOHERTY**, S. KELLER, J. BLAZE, T. ROTH;
Univ. of Delaware, Newark, DE

Abstract: Disruption of infant-caregiver attachment is a major risk factor for the development of aberrant behavior. While the mechanisms by which this disruption affects behavioral outcomes have not been fully elucidated, epigenetic mechanisms have emerged as promising candidates. We have previously reported altered patterns of methylation associated with brain derived neurotrophic factor (*Bdnf*) DNA the in the prefrontal cortex, hippocampus and amygdala of rats following exposure to caregiver maltreatment. Given that *Bdnf* is a known player in developmental processes and that its dysregulation has been implicated in a variety of behavioral disorders, aberrant methylation of this gene may underlie some of the behavioral abnormalities associated with early-life stress. One aim of this work was to measure behavioral outcomes (through assessment of cognitive and anxiety- and depressive-like behaviors) in adolescence and adulthood in rats exposed to maltreatment in infancy. The second aim of this work was to assess the ability of epigenome modifiers (for example, sodium butyrate) to prevent the altered methylation patterns previously found in maltreated rats. Infant male and female Long Evans rats were subjected to either nurturing care (from their biological mother or a foster dam) or

maltreatment from a foster dam for 30 minutes daily from postnatal days (PN) 1 to 7. Performance was then assessed with several behavioral tasks at PN30 and PN90, with task- and sex-dependent deficits found in the maltreatment group. For aim 2, drug was administered daily to each group prior to caregiving manipulations and brains were extracted at PN8 for methylation assays. Results will be discussed in the framework of mechanisms and interventions in early-life stress. Future work will focus on the utility of epigenome modifiers to prevent behavioral outcomes associated with maltreatment.

Disclosures: **T.S. Doherty:** None. **S. Keller:** None. **J. Blaze:** None. **T. Roth:** None.

Nanosymposium

487. Enduring Consequences of Early Stress I

Location: SDCC 5B

Time: Tuesday, November 15, 2016, 8:00 AM - 11:30 AM

Presentation Number: 487.10

Topic: F.04. Stress and the Brain

Title: Absence of Tau prevents stress-induced neuronal atrophy: the role of synaptic mitochondria

Authors: I. SOTIROPOULOS[1,2], S. LOPES[1,2], L. TEPLYTSKA[3], J. VAZ-SILVA[1,2], C. DIOLI[1,2], M. MORAIS[1,2], *J. A. PALHA[4], C. WEBHOFER[3], O. F. X. ALMEIDA[3], C. W. TURCK[3], N. SOUSA[1,2], M. D. FILIOU[3];
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Abstract: Tau protein in dendrites and synapses has been recently implicated in synaptic degeneration and neuronal malfunction. Chronic stress, a well-known inducer of neuronal and synaptic atrophy, triggers hyperphosphorylation of Tau protein and cognitive deficits. However, the cause-effect relationship between these events remains to be established. To test the involvement of Tau in stress-induced impairments of cognition, we investigated the impact of stress on cognitive behavior and neuronal structure as well as on the synaptic proteome in the prefrontal cortex (PFC) of Tau knock-out (Tau-KO) and wild-type (WT) mice. Whereas exposure to chronic stress resulted in atrophy of apical dendrites and spine loss in PFC neurons and significant impairments in working memory in WT mice, such changes were absent in Tau-KO animals. Quantitative proteomic analysis of PFC synaptosomal fractions, combined with transmission electron microscopy analysis, suggested a prominent role for mitochondria in the mediation of the effects of stress. Specifically, chronically stressed animals exhibit Tau-dependent alterations in the levels of proteins involved in mitochondrial transport and oxidative

phosphorylation as well as in the synaptic localization of mitochondria in PFC. These findings provide evidence for a causal role of Tau in mediation of stress-elicited neuronal atrophy in PFC and related cognitive deficits indicating that stress and Tau may exert its synaptotoxic effects through mitochondria.

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Nanosymposium

487. Enduring Consequences of Early Stress I

Location: SDCC 5B

Time: Tuesday, November 15, 2016, 8:00 AM - 11:30 AM

Presentation Number: 487.11

Topic: G.06. Post-traumatic Stress Disorder

Support: NIH Grant NS073899

NIH Grant MH103848

Title: High FKBP5 expression alters learning and memory

Authors: ***L. J. BLAIR**[1], X. WANG[2], D. ZHENG[1], J. J. SABBAGH[1], E. J. WEEBER[2], C. A. DICKEY[1];
[1]Mol. Med., [2]Pharmacol. and Physiol., USF Byrd Inst., Tampa, FL

Abstract: FK506-binding protein 5 (FKBP5) has been shown to contain single nucleotide polymorphisms (SNPs) which increases risk of psychiatric diseases, like post-traumatic stress disorder (PTSD), when combined with environmental factors. While mechanisms of FKBP5 contribution to this increased risk are still under investigation, it has been shown that many of these SNPs increase FKBP5 expression through decreased FKBP5 DNA methylation. To evaluate the consequences of this enhanced expression, we generated a novel mouse model using targeted insertion of a single copy of the *FKBP5* gene at the Hipp11 locus. The inserted FKBP5 contained a Tetracycline operator, which allowed for high expression throughout the forebrain when crossed with an activator line. Evaluation of this model was done using behavioral, electrophysiological, and biochemical analysis. Overall, we have found that high expression of FKBP5 in the brain does alter normal learning and memory. Importantly, this alteration was detectable in the absence of stress and other environmental factors. Further studies in this model may help reveal additional mechanisms by which FKBP5 contributes to PTSD and other psychiatric disorders.

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Nanosymposium

487. Enduring Consequences of Early Stress I

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Topic: F.04. Stress and the Brain

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Ministero della Salute GR-2011-02352187

Università Cattolica intramural grant Linea D3.2 2015

Title: The transgenerational effects of high fat diet and novel enrichment environment on Bdnf expression and cognitive function

Authors: ***S. FUSCO**[1,2], A. MASTRODONATO[1], M. SPINELLI[1], S. COCCO[1], C. RIPOLI[1], S. BARBATI[1], R. PIACENTINI[1], C. GRASSI[1];
[1]Inst. of Human Physiol., Universita' Cattolica Med. Sch., Rome, Italy; [2]IRCCS San Raffaele Pisana, Rome, Italy

Abstract: It is well known that early life experiences induce long-term modifications. Here we checked whether maternal dysmetabolic environment affects cognitive performance and key neuronal gene expression in the brain of descendants via epigenetic mechanisms. C57 adult female mice (F0) were fed with either standard or high-fat diet (SD or HFD) from 4 weeks before mating until the 3rd week of suckling. The first generation of HFD-fed mice, hereinafter referred as F1-HFD, and their descendants (F2-HFD and F3-HFD, respectively) were all fed with SD and were tested by behavioral, electrophysiological and molecular analyses. Our findings demonstrate that maternal overnutrition alters learning and memory in the offspring and next generations. All HFD-descendant mice showed a lower discrimination index than the SD mice in a standard novel object recognition paradigm (F1-HFD = 56.5 ± 1.1%, F2-HFD = 54.6 ± 1.5%, F3-HFD = 55.9 ± 0.5% vs SD = 68.9 ± 0.6%; n=8 , $p<0.01$ for each group). These effects were associated with a significant impairment of spatial learning and memory in the Morris water maze. Time spent to reach the platform at the 3rd and the 4th training days was increased by +71.4% and +99.6%, respectively in F1-HFD; +78.1% and +91.3% in F2-HFD; +70.3% and +58.6% in F3-HFD, when compared to SD mice (n=8 , $p<0.01$ per each group). Accordingly, electrophysiological analyses on hippocampal brain slices of F1-, F2- and F3-HFD mice revealed

severe deficits of long-term potentiation at CA3-CA1 synapses (ranging from -37.3% in F1-HFD to -52.2% in F3-HFD; n = 11 slices from 4 mice per group, $p<0.05$). Finally, maternal HFD reduced multiple BDNF transcripts and its expression at the protein level via specific epigenetic mechanisms involving BDNF regulatory sequences. More importantly, in caudal epididymis of HFD-descendants male mice we found the same epigenetic marks observed in their hippocampi. Notably, the exposure of F1-HFD male mice to novel enrichment environment before mating counteracted the transmission of both behavioral and molecular changes to the third generation. Collectively, our data suggest that maternal HFD alters BDNF expression and cognitive performances in the descendants via transgenerational epigenetic changes susceptible to the lifestyle.

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Nanosymposium

487. Enduring Consequences of Early Stress I

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Time: Tuesday, November 15, 2016, 8:00 AM - 11:30 AM

Presentation Number: 487.13

Topic: F.04. Stress and the Brain

Support: Samarbeidsorganet HMN-NTNU project number 46056907

Research Council of Norway's FRIMED program project number 204935/F20

Title: Effects of preterm birth and very low birth weight on cortical and subcortical development: A longitudinal MRI study in Norway

Authors: ***K. SRIPADA**[1], K. J. BJULAND[1], A. E. SØLSNES[1], A. K. HÅBERG[1,2], K. GRUNEWALDT[1], G. C. LØHAUGEN[1,3], L. M. RIMOL[1], J. SKRANES[1,3];
[1]Norwegian Univ. of Sci. and Technol., Trondheim, Norway; [2]St. Olav Univ. Hosp., Trondheim, Norway; [3]Sørlandet Hosp., Arendal, Norway

Abstract: [Background] Neurodevelopmental challenges including lower IQ, poorer visual and motor skills, and behavioral problems have been associated with preterm birth (gestational age ≤ 37 weeks) and very low birth weight (VLBW, birth weight ≤ 1500 g). Longitudinal neuroimaging is the only way to truly assess growth and differences in growth and can identify divergent structural and cognitive developmental trajectories following preterm birth. [Methods] Preterm-born VLBW subjects born between 2003 and 2007 were recruited based on admittance to the Neonatal Intensive Care Unit at St. Olav's University Hospital in Trondheim, Norway

(n=46). Term-born control subjects were recruited from the national Norwegian Mother and Child Cohort Study (MoBa) from the Trøndelag region in Norway (n=134). MRI and cognitive data were collected at two timepoints in childhood: first at mean age 8.0 years (range: 4.9-10.6) then at mean age 9.1 years (range: 6.1-12.0). 132 participants had two successful MRI scans, and 48 with only one successful scan were also included.MRI data were collected on a 1.5 T Siemens Avanto scanner using 3D T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) scans at both timepoints. Each MPRAGE series was visually inspected, and only scans with no or minimal movement artifacts were included. FreeSurfer version 5.3.0 was used for morphometric analyses. [Results] This study investigates possible changes in subcortical volumes, cortical thickness, and cortical surface area longitudinally. Comparing the VLBW children to term-born peers, VLBW children had smaller volumes of subcortical gray matter structures, smaller cortical surface area bilaterally in frontal, temporal, and parietal lobes, thicker cortex in the frontal and occipital regions, thinner cortex in the posterior parietal region bilaterally, and minor group x time longitudinal effects in cortical measures using linear mixed effects models. The study will also assess relationships between cognitive measures including IQ scores and age-related changes in gray matter structures in the two populations. [Discussion] Differences in cortical and subcortical measures by middle childhood can reflect both lasting effects effects of preterm birth with VLBW as well as possible differential postnatal developmental trajectories in the brain. The structures implicated in this study may suggest specific brain areas sensitive to early life pathology that persist through childhood.

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Nanosymposium

487. Enduring Consequences of Early Stress I

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Time: Tuesday, November 15, 2016, 8:00 AM - 11:30 AM

Presentation Number: 487.14

Topic: C.09. Brain Injury and Trauma

Support: NIMH R01 MH096093

NIMH R01 MH087660

Title: Impact of early life stress on functional circuitry in mouse models of PTSD: A neuroimaging study

Authors: *E. L. BEARER[1], V. CALHOUN[2], R. E. JACOBS[3];
[1]Dept. of Pathology, UNM Sch. of Med., Albuquerque, NM; [2]Image Analysis and MR, The Mind Res. Network, Albuquerque, NM; [3]Beckman Inst., Caltech, Pasadena, CA

Abstract: Childhood adversity profoundly impacts physical and emotional well-being throughout the life-span. Brain regions consistently altered by childhood maltreatment are those involved in the limbic system: the medial prefrontal and anterior cingulate cortex (mPFC and ACC), as well as hippocampus, amygdala and deeper dopaminergic and serotonergic nuclei. Epigenetic changes, such as DNA methylation, are also emerging as crucial mediators of long-term embedding of childhood maltreatment. Yet it is not known how circuitry modification is linked to maltreatment, nor its developmental vulnerability and time course biologically. Animal models in which experimental evidence testing these questions are critically needed, as is the methodology to discover the biological mechanism(s) that result in the enduring impact of childhood adversity. With this information we can design post-trauma interventions that will move towards improved life-span outcomes. We have tested whether the fragmented care mouse model of early life stress induces altered pre-limbic circuitry in wild-type and SERT knock-out mice by manganese-enhanced magnetic resonance imaging. We used our published mPFC injection procedure to introduce Mn^{2+} into the prelimbic circuit and imaged circuitry over time at high field MR in an 11.7T Bruker scanner. We compared Early Life Stress (ELS) - treated mice with mice having no ELS with and without the SERT gene (n=12 in each group). Images are skull-stripped, align-warped, and then statistical parametric maps prepared using SPM. We begin with flexible factorial comparisons for effect of condition across all groups, and then perform within-group between time-points. Our preliminary analysis demonstrates large differences between the anatomy over time of the functional circuitry from forebrain into deeper limbic structures ($p=<0.05$ FDR). ELS and SERT-KO mice also display enhanced and prolonged anxiety behavior after exposure to predator odor, a naturally occurring unconditioned fear response, as measured by time spent in the dark of the light-dark box and freezing in the open field behavioral paradigms. Preliminary analysis of forebrain DNA methylation patterns of 38,000 sites correlating to human meDNA using the hm450k Illumina Bead Chip revealed both hyper- and hypo-methylation in the experimental groups compared to wildtype. In all cases, the ELS exposed mice were more affected than even the SERT-KO, which is one of only four validated rodent models of PTSD. A next step will be to discover the timing of methylation events, and whether/which precede or depend on circuitry alterations.
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Nanosymposium

488. Schizophrenia: Genetics and Genomics

Location: SDCC 7B

Time: Tuesday, November 15, 2016, 8:00 AM - 9:30 AM

Presentation Number: 488.01

Topic: H.03. Schizophrenia

Title: Novel circular RNA transcripts identified in differentiated human SH-SY5Y neuroblasts using RNA-Seq

Authors: *E. MAHMOUDI[1], M. GEAGHAN[1], M. CAIRNS[1,2];
[1]Sch. of Biomed. Sci. & Pharm., Univ. of Newcastle, Newcastle, Australia; [2]Schizophrenia Res. Inst., Sydney, Australia

Abstract: Circular RNA (circRNA) is a novel class of long non-coding RNA generated by non-sequential back-splicing of pre-mRNA transcripts. CircRNA have been shown to have cellular function, for example as microRNA sponges, RNA-binding protein magnets and transcription promoter, suggesting they provide an additional mechanism in gene regulation. CircRNAs have been discovered in several different species and many tissues, particularly the brain where circRNAs are highly enriched relative to other tissues. In the current study we profiled rare endogenous circRNAs in differentiated human neuron-like SH-SY5Y cells, by performing high-throughput sequencing (RNA-seq) of libraries prepared from total RNA depleted of linear RNA. The depletion step was achieved in addition to ribosomal RNA removal by RNA-exonuclease digestion with RNase R. The libraries were sequenced on an illumina NextSeq500 to a read density of 55 million reads per sample. This revealed thousands of circRNA including many novel species, further supporting the abundance of this type of RNA in brain tissue. Further analysis of circRNA alteration after KCl-induced depolarization identified several differentially expressed circRNAs including circ-PEX13, circ-DLG2 and circ-R3HDM1 (all above 3.2-fold change, $P < 0.05$) Analysis of the differentially expressed circRNAs indicated their host genes were mostly implicated in brain-related biological processes such as synaptic remodeling, supporting a role for circRNA in neuronal function. These findings provide more evidence for the abundance of circular RNA in neurons and support a potential role for these molecules in gene regulation of the nervous system.

Disclosures: **E. Mahmoudi:** None. **M. Geaghan:** None. **M. Cairns:** None.

Nanosymposium

488. Schizophrenia: Genetics and Genomics

Location: SDCC 7B

Time: Tuesday, November 15, 2016, 8:00 AM - 9:30 AM

Presentation Number: 488.02

Topic: H.03. Schizophrenia

Title: *In vitro* characterization of the mouse model of the human 15q13.3 microdeletion associated to schizophrenia

Authors: *T. N. JØRGENSEN, I. V. KLEWE, L. ARVASTSON, D. CLAUSEN, T. BENNED-JENSEN, J. NIELSEN;
Synaptic Transmission, H. Lundbeck, Valby, Denmark

Abstract: The 15q13.3 microdeletion is a copy number variant (CNV) with hemizygous deletion of seven genes (MTMR15, MTMR10, TRPM 1, MIR211, KLF13, OTUD7A, and CHRNA7) on chromosome 15. The microdeletion is associated with a ~10-fold increase in the risk of schizophrenia, which makes it one of the strongest known genetic risk factors for the disorder. We have previously characterized a hemizygous mouse model of the human 15q13.3 microdeletion, and identified several schizophrenia-relevant in vivo phenotypes supporting the translational value of this genetic mouse model (Fejgin et al. Biol Psychiatry (2014);76:128-137). In the present study, we aim to investigate the biological alterations of the 15q13.3 model using primary cortical cultures from 15q13.3 mice. Based on calcium imaging of neuronal populations in culture combined with a multivariate analysis of the spontaneous firing pattern in the cultures, we identified an altered network activity in 15q13.3 compared to cultures from wild type littermates. Furthermore, an increase in GABA transporter 1 (GAT-1) mediated GABA uptake was observed for 15q13.3 cultures. The latter was in accordance with an increase in the mRNA levels of the SLC6A1 (GAT-1) transcript and a decrease in the levels of GABA in the culture media. These findings advance the mechanistic understanding of the 15q13.3 microsomal deletion associated to schizophrenia and provide an in vitro model for further dissection of the molecular mechanisms behind the disorders.

Disclosures: **T.N. Jørgensen:** A. Employment/Salary (full or part-time): H. Lundbeck A/S. **I.V. Klewe:** A. Employment/Salary (full or part-time): H. Lundbeck A/S. **L. Arvastson:** A. Employment/Salary (full or part-time): H. Lundbeck A/S. **D. Clausen:** A. Employment/Salary (full or part-time): H. Lundbeck A/S. **T. Benned-Jensen:** A. Employment/Salary (full or part-time): H. Lundbeck A/S. **J. Nielsen:** A. Employment/Salary (full or part-time): H. Lundbeck A/S.

Nanosymposium

488. Schizophrenia: Genetics and Genomics

Location: SDCC 7B

Time: Tuesday, November 15, 2016, 8:00 AM - 9:30 AM

Presentation Number: 488.03

Topic: H.03. Schizophrenia

Title: Sex differences in a population with familial high risk for psychosis: analysis of neuroanatomical and symptom sexual dimorphisms

Authors: *E. GUMA[1], G. A. DEVENYI[2], J. GERMANN[2], M. CHAKRAVARTY[2]; [1]Douglas Res. Ctr. - McGill Univ., Verdun, QC, Canada; [2]Douglas Res. Ctr. - McGill Univ., Montreal, QC, Canada

Abstract: Sex differences exist in the clinical manifestation of schizophrenia (SZ). Males typically have early age-of-onset, increased negative symptom burden, and impaired social functioning. Conversely, females have higher affective symptom burden, but better outcomes. Neuroanatomical sexual dimorphism has also been observed in SZ; males have larger ventricular, smaller temporal lobe and hippocampal volumes, whereas females have smaller cingulate and orbital cortices. Since sex differences are present in SZ, we expect similar dimorphisms in those at high-risk. Having a first-degree relative with SZ (ie: familial risk) is a risk factors for SZ. To better understand neuroanatomical sexual dimorphism in individuals with familial risk we investigated SZ patients (171;114 male), their unaffected siblings (44;21 male), and controls (170;86 male) (data from NUSDAST). The CIVET processing pipeline (1.1.13) and MAGeTBrain algorithm were used for cortical and subcortical analyses, respectively. We observed sexual dimorphism in total cortical surface area (SA) in siblings (males>females) in the right inferior and left middle frontal gyri (5% False Discovery Rate [FDR]; FigAB). The same pattern of sexual dimorphism was observed in the SZ group in total SA of the right superior frontal gyrus (5% FDR; FigB). The opposite pattern was found for subcortical structures in siblings (females>males); significant sex*group interactions were observed in the striatum ($p=0.02$; FigC), globus pallidus ($p=0.07$; FigE), and thalamus ($p=0.05$; FigD). Males have more severe negative symptom (Scale of Assessment of Negative Symptoms; $p=0.04$; FigF). Finally, a significant score*sex interaction was found in Brodmann area 9 for affective flattening, by which SA in males increased with symptom score, while it decreased in females (SZ and sibling groups only; FigG). Thus, brain anatomy in unaffected sibling populations of SZ are present and possibly associated with symptom measures; an improved understanding of the genetic basis for this sexual dimorphism will improve our understanding of SZ etiology.

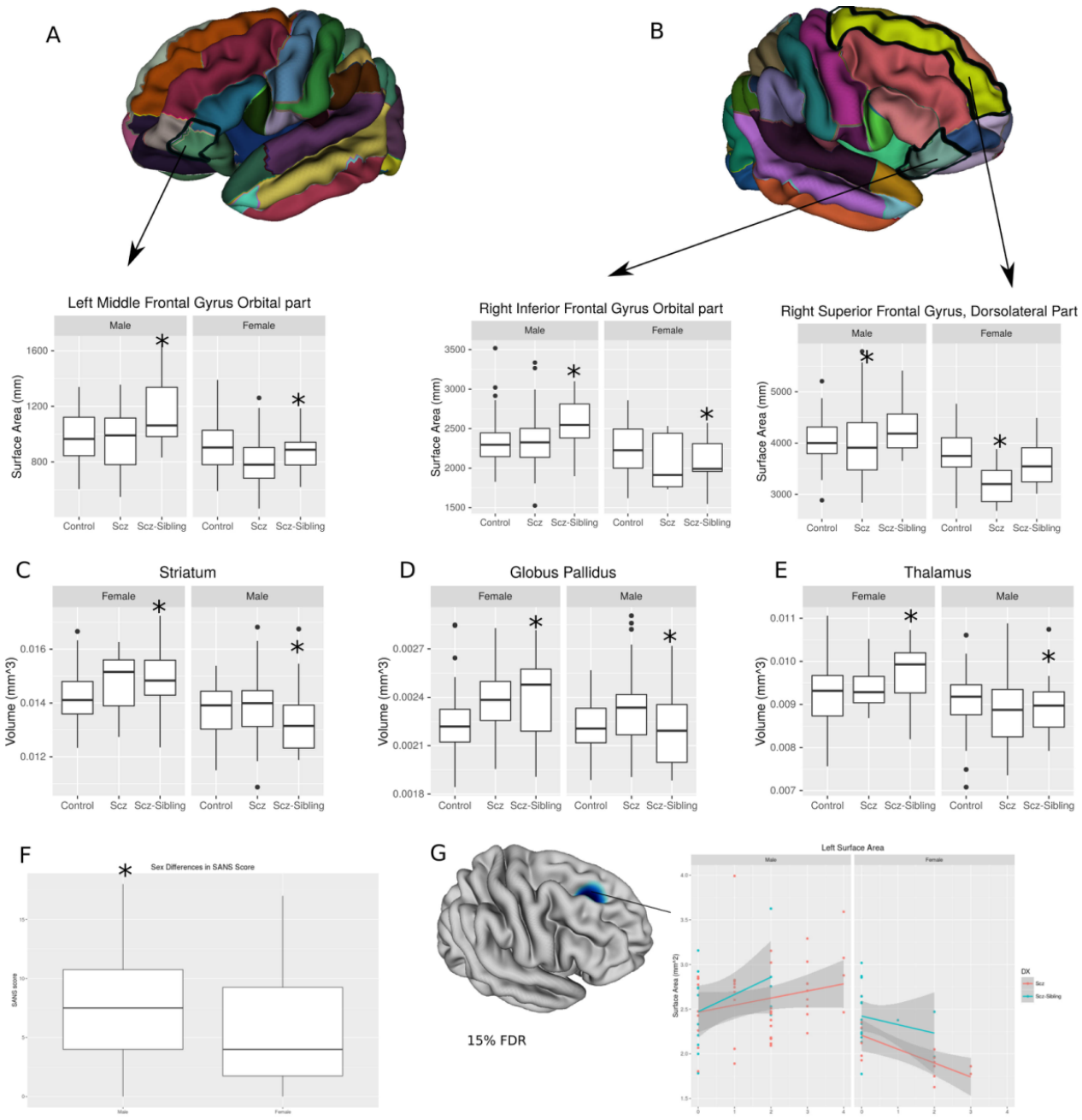

Figure 1. Sexual dimorphism in individuals with schizophrenia and their unaffected siblings. A. Sexual dimorphism in cortical surface area measures of the left middle frontal gyrus orbital part of unaffected siblings (sex*diagnosis survises 5% FDR). B. Sexual dimorphism in cortical surface area in the right inferior frontal gyrus orbital part in the unaffected siblings (sex*diagnosis interaction; 5%FDR), and in the right superior frontal gyrus, dorsolateral part of the schizophrenia group (sex*diagnosis interaction; 5%FDR). C. Significant sex*diagnosis interaction for striatal volume of the unaffected siblings (p=0.02). D. Significant sex*diagnosis interaction for globus pallidus volume of the unaffected siblings (p=0.07). E. Significant sex*diagnosis interaction for thalamic volume of the unaffected siblings (p=0.05). F. Males have significantly higher Globals SANS score than females (p=0.04). G. Significant score* sex interaction for affective flattening (15% FDR), in Brodmann Area 9 surface area.

Disclosures: E. Guma: None. **G.A. Devenyi:** None. **J. Germann:** None. **M. Chakravarty:** None.

Nanosymposium

488. Schizophrenia: Genetics and Genomics

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Presentation Number: 488.04

Topic: H.03. Schizophrenia

Support: Alexander von Humboldt Foundation (Feodor Lynen Return Fellowship)

DFG (FR3420/2-1)

DFG (RE1632/5-1)

DFG (RTG1253)

Title: Disruption of neuronal nitric oxide synthase postsynaptic density protein 95 (PSD95) /discs large 1/zona occludens 1 (PDZ) interactions results in schizophrenia-like behavior

Authors: *E. CANDEMIR[1,2], A. O'LEARY[1], L. GRÜNEWALD[1], A. REIF[1], F. FREUDENBERG[1];
[1]Dept. of Psychiatry, Psychosomatic Med. and Psychotherapy, Lab. o, Johann Wolfgang Goethe Univ. Hosp., Frankfurt, Germany; [2]Grad. Sch. of Life Sci., Univ. of Würzburg, Würzburg, Germany

Abstract: Neuronal nitric oxide synthase (NOS-I) and its adaptor protein (NOS1AP) have been associated with schizophrenia [1]. NOS1AP directly compete with PSD95 for interaction with NOS-I PDZ domain. Disruption of NOS-I PDZ interaction results in schizophrenia related dendritic alterations [2]. Here, we further investigated whether integrity of this interaction leads to schizophrenia-like behavior.
Recombinant adeno-associated virus expressing full length murine NOS1AP (NOS1AP), residues 396-503 of NOS1AP ($NOS1AP_{396-503}$) encoding the NOS-I interaction motif, N terminal 133 amino acids of NOS-I ($NOSI_{N133}$) containing the PDZ domain and mCherry control vector [2] were stereotaxically delivered to the dorsal hippocampus of 7 weeks old male C57Bl/6J mice (n=8/group). After 4 weeks, mice were subjected to a comprehensive behavioral analysis. One-way ANOVA was used for statistical analysis.
Locomotor activity in the open field was increased in mice overexpressing $NOSI_{N133}$ (p=0.04). Mice overexpressing NOS1AP (p=0.067) or NOS-I_{N133} (p=0.073) showed a trend towards reduced time in the center of the open field. Upon inclusion of a novel object, all groups (NOS1AP p=0.038; $NOS1AP_{396-503}$ p=0.01; NOS-I_{N133} p=0.03) showed reduced exploration of this object. In elevated zero maze, NOS1AP mice showed a tendency (p=0.066) towards reduced time in the open segments. Results from these tests suggest increased anxiety upon disruption of NOS-I PDZ interaction. Disruption of the NOS-I PDZ interaction did not have an effect on PPI

of the acoustic startle reflex (p=0.521), suggesting intact sensorimotor gating. Social interaction with a 4 weeks old male mouse was not affected in any group (p=0.331). However, social recognition was impaired in mice overexpressing NOS1AP (p=0.049), $NOS1AP_{396-503}$ (p<0.001) or $NOS\text{-}I_{N133}$ (p=0.011), which showed no preference for a novel mouse over the familiar mouse. Mice overexpressing $NOS1AP_{396-503}$ (p=0.006) or $NOS\text{-}I_{N133}$ (p<0.001) showed impaired working memory in T-maze forced alternation task, but only $NOS\text{-}I_{N133}$ mice showed impaired spatial reference memory in Y-maze (p=0.002).
Our data suggests that disruption of NOS-I PDZ interaction results in several phenotype related to schizophrenia and its comorbidities. Our findings may aid to understand the molecular mechanisms involved in schizophrenia and to develop more direct treatment strategies.
References
[1] Brzustowicz LM. 2008. NOS1AP in schizophrenia. Curr. Psychiatry Rep. 10:158–63.
[2] Candemir E. et al. 2016. Interaction of NOS1AP with the NOS-I PDZ domain: Implications for schizophrenia-related alterations in dendritic morphology. Eur. Neuropsychophar. 26, 741–755.

Disclosures: **E. Candemir:** 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; Alexander von Humboldt Foundation (Feodor Lynen Return Fellowship to FF), DFG (FR3420/2-1 to FF and RE1632/5-1 and RTG1253 to AR), European Community‘s Seventh Framework Programme (FP7/2007– 2013, Aggressotype) under grant agreement n° 602805 (AR). **A. O’Leary:** None. **L. Grünewald:** None. **A. Reif:** 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; DFG (RE1632/5-1 and RTG1253 to AR). **F. Freudenberg:** 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; Alexander von Humboldt Foundation (Feodor Lynen Return Fellowship), DFG (FR3420/2-1).

Nanosymposium

488. Schizophrenia: Genetics and Genomics

Location: SDCC 7B

Time: Tuesday, November 15, 2016, 8:00 AM - 9:30 AM

Presentation Number: 488.05

Topic: H.03. Schizophrenia

Support: CAPES BEX 1279-13-0

Title: Global gene expression profile of the knockdown of the schizophrenia-susceptibility gene NT5C2 in a human neural stem-cell line

Authors: *R. R. R. DUARTE[1], N. J. BRAY[2], R. M. MURRAY[1], D. P. SRIVASTAVA[1]; [1]King's Col. London, London, United Kingdom; [2]Cardiff Univ. Sch. of Med., Cardiff, United Kingdom

Abstract: Chromosome 10q24 is one of the best supported genetic risk loci to emerge from large-scale genome-wide association studies (GWAS) of schizophrenia (Ripke et al., 2011, 2013, 2014). It is also genome-wide significant for the five disorders of the Psychiatric Genomics Consortium combined (schizophrenia, ADHD, autism, depression and bipolar disorders), suggesting it increases risk to psychiatric illness in general (Cross-Disorder Group of the PGC Consortium, 2013). A recent study published by our group (Duarte et al., 2016) revealed *NT5C2* expression in different brain areas in robust association with genotype at the 2 top risk variants on chr10q24 (SNP rs11191419 and indel ch10_104957618_I), with individual risk alleles reducing expression of *NT5C2* by 7-15% depending on the brain region tested (DLPFC, hippocampus, caudate and whole foetal brain). This work provides a strong rationale for now exploring *NT5C2* as a genuine susceptibility gene for psychiatric disease. Here, we experimentally reduced the endogenous expression of NT5C2 in a neural progenitor cell line derived from human fetal brain using RNA interference. Effects on global gene expression were assessed using microarray, followed by pathway analyses to explore molecular changes through which developmental reductions in neural NT5C2 expression could predispose to schizophrenia.

Disclosures: R.R.R. Duarte: None. **N.J. Bray:** None. **R.M. Murray:** None. **D.P. Srivastava:** None.

Nanosymposium

488. Schizophrenia: Genetics and Genomics

Location: SDCC 7B

Time: Tuesday, November 15, 2016, 8:00 AM - 9:30 AM

Presentation Number: 488.06

Topic: G.07. Other Psychiatric Disorders

Support: NIMH Grant MH100228

Title: Gene expression profile in the dorsolateral prefrontal cortex in schizophrenia: differences associated with adolescent cannabis use

Authors: *S. MUKHERJEE[1], D. DURAKOGLUGIL[3], S. PAWAR[1], S. PARK[2], K. GLEASON[1], T.-H. HWANG[2], C. TAMMINGA[1], S. GHOSE[1];

[1]Dept. of Psychiatry, [2]Dept. of Clin. Sci., UT Southwestern Med. Ctr., Dallas, TX;
[3]UTSouthwestern, Dallas, TX

Abstract: Adolescent cannabis use (ACU) is an environmental risk factor repeatedly implicated in the pathophysiology of schizophrenia. The mechanism by which ACU might predispose the brain to schizophrenia is unknown. One plausible hypothesis is that the use of cannabis during a period of brain maturation leads to persistent neural changes that lead to the development of schizophrenia. Further, there is evidence that individuals with schizophrenia and a history of ACU (ACU+SCZ) compared to schizophrenia without a history of ACU (ACU-SCZ) exhibit distinct clinical profile in terms of onset of illness, severity of psychosis and cognitive function. To investigate this hypothesis, we assembled a human post mortem cohort of DLPFC (BA 9) tissue from cases of schizophrenia divided into those with and without ACU (n=10 per group). DLPFC whole transcriptome sequencing was conducted in these cases. We performed group comparison analysis and co-expression network analysis to identify sub-networks differentially expressed between the two Gene-set enrichment analyses was performed to discover biological processes involved with each sub-network. Our preliminary analyses reveal 1851 genes are differentially expressed in between the two schizophrenia groups. These include genes in the extracellular matrix, MAPK signaling pathway, axon guidance and Toll receptor signaling pathway. The co-expression network analysis revealed several network modules. Two of the most significantly changed modules include genes from cholecystokinin (CCK) -related the metalloproteinase pathway. We selected ADAMTS9, ITGAV, CXCL5, LIMK2 from up-regulated and CCK, NNAT, EFHD2, CDK5, ACOT7, RAB26, KCNIP3, NDUFAB1 genes from down-regulated network for further analyses. Molecular characterization of DLPFC at the mRNA and protein level and cellular localization studies of these genes are ongoing. 'These transcriptome data suggest that ACU+SCZ and ACU-SCZ may have distinct underlying pathophysiology.

Disclosures: **S. Mukherjee:** None. **D. Durakoglugil:** None. **S. Pawar:** None. **S. Park:** None. **K. Gleason:** None. **T. Hwang:** None. **C. Tamminga:** None. **S. Ghose:** None.

Nanosymposium

489. Biomarker and Drug Discovery Approaches

Location: SDCC 1B

Time: Tuesday, November 15, 2016, 8:00 AM - 10:30 AM

Presentation Number: 489.01

Topic: I.05. Biomarker and Drug Discovery

Support: NIH Grant R01 GM076990

Title: Deciphering the cell-type specific component in the pathophysiology of brain-related disorders

Authors: *L. TOKER, O. B. MANCARCI, S. TRIPATHY, P. PAVLIDIS;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Neuropsychiatric/neurodevelopmental disorders are characterized by genetic heterogeneity. High-throughput methods, such as microarrays, RNAseq, and genome sequencing are often used to study the underlying biological mechanisms and pathways. A major challenge in interpreting these studies is understanding the biological impact of the identified genes (variants). Researchers often struggle with questions such as - which cells are expressing the affected genes? Are the observed changes cell type specific? Furthermore, it is unclear what part of the transcriptional pattern is driven by changes in cell type densities (e,g, cellular death or neuroinflammation, often reported in subjects with neuropsychiatric disorders).Currently, these questions are difficult to answer since cellular expression patterns are largely unknown for the majority of genes in the brain. Moreover, cell type specific changes are likely to be overlooked if the prevalence of the cell type in the examined tissue is low.To address these issues, we created Neuroexpresso - a rigorously curated database of gene expression data from the major cell-types in the brain. The database contains high-throughput expression data from ~35 neuronal and glial cell types spanning all major brain regions. We used the expression data to identify novel cellular markers and evaluate the specificity of the existing ones. For example, we identified *Cox6a2,* a gene currently considered to be not expressed in the brain, as a marker of fast spiking parvalbumin interneurons, and demonstrated that the commonly used neuronal marker - NeuN (encoded by *Rbfox3* gene), is expressed in some populations of glial cells while being absent from several neuronal populations.We next used the marker genes to identify changes in specific cellular populations based on bulk tissue samples from psychiatric patients. We robustly inferred changes in several cellular populations including fast spiking basket cells in three cohorts of bipolar disorder and schizophrenic patients.
Altogether we show that Neuroexpresso can and should be used to gain a better understanding of brain-related disorders and increase our knowledge of the different cell types in the brain.

Disclosures: L. Toker: None. **O.B. Mancarci:** None. **S. Tripathy:** None. **P. Pavlidis:** None.

Nanosymposium

489. Biomarker and Drug Discovery Approaches

Location: SDCC 1B

Time: Tuesday, November 15, 2016, 8:00 AM - 10:30 AM

Presentation Number: 489.02

Topic: I.05. Biomarker and Drug Discovery

Title: Functional mapping of anti-microRNA oligonucleotide delivery in the central nervous system

Authors: *K. FISCHER, C. MANALO, Y. TUFAIL, H. ESTRELLA, T. OWEN, M. ONORATO, S. PHILLIPS, M. KIM, R. PAGARIGAN, V. KAIMAL, A. PAVLICEK; Regulus Therapeutics, Inc., San Diego, CA

Abstract: Oligonucleotides (OGNs) represent a class of therapeutics, enabling precise targeting of RNA through sequence complementarity. MicroRNAs represent a novel class of targets that can be modulated by OGNs. Numerous studies have identified a number of differentially expressed microRNAs (miR) in central nervous system (CNS) disorders that exacerbate disease pathology. This dysregulation provides an opportunity to use anti-miRs as a novel therapeutic strategy to antagonize aberrant miR function and restore the balance of normal gene regulation inside the cell. One challenge to anti-miR therapy remains the limited understanding of productive uptake in tissue and cell types throughout the CNS. Using a tool anti-miR we evaluated functional delivery and distribution in CNS regions and cell types after local delivery. We generated a tool anti-miR (anti-Let7a) and a specific gene signature to monitor functional inhibition of the Let7 family in various mouse tissues and cell types. After a single intracerebral ventricle (ICV) bolus dose of anti-Let7 in mice, robust and statistically significant let-7 modulation was demonstrated over diverse regions of the CNS including the spinal cord. To further investigate functional delivery within specific CNS cell types, neurons and glial cells were isolated from anti-Let7a ICV dosed mice. Functional delivery was confirmed by both gene regulation and histology in neurons, microglia, and astrocytes. We also observed that the anti-Let7a OGN had a long tissue half-life of approximately 30 days in various regions of the CNS. The long compound half-life resulted in very long duration of the let-7 family inhibition lasting over 4 months in most CNS regions.
We have developed a tool to map functional inhibition of the ubiquitously expressed let-7 microRNA family in mice CNS. Our results demonstrate anti-miRs are stable and functionally active in most regions and cell types within the CNS. Importantly, anti-miRs have a long duration of action which may enable infrequent local dosing once per several months. Utilization of the tool anti-miR helps to build the understanding of pharmacological microRNA inhibition to guide anti-miR therapeutic programs in the CNS therapeutic area.

Disclosures: **K. Fischer:** A. Employment/Salary (full or part-time): Regulus Therapeutics, Inc. **C. Manalo:** A. Employment/Salary (full or part-time): Regulus Therapeutics, Inc. **Y. Tufail:** A. Employment/Salary (full or part-time): Regulus Therapeutics, Inc. **H. Estrella:** A. Employment/Salary (full or part-time): Regulus Therapeutics, Inc. **T. Owen:** A. Employment/Salary (full or part-time): Regulus Therapeutics, Inc. **M. Onorato:** A. Employment/Salary (full or part-time): Regulus Therapeutics, Inc. **S. Phillips:** A. Employment/Salary (full or part-time): Regulus Therapeutics, Inc. **M. Kim:** A. Employment/Salary (full or part-time): Regulus Therapeutics, Inc. **R. pagarigan:** A. Employment/Salary (full or part-time): Regulus Therapeutics, Inc. **V. Kaimal:** A. Employment/Salary (full or part-time): Regulus Therapeutics, Inc. **A. Pavlicek:** A. Employment/Salary (full or part-time): Regulus Therapeutics, Inc..

Nanosymposium

489. Biomarker and Drug Discovery Approaches

Location: SDCC 1B

Time: Tuesday, November 15, 2016, 8:00 AM - 10:30 AM

Presentation Number: 489.03

Topic: I.05. Biomarker and Drug Discovery

Support: NIH RO1 #DC013080

NIH NRSA #DC03665

NSF Grant #1058957

FSU Legacy Fellowship

Tallahassee Memorial Hospital Bryan Robinson Endowment

Title: Margatoxin-bound quantum dots as a novel inhibitor of the voltage-gated ion channel Kv1.3

Authors: *A. SCHWARTZ, A. KAPUR, W. WANG, Z. HUANG, E. FARDONE, H. MATTOUSSI, D. A. FADOOL;
Florida State Univ., Tallahassee, FL

Abstract: Venom-derived ion channel inhibitors have strong channel selectivity, potency and stability, however, like most drug molecules, targeting to a specific location can be problematic. Kv1.3 is a voltage-gated potassium channel (K*v*) that has select distribution and is well characterized for its role in immunity, glucose metabolism and olfaction. Our interests lie in Kv1.3's ability to regulate excitability in mitral cells of the olfactory bulb (OB), where it has the potential to serve as a metabolic target to balance body weight and enhance olfactory ability. To aid in targeted delivery, we utilized the venom-derived Kv1.3 inhibitor margatoxin (MgTx) and developed a protocol using carbodiimide crosslinker chemistry for effective conjugation to luminescent quantum dots (QDs). We screened both unconjugated (MgTx) and conjugated MgTx (QD-MgTx) for their ability to inhibit *Shaker* channels Kv1.1 to Kv1.7 using patch-clamp electrophysiology in a heterologous expression system (HEK 293 cells). Our data indicate that MgTx inhibits 79% of the outward current in Kv1.3-transfected cells and that the QD-MgTx conjugate is able to achieve a similar level of block, albeit a slightly reduced efficacy (66%) and at a slower time course or k_{on} (50% block by 10.7 $\pm$ 1.1 min, n=16, MgTx; vs. 14.5 $\pm$ 1.1 min, n=36, QD-MgTx). While it is a potent inhibitor of Kv1.3, MgTx has been demonstrated to block other K*v* channels at higher concentrations. At 1 nM, MgTx inhibited both Kv1.3- and Kv1.2-transfected cells, but had no effect on other *Shaker* channels. The QD-MgTx conjugate displayed a similar pattern of selectivity. Because MgTx interacts with the pore of the channel, it is logical that properties of voltage dependence may be affected. To study this, we examined the I-V

relation following inhibition of Kv1.3 in transfected cells. Inhibition by MgTx yielded a shift in activation voltage by as much as 30 mV and a flattening of the Boltzman relation for plotted tail currents, indicating an additional change in the steepness of voltage dependence (κ). The QD-MgTx conjugate yielded a similar voltage shift and change in κ, with the addition of the QD only modestly reducing this effect. We also used electrophysiology to test the ability of QD-MgTx to excite mitral cells in native Kv1.3 in OB slices and found that the conjugate had a similar ability as MgTx in enhancing action potential firing frequency (6.2 $\pm$ 2.6 fold change vs control; n=7). Our data demonstrate a retention of known biophysical properties associated with block of the vestibule of Kv1.3 by the QD-MgTx conjugate compared to that of MgTx, inferring that this may be a useful tool to deliver ion channel inhibitors to targeted tissues *in vivo*.

Disclosures: **A. Schwartz:** None. **A. Kapur:** None. **W. Wang:** None. **Z. Huang:** None. **E. Fardone:** None. **H. Mattoussi:** None. **D.A. Fadool:** None.

Nanosymposium

489. Biomarker and Drug Discovery Approaches

Location: SDCC 1B

Time: Tuesday, November 15, 2016, 8:00 AM - 10:30 AM

Presentation Number: 489.04

Topic: I.05. Biomarker and Drug Discovery

Title: Controlled Release of Nerve Growth Factor for long term treatment

Authors: ***N. ZILONY**[1], M. ROSENBERG[2], E. SEGAL[2], O. SHEFI[1];
[1]Fac. of Engin., Bar-Ilan Univ., Ramat Gan, Israel; [2]Dept. of Biotech. and Food Engin., Technion, Haifa, Israel

Abstract: Nerve growth factor (NGF) is a well characterized protein and an essential contributor to neuronal differentiation. NGF has shown high pharmacological potential in several models of neurodegenerative diseases as Alzheimer. However, growth factors undergo rapid degradation which leads to a short biological half-life, limiting the effectiveness in therapeutics. Recent work done in our lab has shown an enhancement in the NGF activity due to covalent conjugation of NGF to iron oxide nanoparticles. Recently, we have designed and examined high porous chips made of silicon as drug carriers. The nanostructured porous Silicon (PSi) is characterized by tunable properties predestining it for design of drug delivery systems, including high surface area, biocompatibility and degradability in a physiological environment. The delivery system we have developed, composed of PSi chips and growth factor, allows sustained and controlled release of NGF. Different PSi nanostructures (that vary in size and depth) were fabricated by anodic electrochemical etching of single-crystalline Si wafers and the synthesis conditions were adjusted to allow efficient loading of NGF by physical adsorption. The NGF release profile was

examined demonstrating a sustained release during a prolong period of a month. To study the effect of the combined complex, we used PC12 cell culture, a common model for neuronal differentiation, and *ex-vivo* DRG explants. NGF is an essential factor for the survival and differentiation of PC12 cells in culture and a key factor in the acceleration of neurite elongation of DRG neurons. The NGF-chips were introduced to PC12 cells and the cells began to differentiate and outgrow neurites. The differentiation process was determined by the number of neurites, neurites total length, number of branching points and by molecular markers. The culture was maintained with the NGF-chips up to 14 days. In addition, the chips were introduced to DRG explants grown in a 3D model of gel matrix. Our work aims to develop new PSi-based carriers for the controlled release of NGF. We demonstrate that NGF entrapment within the PSi allows for its sustained delivery constantly over time without any need of external supplement. This proof of concept leads us to study the therapeutic effects of our novel system on neurodegenerative diseases. It holds the promise to develop a new strategy of treatment that will further discussed.

Disclosures: **N. Zilony:** None. **M. Rosenberg:** None. **E. Segal:** None. **O. shefi:** None.

Nanosymposium

489. Biomarker and Drug Discovery Approaches

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Presentation Number: 489.05

Topic: I.05. Biomarker and Drug Discovery

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Huffington Foundation

Robert A. and Renée E. Belfer Family Foundation

Title: Automated *In vivo* screen of genetic modifiers and small molecules in *Drosophila* models of neurological disorders.

Authors: *L. LI[1,2], C. J. CUMMINGS[1,2], D. DO[1,2], J. BOTAS[1,2];
[1]Jan and Dan Duncan Neurolog. Res. Inst., Houston, TX; [2]Baylor Col. of Med., Houston, TX

Abstract: Do you sit on human GWAS data, not knowing how you will ever have enough manpower to validate it? Do you struggle with sequencing data and wonder how you can perform a functional analysis *in vivo*? Or is there a collection of small molecules you wish screen? Whatever your interest may be, we may be able to assist you with our newly established automated *in vivo* screening core facility and existing *Drosophila* models of neurological

disorders: AD, FTDP-17, PD, HD, DM1, SCA1, SCA2, SCA7, several LSDs etc. If not available, we can also help you establish a model. Our core facility is equipped to perform large-scale genetic or chemical screens *in vivo* using neuronal function/dysfunction as readout. Genetic screens can include but are not limited to testing candidate genes from human or model system studies such as GWAS, sequencing, and gene expression profiles. Chemical screens may include testing small chemical compounds for efficacy, toxicity and bioavailability in order to prioritize expensive and time-consuming mouse trials. The facility includes a number of integrated instruments make it unique. A custom-made robotic instrument is designed for automated assessment of movement impairments caused by neuronal dysfunction. This central assay robot is fed by other pieces of automation designed to manipulate and transfer animals from vial to vial, sort embryos according to genotypes, and dispense small chemicals into *Drosophila* media. Custom-built video analysis software precisely records over 120 metrics including the average speed, trajectory, and percentage of animals that achieve different performance milestones. The software also automatically performs statistical analysis and suggests the significant suppressor or enhancer of disease of interest. We have used the instruments and software to successfully identify genetic modifiers and/or small chemicals ameliorating pathogenesis in models of AD, PD, HD, SCA1 etc. This facility (http://nri.texaschildrens.org/core-facilities/high-throughput-behavioral-screening-core.aspx) is now available to the neuroscience research community at an affordable cost. We are located in the Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital Houston, Texas. We have experience in these collaborative-based efforts, and we will provide support for users, which include a facility manager to provide maintenance and training, a data center for data storage, and a data manager to help with customized data analysis and research assistants for general screening support.

Disclosures: **L. Li:** None. **C.J. Cummings:** None. **D. Do:** None. **J. Botas:** None.

Nanosymposium

489. Biomarker and Drug Discovery Approaches

Location: SDCC 1B

Time: Tuesday, November 15, 2016, 8:00 AM - 10:30 AM

Presentation Number: 489.06

Topic: I.05. Biomarker and Drug Discovery

Title: Blood brain barrier bbb-chip: a novel, physiologically relevant ipsc-based micro-engineered platform.

Authors: *G. D. VATINE[1], S. SANCES[1], R. BARRILE[2], B. BARRIGA, 90405[1], A. LAPERLE[1], C. LUCCHESI[2], N. WEN[2], C. HINOJOSA[2], J. KERNS[2], G. A. HAMILTON[2], C.

N. SVENDSEN[1];
[1]Cedars-Sinai Med. Ctr., West Hollywood, CA; [2]Emulate, Inc., Boston, MA

Abstract: The blood brain barrier (BBB) is an important neurovascular unit that protects the central nervous system (CNS) from a range of factors found in blood that may disrupt delicate brain functioning. Therefore, the BBB is a major obstacle in delivering brain-acting drugs to the CNS and faithful models are critical for drug development. Here, we have combined micro-engineered technology with iPSC differentiation protocols to accurately model the in vivo environment. We show that brain microvascular endothelial cells (BMECs) and neural cells derived from iPSCs can coat each side of a porous membrane encased within a two channel, Organ-Chip. Media flow across each channel allows constant exchange of fluid. The BMECs, astrocytes and neurons within the BBB-Chip co-survive, differentiate and mature in harmony. Astrocytes send end feet through the membrane pores to interact with BMECs in a separate compartment. This model provides a unique platform to study the interactions between endothelial cells and neural tissue, forms the basis for a new BBB-Chip model, and moves towards disease specific chip models using iPSCs derived from patients with neurological disorders.

Disclosures: **G.D. Vatine:** A. Employment/Salary (full or part-time): Cedars Sinai. **S. Sances:** None. **R. Barrile:** None. **B. Barriga:** None. **A. Laperle:** None. **C. Lucchesi:** None. **N. Wen:** None. **C. Hinojosa:** None. **J. Kerns:** None. **G.A. Hamilton:** None. **C.N. Svendsen:** None.

Nanosymposium

489. Biomarker and Drug Discovery Approaches

Location: SDCC 1B

Time: Tuesday, November 15, 2016, 8:00 AM - 10:30 AM

Presentation Number: 489.07

Topic: I.05. Biomarker and Drug Discovery

Support: SNF 320030_156029

Title: Low-noise amplifier improves automated Fast Ripple (FR) detection in ECoG during epilepsy surgery

Authors: ***T. FEDELE**[1], G. CURIO[2], S. BURNOS[1], E. BORAN[1], P. HILFIKER[3], T. GRUNWALD[3], N. KRAYENBÜHL[1], J. SARNTHEIN[1];
[1]Unispital Zürich, Zürich, Switzerland; [2]Charite, Berlin, Germany; [3]Swiss Epilepsy Ctr., Zurich, Switzerland

Abstract: Fast ripples (FR) in the intraoperative electrocorticogram (ECoG) have recently been shown to be specific predictors of outcome of epilepsy surgery. FR detection is restricted by their low signal-to-noise ratio and time consuming visual marking. We present here the combination of a low-noise recording system with semi-automatic detection of high-frequency oscillations (HFO).
We recorded intraoperative ECoG (N = 9 patients, 5722 minute recordings, 135 channels) simultaneously with a commercial device (CD, input noise level 21 nV/√Hz) and with a custom-made low-noise amplifier (LNA, input noise level 2.3 nV/√Hz). The HFO analysis was conducted separately for Ripples (80-250 Hz) and FR (250-500 Hz). The automatic detector performed first an entropy-based computation of baseline amplitude and then a validation of events in the time-frequency domain. Events were visually validated by three independent reviewers. Channels with >1 event/min were counted as indicative of poor outcome.
Over all channels, the baseline amplitude was 4.6±3.0 µV for Ripples and 2.0±1.4 µV for FR in CD and 4.8±7.1 µV for Ripples and 1.3±0.8 µV for FR in LNA. Ripple rates were 4.4± 6.8 events/minute in CD and 7.9± 15.9 in LNA ($p < 0.001$). FR rates were 0.2± 0.5 in CD and 0.9±1.5 in LNA ($p<0.001$). Across patients, the similarity of spatial patterns between CD and LNA was 0.76±0.3 for Ripples and 0.2±0.4 for FR. In post-resection ECoG of the 7 patients with available outcome, FR were found in 2/7 patients with LNA recordings, while no FR were found in CD recordings. This resulted in PPV_{FR} = 0% and NPV_{FR} = 57% in CD, and PPV_{FR} = 100%, CI [15 100] and NPV_{FR} = 80%, CI [28 99] in LNA.
In conclusion, low-noise recordings enhance the signal-to-noise ratio in the FR spectral range. The combination of an optimized acquisition system with a semi-supervised HFO detector improved FR detection. The opportunity to detect a higher amount of FR represents a critical advance in evaluating the benefit of FR in clinical application.

Disclosures: **T. Fedele:** None. **G. Curio:** None. **S. Burnos:** None. **E. Boran:** None. **P. Hilfiker:** None. **T. Grunwald:** None. **N. Krayenbühl:** None. **J. Sarnthein:** None.

Nanosymposium

489. Biomarker and Drug Discovery Approaches

Location: SDCC 1B

Time: Tuesday, November 15, 2016, 8:00 AM - 10:30 AM

Presentation Number: 489.08

Topic: I.05. Biomarker and Drug Discovery

Support: Michael J. Fox Foundation

JSPS KAKENHI Grant Number 70357060

Title: Oxidation and interaction of DJ-1 with 20S proteasome in the erythrocytes of early stage Parkinson's disease patients

Authors: *Y. SAITO, K. SUTOU, M. KOBAYASHI, Y. MITA, N. NOGUCHI;
Doshisha Univ., Kyotanabe/ Kyoto, Japan

Abstract: Parkinson's disease (PD) is a progressive, age-related, neurodegenerative disorder, and oxidative stress is an important mediator in its pathogenesis. The identification of a biomarker for PD in its early phase is vital for overcoming PD, since more than half of the dopamine neurons in the substantia nigra of the midbrain have been lost by the time the patient is diagnosed with PD. DJ-1, the product of the causative gene of a familial form of PD, plays a significant role in anti-oxidative defence to protect cells from oxidative stress. DJ-1 undergoes preferential oxidation at the cysteine residue at position 106 (Cys-106) under oxidative stress. The critical role of this cysteine residue in the biological functioning of DJ-1 has been demonstrated. DJ-1 acts as an oxidative stress sensor, detecting cellular redox status through the oxidation of Cys-106 and altering the activity of signal mediators and the expression levels of genes involved in anti-oxidative defense. Thus, oxidized DJ-1 could be a promising candidate as a biomarker for oxidative stress in PD. We have previously developed specific antibodies against Cys-106-oxidized DJ-1 (oxDJ-1). Immunohistochemical analysis using oxDJ-1 specific antibodies suggests that, in the substantia nigra of PD patients, oxDJ-1 levels increase in the early phases of PD (J. Neuropath. Exp. Neurol.73, 714-728, 2014). Using the enzyme-linked immunosorbent assay (ELISA) for oxDJ-1, it was found that the levels of oxDJ-1 in the erythrocytes of unmedicated PD patients (n = 88) were higher than in those of medicated PD patients (n = 62) and healthy control subjects (n = 33). "Unmedicated PD patients" are those diagnosed with PD but not yet started on medications such as L-DOPA. Thus, the evidence suggests that DJ-1 oxidation in erythrocytes occurs in PD patients, particularly during the early phases. Elevated oxDJ-1 levels were also observed in a non-human primate PD model. Biochemical analysis of oxDJ-1 in erythrocyte lysates showed that oxDJ-1 formed dimer and polymer forms, and that the latter interacts with 20S proteasome. It has been reported that DJ-1 binds the 20S proteasome and inhibits its activity. Several studies have reported that unusual proteins such as α-synuclein oligomer and protein carbonyls are accumulated in the erythrocytes of PD patients. Our observations suggest that oxDJ-1 interacts with the 20S proteasome, inhibits its activity, and is related to the accumulation of unusual proteins in the erythrocytes of PD patients. Collectively, these results clearly indicate a biochemical alteration in the blood of PD patients, which could be utilized as an early diagnosis marker for PD.

Disclosures: **Y. Saito:** None. **K. Sutou:** None. **M. Kobayashi:** None. **Y. Mita:** None. **N. Noguchi:** None.

Nanosymposium

489. Biomarker and Drug Discovery Approaches

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Time: Tuesday, November 15, 2016, 8:00 AM - 10:30 AM

Presentation Number: 489.09

Topic: I.05. Biomarker and Drug Discovery

Support: Marie Sklodowska-Curie Individual Fellowships, MSCA- IF-2015

University Medical Center Hamburg-Eppendorf, FFM Postdoctoral Fellowship

Title: Clinical usability of *In vivo* MR g-ratio mapping methods

Authors: *I. ELLERBROCK, S. MOHAMMADI;
Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: Non-invasive quantitative MRI biomarkers are becoming increasingly important for diagnosing and monitoring patients with multiple sclerosis (MS). The myelin g-ratio, quantifying the ratio between the inner and outer diameters of a fiber, is expected to be particularly specific to pathological changes in MS (e.g. re- and de-myelination processes or old and new lesions in white matter). Recently, a novel in vivo MRI method has been proposed to measure the aggregated MR g-ratio on a voxel-by-voxel basis from two established quantitative MRI biomarkers, the myelin-volume and fiber-volume fraction (MVF and FVF) (Stikov et al. 2015). Although the MR g-ratio is potentially a valuable biomarker, it is important to assess its reproducibility before applying it in clinical studies. Different MR g-ratio methods have been proposed (Mohammadi et al., 2015; Stikov et al., 2015), using different techniques to measure the underlying MVF and FVF. Here, we tested the reproducibility of four proposed g-ratio methods using scan-rescan measurements in healthy volunteers. We used an extensive quantitative MRI protocol with, respectively, two distinct acquisition techniques for MVF: (i) proton density imaging (Mezer et al., 2013) and (ii) magnetization transfer rate imaging (Helms et al., 2008), and two techniques for FVF: (i) neurite orientation dispersion and density imaging (NODDI) (Stikov et al., 2015) and (ii) tract-fiber density (TFD) (Reisert et al., 2013). The two techniques for MVF mapping provided similar results, whereas NODDI was mostly better than TFD for FVF measurement (Fig. 1). Overall the reproducibility of all g-ratio metrics was acceptable for clinical usage (87-94%). In summary, this study demonstrated stability of a novel MRI biomarker for MS, encouraging adaptation for clinical studies.

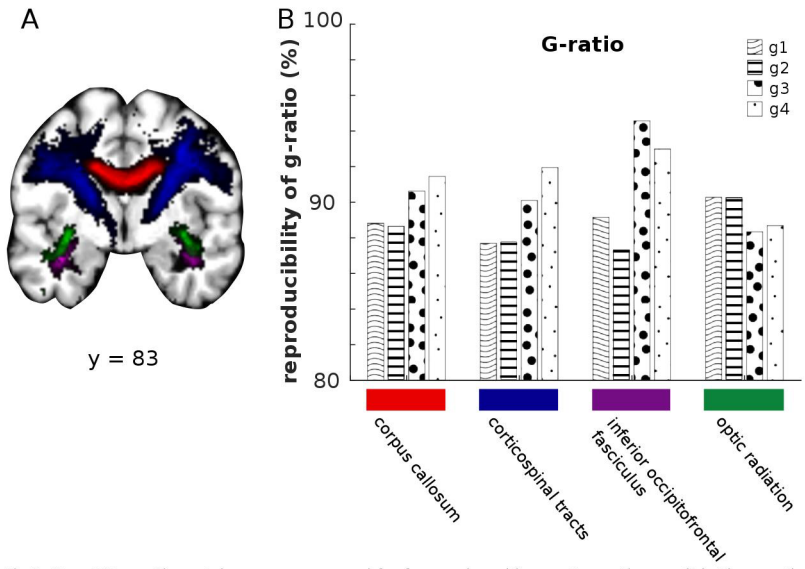

Fig.1: Four MR g-ratio metrics were compared for four major white-matter pathways (A). The g-ratios were estimated using the following combination of MRI techniques to estimate myelin-volume fraction (MVF) and fiber-volume fraction (FVF): (g1) MVF: Helms / FVF: Reisert, (g2) MVF: Mezer / FVF: Reisert, (g3) MVF: Helms / FVF: Stikov, and (g4) MVF: Mezer / FVF: Stikov. (B) All g-ratio metrics demonstrated high reproducibility, ranging between 87-94 %.

Disclosures: I. Ellerbrock: None. **S. Mohammadi:** None.

Nanosymposium

489. Biomarker and Drug Discovery Approaches

Location: SDCC 1B

Time: Tuesday, November 15, 2016, 8:00 AM - 10:30 AM

Presentation Number: 489.10

Topic: I.05. Biomarker and Drug Discovery

Support: DFG Grant

Title: Cortical hyperactivity beyond the immune attack: starting point of neurodegeneration

Authors: *G. K. PRAMANIK[1,2], E. ELLWARDT[3], E. R. JUBAL[2], T. NOVKOVIC[4], Z. BERGER[6], D. LUCHTMANN[3], M. SCHMALZ[3], T. MITTMANN[5], F. ZIPP[3], A. STROH[2]; [1]University-Medicine of Johannes Gutenberg Universi, Mainz, Germany; [2]Inst. of Microscopy, Anat. and Neurobio., [3]Dept. of Neurol., [4]Inst. of Physiol., [5]4Institute of Physiol., University-medicine of Johannes Gutenberg-University Mainz, Mainz, Germany; [6]Dept. of Biol., Univ. of Washington, Seattle, WA

Abstract: Multiple sclerosis patients exhibit cortical neurodegeneration despite the immune attack occurring predominantly in white matter and spinal cord. To unravel the impact of

peripheral immune attack on cortical networks, we probed the association between progressive disease stages identified by clinical phenotype with cortical microcircuit activity *in vivo* in experimental autoimmune encephalomyelitis (EAE) animals. Using two-photon Ca^{2+} imaging, we identified a hyperactive neuronal phenotype in layer II/III primary visual cortex (V1) in remission and not in the disease peak. This was further corroborated by increased in spontaneous EPSC frequency from cortical slices of remission phase animals. Notably, we did not observe any cortical demyelination or ongoing inflammation. In remission animals, cortical TNF-α was causally associated with increase in cortical hyperactivity and inhibiting TNF-α binding to its receptors by intraventricular injection of infliximab restored spontaneous EPSC frequency in cortical slices. We report a link between elevation of cortical TNF-α and enhanced cortical network activity, indicating that early emergence of hyper-excitation could potentially lead the network towards neurodegeneration in a primarily unaffected cortex.

Disclosures: **G.K. Pramanik:** None. **E. Ellwardt:** None. **E.R. Jubal:** None. **T. Novkovic:** None. **Z. Berger:** None. **D. Luchtmann:** None. **M. Schmalz:** None. **T. Mittmann:** None. **F. Zipp:** None. **A. Stroh:** None.

Nanosymposium

490. Models of Memory and Anticipatory Coding

Location: SDCC 2

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 490.01

Topic: I.06. Computation, Modeling, and Simulation

Title: Signatures of negative group delay dynamics in neuroscience data

Authors: *H. U. VOSS[1], N. D. STEPP[2];
[1]Radiology, Weill Cornell Med. Col., New York, NY; [2]Information and Syst. Sci. Lab., HRL Laboratories, LLC, Malibu, CA

Abstract: Objectives:
We propose that the physical concept of negative group delay (NGD) provides a parsimonious yet efficient mechanism for the real-time prediction of signals for which no explicit model exists. NGD can be understood by the entirely causal linear response characteristics of certain input-output systems. In the realm of physics, NGD has been observed as an apparent advancement of incoming signal peaks in media with anomalous dispersion, simple electronic filters, and metamaterials. Our previous work showed how *delay-induced* NGD, which might arise from neuronal delays, could be a plausible way of real-time prediction of smooth signals [1,2]. Here we discuss possible signatures of NGD dynamics in experimental data. These concepts are then applied to a feedback-delayed manual tracking experiment [3] under the hypothesis that the

human subject's performance can be modeled by a linear system with delay-induced NGD.

Methods:

The prediction y(t) is given by an anticipatory relaxation system with smooth external input signal x(t) and a time-delayed feedback y(t - τ) [2], i.e.,

$dy(t)/dt = - a\, y(t) + b\, x(t) - c\, y(t-\tau)$ ($a, c \geq 0$, $b > 0$; $\tau > 0$ is the feedback delay). (*)

For the example of delayed manual tracking experiments, x is the coordinate of the target on the screen, y the normalized horizontal handle/stylus coordinate, and y(t - τ) the delayed coordinate of the marker on the screen, which is supposed to continuously track the target. Therefore, in order to track the target at position x, subjects have to continuously predict where its position will be at a time τ later. The output of model (*) delivers this prediction.

Results:

We demonstrate on numerical simulations of various dynamic scenarios that delay-induced NGD dynamics (*) leads to characteristic signatures in the time and frequency domain of the data, and review literature where parts of the observations could be explained by the theory of NGD. Specifically for the experiment [3] we find that delay-induced NGD explains key components of experimental observations, including the transition from reactive to predictive behavior with increasing feedback delay.

Discussion:

In contrast to most other known prediction schemes, which use past *signal* values for prediction, prediction by delay-induced NGD via model (*) relies on *past values of the predicted signal*. Therefore, this mechanism does not require a memory of past signal values, only of the already internalized predicted values, which might be advantageous for neural coding schemes.

[1] H. U. Voss, Neural Comput**, in press** (2016).
[2] H. U. Voss, Phys Rev E **93**, 030201(R) (2016).
[3] N. Stepp, Exp Brain Res **198**, 521 (2009).

Disclosures: H.U. Voss: None. **N.D. Stepp:** None.

Nanosymposium

490. Models of Memory and Anticipatory Coding

Location: SDCC 2

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 490.02

Topic: I.06. Computation, Modeling, and Simulation

Support: CNPq Grant 480053/2013-8

CNPq Grant 310712/2014-9

CAPES Grant PVE 88881.068077/2014-01

Title: Inhibitory loop robustly induces anticipated synchronization in neuronal microcircuits

Authors: *M. COPELLI[1], F. S. MATIAS[3], P. CARELLI[2], C. MIRASSO[4];
[2]Physics, [1]Fed. Univ. Pernambuco (UFPE), Recife, Brazil; [3]Fed. Univ. Alagoas (UFAL), Maceio, Brazil; [4]IFISC, CSIC-UIB, Palma de Mallorca, Spain

Abstract: We investigate *in silico* the synchronization properties between two excitatory coupled neurons in the presence of an inhibitory loop mediated by an interneuron. Inhibition and noise (independently applied to each neuron) provide phase diversity in the dynamics of the neuronal motif. We show that the interplay between the coupling strengths and noise controls the phase relations between the neurons in a counter-intuitive way. For a master-slave configuration (unidirectional coupling) we find that the slave can anticipate the master, on average, if the slave is additionally subject to inhibitory feedback. In this unusual regime, called anticipated synchronization (AS), the phase of the post-synaptic neuron is advanced with respect with that of the pre-synaptic neuron. We also show that the AS regime survives even in the presence of unbalanced bidirectional excitatory coupling. Moreover, for the symmetric mutually coupled situation, the neuron participating in the inhibitory loop is the leader in phase. In the absence of noise, the problem can be extended to more general motifs under an analytic approach whose results agree well with numerical simulations.

Disclosures: **M. Copelli:** None. **F.S. Matias:** None. **P. Carelli:** None. **C. Mirasso:** None.

Nanosymposium

490. Models of Memory and Anticipatory Coding

Location: SDCC 2

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 490.03

Topic: I.06. Computation, Modeling, and Simulation

Title: Anticipatory top-down coupling of V1 and extrastriate cortex in visual expectation

Authors: *S. L. BRESSLER;
Florida Atlantic Univ., Boca Raton, FL

Abstract: Top-down processing in the visual cortex underlies important cognitive anticipatory functions such as visual predictive coding and attentional set (Bressler & Richter 2015). Experimental evidence from the visual cortex of awake behaving macaque monkeys indicates that extrastriate cortex employs beta-frequency synchronization to impose anticipatory top-down influences on primary visual cortex (V1) as the monkey expects an impending visual stimulus while performing a visuomotor pattern discrimination task. Furthermore, pattern discrimination

analysis of the spatial pattern of top-down influence shows that behavioral context is conveyed to low-level sensory neurons from higher levels of the visual hierarchy. References: Bressler, S.L., Richter, C.G. (2015) Interareal oscillatory synchronization in top-down neocortical processing. Current Opinion in Neurobiology 31:62-66.

Disclosures: S.L. Bressler: None.

Nanosymposium

490. Models of Memory and Anticipatory Coding

Location: SDCC 2

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Presentation Number: 490.04

Topic: I.06. Computation, Modeling, and Simulation

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CNPq Grant 480053/2013-8

CNPq Grant 310712/2014-9

FACEPE APQ-0826- 1.05/15

Title: Anticipated Synchronization in neuronal populations: the case of the cat primary visual system

Authors: *C. MIRASSO[1], F. S. MATIAS[2], M. COPELLI[3], P. V. CARELLI[3], L. MARTINEZ[4]; [1]IFISC, Univ. de les Illes Balears, Palma de Mallorca, Spain; [2]Inst. de Física, Univ. Federal de Alagoas, Maceió, Brazil; [3]Dept. de Física, Univ. Federal de Pernambuco, Recife, Brazil; [4]Inst. de Neurociencias de Alicante, Alicante, Spain

Abstract: Anticipated Synchronization (AS) can emerge when two or more dynamical systems are coupled. In the AS regime the influence is predominantly transmitted from a sender to a receiver, but the receiver leads the sender in time. This counterintuitive regime has been found in many dynamical systems including oscillators, lasers and, more recently, in neuron microcircuits and neuron populations. We will discuss how AS can emerge in coupled neuronal populations, focusing on its dependence on the excitation-inhibition balance. As an example, we will develop a model for the cat primary visual system to find under which conditions AS can emerge in this circuit. We will show that our results agree with experimental observations.

Disclosures: C. Mirasso: None. **F.S. Matias:** None. **M. Copelli:** None. **P.V. Carelli:** None. **L. Martinez:** None.

Nanosymposium

490. Models of Memory and Anticipatory Coding

Location: SDCC 2

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 490.05

Topic: I.06. Computation, Modeling, and Simulation

Support: 1K23MH101637

5P50MH086404

Title: Predictive coding and mechanisms of psychosis

Authors: *G. HORGA, C. M. CASSIDY, A. ABI-DARGHAM;
Psychiatry, Columbia Univ. Med. Ctr., New York, NY

Abstract: Predictive-coding and related models of perceptual inference provide a unifying framework to understand how expectations are integrated into perception. At the core of these models is the assumption that the brain is an inference machine that learns environmental regularities to form an internal model of the world. This internal model would serve to efficiently anticipate, filter, and interpret sensory events in a way that can also bias our perception. Modern models of psychosis propose that abnormalities in predictive coding could explain the perceptual disturbances (i.e., hallucinations) and delusional beliefs that characterize this clinical syndrome, although empirical evidence in this respect has been incomplete. Here, I will present recent data in support of the idea that abnormalities in predictive coding are a core mechanism of psychosis and that these abnormalities might relate to other well-established neural phenotypes of psychosis, such as striatal dysregulation in dopamine release. I will present recent functional magnetic resonance imaging (fMRI) data suggesting that patients with active auditory hallucinations display an abnormality in predictive-coding signals (in particular in sensory prediction errors) in voice-sensitive regions of the auditory cortex, an abnormality that is associated with other neural phenotypes thought to represent downstream determinants of auditory hallucinations. I will also present resting-state fMRI and molecular positron emission tomography (PET) imaging data suggesting that a disruption in functional connectivity between the striatum and associative cortices, including some areas of the auditory cortex, is related to cortical dopamine function and psychosis. Finally, I will present recent behavioral and PET imaging data before and after an amphetamine challenge that suggest striatal dopamine release is specifically related to context-dependent modulation of subjective perception under uncertainty, a potential cognitive mechanism mediating the effects of dopamine dysregulation on the generation of auditory hallucinations in psychosis. Together, these findings support a predictive coding account of psychosis and may provide new insights on the neurobiological and computational mechanisms of auditory hallucinations in psychosis. More generally, these data

attest to the broad utility of predictive-coding and related models to understand a wide array of phenomena associated with normal and pathological perceptual experiences.

Disclosures: G. Horga: None. **C.M. Cassidy:** None. **A. Abi-Dargham:** None.

Nanosymposium

490. Models of Memory and Anticipatory Coding

Location: SDCC 2

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 490.06

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant R01GM105045

Title: Sensorimotor feedback delays and anticipatory coupling of chaotic human behavior

Authors: *A. WASHBURN[1], C. A. COEY[3], R. W. KALLEN[2], K. SHOCKLEY[2], M. J. RICHARDSON[2];
[1]Psychology, [2]Univ. of Cincinnati, Cincinnati, OH; [3]Col. of the Holy Cross, Boston, MA

Abstract: The ability to coordinate with ongoing, ever-changing environmental events is fundamental to the performance of many everyday human tasks. Individuals can anticipate the evolution of potentially chaotic events, and prospectively control their behavior accordingly. Previous work investigating the mechanisms that support such anticipation has focused on hypotheses about neural simulation processes, or feed-forward internal models and motor programs. These constructs are meant to account for the human nervous system's ability to compensate for temporal delays that inherently occur between the production of a movement and the perception of its outcome. The traditional assumption, using linear systems theory, is that perceptual-motor feedback delays present a problem in coordinating behavior because they amplify errors and lead to instability. In contrast, work examining the dynamics of electrical circuits and coupled neurons suggests that small temporal feedback delays might actually enhance the ability of a physical system to synchronize with, and even anticipate, unpredictable, chaotic events. This counterintuitive phenomenon (i.e., strong anticipation or self-organized anticipatory synchronization) emerges when a "slave" system is coupled to a chaotically behaving "master" system. As the slave system begins to synchronize with the chaotic behavior of the master system the introduction of small temporal feedback delays results in the slave system anticipating the ongoing behavior of the master. By asking individuals to synchronize their arm movements with visually displayed, pre-recorded chaotic human movement sequences the current study demonstrated that: 1) a human actor can exhibit anticipatory synchronization with respect to natural, chaotic human movement; and 2) small temporal delays in coupling

enhance coordination with such sequences, as has been seen in other physical systems. These experimentally introduced delays are superimposed on top of those inherent to the human nervous system and therefore provide an exaggerated view of naturally occurring anticipatory synchronization processes. In fact, the ability of an actor to coordinate with chaotic behaviors at a very short temporal lag, and in the absence of any experimentally introduced feedback delay, can be understood as evidence for naturally occurring anticipatory synchronization. Experimental examination of the relationship between the length of perceptual- motor feedback delays and the degree of associated anticipation allows for a better understanding of how delays inherent to the human nervous system relate to behavioral coordination and prospective control.

Disclosures: A. Washburn: None. **C.A. Coey:** None. **R.W. Kallen:** None. **K. Shockley:** None. **M.J. Richardson:** None.

Nanosymposium

490. Models of Memory and Anticipatory Coding

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Presentation Number: 490.07

Topic: I.06. Computation, Modeling, and Simulation

Support: Wellcome Trust Grant

Bernard Wolfe Health Neuroscience Fund

Niels Stensen Foundation

Title: Adaptive coding of prediction errors varies with prefrontal glutamate concentrations and delusional-like thinking in healthy individuals

Authors: *K. M. DIEDEREN, J. HAARSMA, T. SPENCER, W. SCHULTZ, P. FLETCHER; Univ. of Cambridge, Cambridge, United Kingdom

Abstract: The sensitivity of the brain to expected outcomes and rewards is evidenced by its anticipatory neural activity to such outcomes. These anticipatory signals facilitate the detection of violations in our expectations, which generate prediction errors (PEs) that are crucial learning signals. Effective error-driven learning requires neurons to adaptively code PEs relative to their reliability, with more reliable PEs producing a greater facilitation of learning. Importantly, a breakdown of PE adaptation could result in a failure to attenuate learning when PEs are less reliable, and this is thought to underlie delusion-like thinking. Here, we sought to investigate the relationship between adaptive PE coding and delusion-like beliefs in healthy individuals. As alterations in glutamate concentration in the prefrontal cortex and ventral striatum have been

associated with impaired error-driven learning and clinical delusions in psychosis, we also examined the role of glutamate in this relationship. To quantify adaptive PE coding, 25 healthy volunteer participants predicted the magnitude of upcoming rewards drawn from distributions with different degrees of variability during fMRI scanning. On each trial, after making the prediction participants received a reward, thus yielding trial-by-trial PEs. Following fMRI acquisition, glutamate concentrations were measured in the PFC and striatum using MR Spectroscopy. Delusion-like thinking was assessed with the Peters Delusion Inventory. PE slopes in the midbrain and ventral striatum were steeper for PEs occurring in distributions with smaller reward variability, signaling adaptive coding. Increases in adaptive PE coding in the ventral striatum significantly correlated with decreases in delusion-like thinking, as well as with increases in glutamate concentrations in the PFC, but not the striatum. Glutamate concentrations also correlated with learning performance (defined as participants' accuracy predicting the mean of reward distributions), which was impaired in individuals with lower glutamate levels and increased delusion-like thinking. Our results demonstrate a direct relationship between adaptive coding of predictive signals for learning, glutamate levels and delusion-like thinking, as hypothesized previously. Our findings suggest that normal glutamate levels are important for adaptive coding of PEs and efficient learning about variable rewards. In addition, these findings provide early support for the hypothesis that delusion-like thinking in healthy individuals is associated with alterations in adaptive PE coding, and aberrant glutamate signalling.

Disclosures: **K.M. Diederen:** None. **J. Haarsma:** None. **T. Spencer:** None. **W. Schultz:** None. **P. Fletcher:** None.

Nanosymposium

490. Models of Memory and Anticipatory Coding

Location: SDCC 2

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 490.08

Topic: I.06. Computation, Modeling, and Simulation

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CAPES Grant PVE 88881.068077/2014-01

FACEPE APQ-0826- 1.05/15

Title: Anticipated synchronization and spike timing dependent plasticity work synergistically to determine the network dynamics

Authors: *F. S. MATIAS[1], P. V. CARELLI[2], C. R. MIRASSO[3], M. COPELLI[2];
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Abstract: Several cognitive tasks related to learning and memory exhibit synchronization of macroscopic cortical areas together with synaptic plasticity at neuronal level. Therefore, there is a growing effort among computational neuroscientists to understand the underlying mechanisms relating synchrony and plasticity in the brain. Here we numerically study the interplay between spike-timing dependent plasticity (STDP) and anticipated synchronization (AS). AS emerges when a dominant flux of information from one area to another is accompanied by a negative time lag (or phase). This means that the receiver region pulses before the sender does. Here we study the interplay between different synchronization regimes and STDP at the level of three-neuron microcircuits as well as cortical populations. We show that STDP can promote auto-organized zero-lag synchronization in unidirectionally coupled neuronal populations. We also find synchronization regimes with negative phase difference (AS) that are stable against plasticity. Finally, we show that the interplay between negative phase difference and STDP provides limited synaptic weight distribution without the need of imposing artificial boundaries.

Disclosures: F.S. Matias: None. **P.V. Carelli:** None. **C.R. Mirasso:** None. **M. Copelli:** None.

Nanosymposium

490. Models of Memory and Anticipatory Coding

Location: SDCC 2

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Topic: H.01. Animal Cognition and Behavior

Support: CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico

Title: Effect of previous acquired remote memory in subsequent learning: a mechanistic and anatomical study

Authors: *A. P. CRESTANI[1,2], R. O. SIERRA[1], A. MACHADO[3], K. S. MARTIN[1], B. J. WILTGEN[2], L. DE OLIVEIRA ALVARES[1], J. A. QUILLFELDT[1];
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Abstract: Preceding studies have pointed out the importance of previous experience in future learnings. Those studies have shown that a prior learning may cause modifications in the plasticity mechanism transforming the process of memory acquisition independent of NMDAR.

In addition, it has been demonstrated that the first learning requires the integrity of the entire hippocampus, whereas subsequent conditioning can be mediated either by the ventral or the dorsal hippocampus. In other hand, several works have shown a gradual reorganization of brain structures supporting memory, with a decreasing involvement of the hippocampus and an increasing engagement of the cortex over time. In this way, we hypothesized that the gradual decrease o hippocampal dependency could induce a "clearance" phenomenon of hippocampal mechanisms involved in the second learning. If those assumption are true, after systems consolidation of the first leaning, the acquisition of a subsequent similar experience could once again becomes NMDAR-dependent in the hippocampus. Furthermore, memory migration to ACC could change learning molecular mechanisms of second experience, i.e. subsequent learning might occur in a NMDAR-independent way in that brain region. Thereby, the main goal of our experiments was evaluate (i) which brain regions are involved in encoding of subsequent learning after systems consolidation of the previous similar learning and (ii) whether acquisition of this second memory is NMDAR-independent in the hippocampus and in the neocortex. In order to answer these questions, Wistar rats were trained in the first contextual fear conditioning and 40 days later in the second one. GABA agonist muscimol or NMDAR antagonist APV were infused before the second training to assess brain area or receptor dependence in dHPC, vHPC and ACC. Our main findings demonstrated that (i) at least one subregion of the hippocampus (dHPC or vHPC) is necessary to encode subsequent learning and (ii) this learning is NMDAR-independent in the ACC and once again becomes NMDAR-dependent in the entire hippocampus.

Disclosures: **A.P. Crestani:** None. **R.O. Sierra:** None. **A. Machado:** None. **K.S. Martin:** None. **B.J. Wiltgen:** None. **L. de Oliveira Alvares:** None. **J.A. Quillfeldt:** None.

Nanosymposium

490. Models of Memory and Anticipatory Coding

Location: SDCC 2

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Research Initiatives for Science and Engineering (RISE)

Title: Optimal long-term memory in complex synapses with bounded dynamical variables

Authors: *M. K. BENNA, S. FUSI;
Neurosci., Columbia Univ., New York, NY

Abstract: Memory consolidation at the level of synaptic connections relies on a complex network of highly diverse biochemical processes that operate on a wide range of different timescales. Identifying their computational roles and understanding how these intricate networks of interactions support synaptic memory formation and maintenance requires an appropriate theoretical framework.

In this work we construct a broad class of synaptic models with tightly bounded dynamical variables and efficacies that can efficiently harness biological complexity to store and preserve numerous memories. The number of storable memories grows almost linearly with the number of synapses, which constitutes a substantial improvement over the square root scaling of previous models, especially when large neural systems are considered. In addition, the initial memory strength is also high in these models, and scales approximately like the square root of the number of synapses.

These favorable properties are achieved by combining together multiple dynamical processes that operate on different timescales, to ensure the memory strength decays as slowly as the inverse square root of the age of the corresponding synaptic modification. This decay curve implements an optimal compromise between large memory strengths and long lifetimes, maximizing the area under the (doubly logarithmic plot of the) signal to noise ratio as a function of time. Memories are initially stored in fast variables and then progressively transferred to slower variables. Importantly, in our case the interactions between fast and slow variables are bidirectional, in contrast to the unidirectional cascades of previous models. Each synapse only requires a small number of variables that can have very limited precision.

The proposed models are robust to perturbations of parameters and can capture several properties of biological memories, which include delayed expression of synaptic potentiation and depression, synaptic metaplasticity, and spacing effects. We discuss predictions for the autocorrelation function of the synaptic efficacy that can be tested in plasticity experiments involving long, balanced sequences of synaptic modifications.

We investigate both perceptron-like classifiers and fully connected recurrent networks incorporating these model synapses, numerically confirming the almost linear scaling of the corresponding capacities, and characterizing their generalization performance. Furthermore, we show that the advantages of complex synapses can be combined with those of sparse coding, leading to memory lifetimes that grow superlinearly with the number of synapses.

Disclosures: **M.K. Benna:** None. **S. Fusi:** None.

Nanosymposium

490. Models of Memory and Anticipatory Coding

Location: SDCC 2

Time: Tuesday, November 15, 2016, 8:00 AM - 11:00 AM

Presentation Number: 490.11

Topic: I.06. Computation, Modeling, and Simulation

Title: A bayesian approach for cortical source localization of EEG signals based on model-posterior probabilities.

Authors: *H. COURELLIS[1,2], J. IVERSEN[2], G. CAUWENBERGHS[1,3];
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Abstract: Spatial localization of cortical current density associated with a cognitive task across multiple subjects using Independent Component Analysis (ICA) of recorded EEG signals presents a challenge because a large number of Independent Components (ICs) need to be manually screened before suitable ICs are selected that exhibit spatial consistency across subjects. ICA algorithms order computed ICs in descending power without any guarantee of spatial IC consistency among subjects. As a result, identification of dipolar ICs in identical anatomical locations across all test subjects in a group is a process that must be done manually. This manual IC identification for each individual within a group quickly becomes intractable as the number of ICs for any given study can reach the thousands. Cortical Low Resolution Electromagnetic Tomography (cLORETA) bypasses these challenges by allowing for the manual selection of cortical regions of interest (ROIs) to which current dipoles are localized. However, the selected ROIs may not necessarily have contributed greatly to the activity of interest within the measured EEG electrical potentials. In such cases, forced localization will yield low SNR current density which will poorly represent brain activity associated with the cognitive task if it is used for further data analysis. A methodology is presented for selecting the most relevant ROIs based on the Bayesian framework upon which cLORETA is implemented. The cortical boundary element model (BEM) used in cLORETA is anatomically segmented, providing a set of ROIs, and the model-posterior probability of each ROI is computed by pruning the electrical lead field and Laplacian matrices to limit estimation of the posterior to each individual ROI given the measured EEG data. This procedure is performed for each subject individually using the subject's recorded EEG and a customized BEM warped to resemble the subject's cortical geometry. Only ROIs with consistently high posterior probabilities across all subjects are used for further analysis. ROIs deemed functionally important for the cognitive task may be assigned additional probability mass through the model (ROI) prior distribution. The proposed ROI selection methodology was first evaluated using simulated cortical dipoles to demonstrate its ability to accurately capture regions of significant current density. The method was then applied

to human EEG data recorded from a group of subjects during a reach-saccade task to identify relevant visuo-motor ROIs. Preliminary results demonstrated the effectiveness of the proposed ROI selection methodology, which provided ROIs with consistently high posterior probability.

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Nanosymposium

490. Models of Memory and Anticipatory Coding

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Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant RO1GM098578

Title: How network topology shapes directionality of information flow in brain networks across different species

Authors: ***J.-Y. MOON**[1], J.-H. KIM[2], T.-W. KO[3], M. KIM[2], Y. MEDINA[4], J.-H. CHOI[5], J. LEE[1], G. MASHOUR[1], U. LEE[1];
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Abstract: To understand how spatially distributed information is integrated in the brain is essential to study higher cognitive brain functions. With the advance of computational and imaging techniques, current progress in the field of cognitive neuroscience has moved from a modular, region-of-interest, and correlative approach, to a network-based description of neural information processing with a focus on causal mechanisms of the network connectivity. In this regard, identification of the relationship between the underlying network topology and the patterns of the information transfer is highly requested. In this work, the mechanism of how global topology shapes the directionality patterns of information flow is presented: the hub nodes (nodes with more connections) will phase lag compared to other nodes, hence becoming the sinks of the directionality, while the peripheral nodes will phase lead, becoming the source of the directionality. First, we derive analytically the mechanism of this relationship using canonical oscillator models (Kuramoto and Stuart-Landau models) on model complex networks. Updating our previous results (Moon et al., Plos Comp Biol 2015), we deduce rigorously the exact phase of each node from the topology of the underlying network, and confirm our analytic results with computational simulations. We proceed to show that the phase lead/lag relationship can be

interpreted as the causal relationship, and demonstrate that such phase lead/lag relationship coincides with other causal measures. Second, we apply our analysis to the real brain structural networks of human, macaque, and mouse, and predict the phase lead/lag patterns of each node of the networks. Lastly, we show that our analytical predictions on different species brain networks are accurately matched by the empirical high-density electroencephalography (EEG) data from different species. Our analysis is general in a sense that it can be applied to different kind of networks. Our work also demonstrates that the brain networks across different species follow similar principles shared by other networks. By showing that the results obtained from studying directionality of the coupled oscillators on the general networks can be applied to brain networks across different species, we open the possibility to reveal other properties of the brain networks by studying general properties of the coupled oscillator networks.

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Nanosymposium

571. Regulation of Adult Neurogenesis

Location: SDCC 25A

Time: Tuesday, November 15, 2016, 1:00 PM - 3:00 PM

Presentation Number: 571.01

Topic: A.02. Postnatal Neurogenesis

Support: Fondazione Istituto Italiano di Tecnologia

Title: Synergic functions of miRNAs determines neuronal fate commitment of adult neural stem cells

Authors: ***M. PONS ESPINAL**[1], E. DE LUCA[1], R. BECKERVORDERSANDFORTH[2], K. FABEL[3,4], G. KEMPERMANN[4,3], D. DE PIETRI TONELLI[1];
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Abstract: Production of new neurons and astrocytes in the adult mammalian hippocampus requires precise control of neuronal *vs.* astrocyte lineage determination of neural stem cells (NSCs). While evidence has been mounting that microRNAs (miRNAs) might critically involved in this step, the set of critical factors and mechanisms of their actions has been unclear. As entry point to address that question we chose the endoribonuclease DICER that is essential for the maturation of nearly all miRNAs and other RNAi-related processes and controls neuronal and

astrocyte lineage fate determination in development. Here, by re-administering miRNAs in Dicer-ablated NSCs, we found that the synergic action of eleven miRNAs (9 of which had not been previously characterized in adult neurogenesis) is sufficient to rescue Dicer-dependent impairment of neurogenesis. Remarkably production of new astrocytes is not impaired upon mIRNA depletion *in vivo and in vitro*. Therefore, our study demonstrates that miRNAs are critical for lineage fate commitment and identifies a new set of miRNAs that, by synergistically enforcement of gene-regulatory networks, allows the acquisition of the neurogenic fate program in aNSCs.

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Nanosymposium

571. Regulation of Adult Neurogenesis

Location: SDCC 25A

Time: Tuesday, November 15, 2016, 1:00 PM - 3:00 PM

Presentation Number: 571.02

Topic: A.02. Postnatal Neurogenesis

Title: ApoE negatively regulates the development of newborn neurons in the adult hippocampus

Authors: ***T.-S. YU**, S. KERNIE;
Pediatrics, Columbia Univ. Med. Sch., New York, NY

Abstract: The presence of specific Apolipoprotein E (ApoE) isoforms in humans strongly correlates with the development of certain forms of dementia-like Alzheimer disease through unknown mechanisms. By performing neural stem cell-specific DNA arrays, we previously identified ApoE as a negative regulator of hippocampal neurogenesis. ApoE is expressed in type 1 nestin- and GFAP-expressing neural progenitors in the dentate gyrus as well as astrocytes throughout the brain in adult mice (Gilley et al, 2011). By using constitutively ApoE-deficient mice, we also demonstrated that neural progenitors proliferate more rapidly at early ages (~4 weeks old), which is accompanied by a decrease in the overall number of neural progenitors at later time points (Yang et al, 2011). Here, we use an eGFP-expressing retrovirus to infect adult neural stem cells and demonstrate that newborn neurons in the adult ApoE-deficient hippocampus have less complicated dendritic morphology upon reaching maturation 4 weeks after infection. To overcome potential developmentally driven confounders in ApoE-deficient mice, we generated an inducible knock-out of ApoE. We crossed ApoE floxed conditional mice with previously characterized nestin-creERT2 mice and administered tamoxifen to trigger cre-mediated recombination specifically in type 1 neural stem cells. By giving tamoxifen to mice from postnatal 7 days to 10 days, the expression of ApoE was decreased noticeably in the

hippocampus in 8-week old mice, and neural progenitor proliferation is altered. In addition, we have directly injected Cre-expressing retrovirus into the hippocampus of ApoE-floxed animals. The infected ApoE-deficient cells developed into mature neurons with less complexity while compared with controls 4 weeks after infection. These studies specifically define the role of ApoE in regulating the proliferation and development of adult newborn neurons in the hippocampus.

Disclosures: T. Yu: None. **S. Kernie:** None.

Nanosymposium

571. Regulation of Adult Neurogenesis

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Presentation Number: 571.03

Topic: A.02. Postnatal Neurogenesis

Support: SNF 143767

Title: Drosha-mediated post-transcriptional regulation of multi-lineage potential of hippocampal stem cells.

Authors: *C. ROLANDO[1], A. ERNI[1], R. BEATTIE[2], A. ENGLER[1], A. GRISON[1], M. MILO[3], P. J. GOKHALE[3], T. WEGLEITER[4], S. JESSBERGER[4], V. TAYLOR[1];
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Abstract: Neural stem cells (NSCs) in the dentate gyrus (DG) of the adult hippocampus are self-renewing and multipotent. DG NSCs are fated to become granule neurons and astrocytes but do not generate oligodendrocytes. This fate restriction is also maintained by DG NSCs in vitro suggesting a cell autonomous mechanism that remains unclear. The miRNA Microprocessor, a multimeric complex of the ribonuclease Drosha and the RNA binding protein DGCR8, binds and cleaves double-stranded hairpins in miRNA primary transcripts to generate precursor miRNA that is exported from the nucleus to the cytoplasm for further processing by Dicer. The Microprocessor also has miRNA-independent functions including targeting and cleaving stem-loop hairpin structures in mRNAs thereby destabilizing the transcripts. We have previously shown that non-canonical target cleavage by Drosha is critical during cortical development. Therefore, we hypothesised that Drosha may regulate NSC activity in the adult nervous system, and examined this by conditionally deleting *Drosha* from DG NSCs. *Drosha* conditional knockout (cKO) reduced NSCs proliferation and number culminating in a decrease in neuroblasts. Surprisingly, *Drosha* cKO NSCs generated oligodendrocytes, a cell type normally

not produced in the adult DG. We showed that this fate change was independent of Dicer and miRNAs. We have identified targets of Drosha responsible for regulating NSC differentiation towards oligodendrocytes. Taken together our findings reveal a novel miRNA-independent mechanism for Drosha in maintaining adult DG NSCs and regulating oligodendrocyte differentiation in the adult hippocampus.

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Nanosymposium

571. Regulation of Adult Neurogenesis

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Title: Adult hippocampal neurogenesis promotes stress resilience by inhibiting ventral dentate gyrus activity

Authors: *C. ANACKER, V. M. LUNA, A. MILLETTE, R. SHORES, D. H. VACCARO, R. HEN;
Columbia Univ., New York, NY

Abstract: Adult hippocampal neurogenesis is necessary to confer antidepressant effects and to prevent stress-induced behavioral abnormalities. However, it is unknown how the small population of adult-born neurons exerts such profound effects on behavior. Here, we investigated how neurogenesis regulates dentate gyrus function to mediate stress resilience.
We used mice with adult-inducible deletion of the pro-apoptotic gene, *Bax*, from neural stem cells and their progeny to increase the number of adult-born neurons by ~2 fold. We then subjected mice to 10 days of social defeat stress and examined neuronal activity in the dentate gyrus using immunohistochemistry of the immediate early gene, c-fos, *in vitro* electrophysiology, and behavioral tests of anxiety.

Chronic social defeat stress increases the number of c-fos+ cells in the ventral dentate gyrus (by 4.6±0.5 fold, n=8, $p<0.001$). This effect was attenuated in mice with increased neurogenesis (2.7±0.5 fold, n=9, $p<0.01$). In addition*, in vitro* electrophysiological recordings showed a 3 fold higher inhibition/ excitation ratio in defeated mice with increased neurogenesis compared to defeated mice with normal levels of neurogenesis (n=9, $p<0.05$). At the behavioral level, defeated mice spend ~40% less time interacting with a novel mouse (Ctrl: 104±5 sec, defeated: 62±11 sec; n=16; $p<0.01$) and ~42% less time in the center of the open field (Ctrl: 43±2 sec, defeated: 18±1 sec; n=14; $p<0.01$). Mice with increased neurogenesis are resilient to social defeat stress and exhibit higher levels of social interaction time (95±9 sec; n=14, $p<0.01$) and center time in the open field (72±8 sec; n=14, $p<0.05$). To determine whether neurogenesis-mediated inhibition of the dentate gyrus is necessary and sufficient to confer stress resilience, we injected the Gi-protein coupled inhibitory designer receptor exclusively activated by designer drugs (DREADD), hM4Di, into the ventral dentate gyrus. We then chronically silenced mature granule cells during social defeat by daily injections of the DREADD-receptor ligand, clozapine-N-oxide (CNO). Indeed, silencing mature granule cells during chronic defeat increases interaction time (VEH: 85±9 sec, CNO: 125±7 sec; n=11; $p<0.01$) and open field center time (VEH: 17±3 sec, CNO: 28±4 sec; n=11; $p<0.05$). Accordingly, stimulating mature granule neurons using the Gq-protein coupled DREADD receptor, hM3Dq, decreases social interaction time (VEH: 74±6 sec, CNO: 37±8 sec; n=8; $p<0.05$) and open field center time (VEH: 42±5 sec, CNO: 23±7 sec; n=8; $p<0.05$).
Our findings demonstrate that hippocampal neurogenesis promotes stress resilience by inhibiting the activity of the ventral dentate gyrus.

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Nanosymposium

571. Regulation of Adult Neurogenesis

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Time: Tuesday, November 15, 2016, 1:00 PM - 3:00 PM

Presentation Number: 571.05

Topic: A.02. Postnatal Neurogenesis

Support: R21MH106939

Title: Long-range septal GABAergic circuits mediate neural stem cell maintenance and neurogenesis via GABA-induced depolarization of local parvalbumin interneurons in the adult brain

Authors: *H. BAO[1,2], B. ASRICAN[1], T. HE[1], I. HANIFF[1], A. QIAO[1], S.-A. LIM[1], W. LI[2], J. SONG[1];
[1]Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; [2]Bio-X Inst., Shanghai Jiao Tong Univ., Shanghai, China

Abstract: Adult neurogenesis arises from neural stem cells within a specialized nich. Neuronal activity and experience, presumably acting upon this local niche, regulate adult neurogenesis process. Our recent studies identified local parvalbumin-expressing (PV^+) interneurons in the adult dentate gyrus as a critical and unique circuitry component in regulating multiple stages of neurogenesis, from neural stem cell activation and fate specification to new neuron integration and survival. The underlying circuitry and signaling mechanisms regulating this critical niche component and subsequent neurogenesis in the adult brain remain unknown. Here we show that long-range GABAergic inputs from medial septum and diagonal band of Broca directly synapse onto dentate PV^+ interneurons to impact adult neural stem cell maintenance and neurogenesis. In contrast to classic mature neurons that are hyperpolarized by GABA, adult dentate PV^+ interneurons exhibit GABA-induced depolarization and Ca^{2+} influx. Our study identifies a novel circuitry and signaling mechanism involving special properties of local PV^+ interneurons that couples long-distance circuit activity to the critical phases of adult hippocampal neurogenesis.

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Nanosymposium

571. Regulation of Adult Neurogenesis

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Presentation Number: 571.06

Topic: A.02. Postnatal Neurogenesis

Title: Age dependent modulation of adult neurogenesis by the voltage gated potassium channel Kv2.1

Authors: *K. D. MURRAY[1], M. YOSHIHARA[2], J. S. TRIMMER[3], H.-J. CHENG[2];
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Abstract: Adult neurogenesis occurs throughout life in the subgranular zone (SGZ) of the hippocampus and the subventricular zone of the lateral ventricle, however in aging brains rates of neurogenesis are severely reduced. In spite of this, remaining newborn neurons maintain the ability to undergo proper morphological maturation and integration into existing neuronal

circuitry. The mechanisms underlying reduced neurogenesis in aging aren't known, but are thought to involve changes in the external cellular environment as well as intrinsic cell autonomous factors. Widespread activation of neuronal circuits dramatically affects adult neurogenesis but whether intrinsic control of neuronal excitation is involved is not known. Here we examined the role of the voltage gated potassium channel Kv2.1, a major regulator of intrinsic neuronal excitability, in maintenance of adult neurogenesis.
To investigate the role of Kv2.1 channels in regulating adult neurogenesis we first determined the effect of Kv2.1 knockout (Kv2.1 KO) on incorporation of thymidine analogue, 5-Ethynyl-2'-deoxyuridine (EdU). We found reduced numbers of labeled cells at short intervals following pulse labeling with EdU in Kv2.1 KO animals. Surprisingly, reduced EdU cell number was age dependent. No difference in EdU cells was observed in early postnatal (0.5 months) or 1 month old brains, but at 3 months, EdU cell numbers were reduced by 41%, and this was even more pronounced in aged brains (95% reduction). To determine whether the typical decrease in neurogenesis seen in aging brain could be due to loss of Kv2.1, we examined expression levels in aged brain by immunofluorescent antibody labeling. Labeling for Kv2.1 was reduced throughout hippocampus (30-50%) and in neocortex (50%) in aged (12-15 month) brain compared with adult (3 month). A reduction in Kv2.1 levels was also observed in aged hippocampus (40%) and neocortex (20%) by immunoblot analysis. To determine whether reduced neurogenesis in Kv2.1 KO mice could be due to intrinsic regulation of the cell cycle in proliferating cells, we examined the expression of Kv2.1 in progenitor cells and their progeny in adult mice using the inducible Gli1CreER mouse line, which conditionally labels neural precursor cells and their progeny. In the SGZ, immunolabeling for Kv2.1 was not observed in neural progenitor cells. Similarly, young newborn hippocampal neurons did not exhibit labeling for Kv2.1 until approximately 4 weeks of age. Together, these results suggest that the decreased expression of the voltage-gated potassium channel Kv2.1 in aged brain may lead to reduced proliferation of newborn neurons in a non-cell autonomous manner.

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Nanosymposium

571. Regulation of Adult Neurogenesis

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Presentation Number: 571.07

Topic: A.02. Postnatal Neurogenesis

Support: AHA 14PRE20460380

Title: Loss of endothelial caveolin-1 results in compromised neurogenesis within the hippocampus of the adult Murine Brain.

Authors: *J. A. BONDS[1], R. D. MINSHALL[2], O. LAZAROV[3];
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Abstract: Neural stem cells (NSCs) exist in discrete microenvironments within the subventricular zone (SVZ) and the dentate gyrus (DG) of the hippocampus in the adult brain. Vascular endothelial cells are thought to play a critical role in supporting, maintaining, and modulating these neurogenic microenvironments. Endothelial Caveolin-1 (Cav-1) is a cholesterol binding protein that resides in cell membrane lipid rafts and is critical for endothelial cell function. Thus we hypothesized that endothelial Cav-1 is critical in regulation of adult neurogenesis. To address this question, we utilized an endothelial specific Cav-1 knock-out (EC-KO) mouse to analyze the number of NSCs in the SVZ and DG. We observe that within both the global and EC-Cav-1 KO the number of NSCs is significantly decreased in the DG 8-weeks of age. We further observed that neurogenesis continues to decline at 6 months of age. Concomitantly, loss of Cav-1 has been shown to result in a dramatic reduction in cerebrovasculature, suggesting that depletion of Cav-1 may compromise the neurovascular niche. This study suggests that endothelial Cav-1 may play an important role in the regulation and homeostasis of the neurogenic vascular niche and that reduction in Cav-1 may underlie the age-dependent decline in neurogenesis.

Disclosures: J.A. Bonds: None. **R.D. Minshall:** None. **O. Lazarov:** None.

Nanosymposium

571. Regulation of Adult Neurogenesis

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Time: Tuesday, November 15, 2016, 1:00 PM - 3:00 PM

Presentation Number: 571.08

Topic: A.02. Postnatal Neurogenesis

Title: A FIP200/p62/Ccl5-Cxcl10 cascade in postnatal neural stem cell triggered non-cell-autonomous inhibition of neurogenesis by microglia

Authors: *C. WANG, S. YEO, J.-L. GUAN;
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Abstract: The fate determination of neural stem/progenitor cells (NSCs) is regulated intrinsically and extrinsically by their local micro-environment. We have shown that conditional

knockout (cKO) of autophagy essential gene FIP200 in postnatal NSCs impaired their maintenance and differentiation. Here, we demonstrated a non-cell-autonomous function of autophagy in postnatal NSCs to regulate their differentiation. First, we found that FIP200-deficient NSCs recruited more microglia, a brain resident immune cell, to the NSC niche within subventricular zone (SVZ). Next, we analyzed gene expression profile of NSCs from control (Ctrl), FIP200 cKO, FIP200/p53 2cKO and p53 cKO mice and found higher expression of Ccl5 and Cxcl10 in FIP200 cKO and FIP200/p53 2cKO NSCs. Then, we found that the increased chemokine expression in FIP200-null SVZ was induced by abnormal p62 aggregates as p62 KO in FIP200 cKO mice rescued the expression levels of Ccl5 and Cxcl10 to those in Ctrl mice. Moreover, we confirmed that re-expression of wild type p62 but not aggregate-formation defective mutant p62 (K7A/D69A) in FIP200/p62 2cKO NSCs restored the expression level of these chemokines to that in FIP200 cKO NSCs in vitro. To investigate the relationship of increased Ccl5 and Cxcl10 with more microglia infiltration in FIP200-null SVZ, we blocked Ccl5 and Cxcl10 by either shRNA knockdown or depleting antibodies and we found both methods abolished the enhanced microglial migration towards conditioned media from FIP200-null NSCs. We noticed that the infiltrated microglia exhibited M1 phenotype with increased expression of proinflammatory cytokines IL-6 and TNF-alpha, along with iNOS in FIP200 cKO SVZ and FIP200/p53 2cKO SVZ. Lastly, we injected TAK-779 to block increased microglia infiltration into FIP200-null SVZ through elevated Ccl5 and Cxcl10 and we also used minocycline to inhibit microglia activation. Both TAK779 and minocycline rescued the defective neurogenesis in FIP200/p53 2cKO mice with normal NSC pool but not in FIP200 cKO mice which were deficient in both NSC maintenance and differentiation. Together, our results suggest that autophagy in postnatal NSCs plays a crucial role in restricting local immune response and regulates neurogenesis through a non-cell-autonomous mechanism.

Disclosures: **C. Wang:** None. **S. Yeo:** None. **J. Guan:** None.

Nanosymposium

572. Autism: Physiology and Behavior

Location: SDCC 23A

Time: Tuesday, November 15, 2016, 1:00 PM - 4:45 PM

Presentation Number: 572.01

Topic: A.07. Developmental Disorders

Support: NIH Grant R01- MH081023

NIH Grant K01-MH097972

Title: Intrinsic functional overconnectivity of the language network in children with autism spectrum disorder

Authors: *Y. GAO[1], M. BERKEBILE[2], A. JAHEDI[3], S. PUNYAMURTHULA[2], W. ZHAO[2], C. H. FONG[2], R.-A. MÜLLER[2], I. FISHMAN[2];
[1]Clin. Psychology, San Diego State University/ UC San Diego, San Diego, CA; [2]Psychology, [3]Mathematics and Statistics, San Diego State Univ., San Diego, CA

Abstract: Background: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that has been linked to atypical brain connectivity. Language delay is one of the first detectable signs of ASD, suggesting a crucial linkage between language development and diagnosis. However, the functional brain connectivity of the language network in ASD is poorly understood. The present study explored intrinsic functional connectivity (iFC) of the language network in children with ASD as compared to typically developing (TD) peers, and its links with ASD symptom severity.
Methods: Low motion 6-minute eyes-open resting state fMRI from 49 ASD and 48 TD youths, ages 8-17 years, were included. The groups were matched on age, non-verbal IQ, head motion, gender, and handedness (all *p*s>.63). Data were preprocessed using nuisance regressors from six motion parameters, white matter and ventricles, and their derivatives. Time points with motion >0.5mm and two subsequent time points were censored. All included subjects had >80% surviving time points. IFC analyses were performed with regions of interest (ROIs) selected from an Activation Likelihood Estimation meta-analysis of 54 language studies (Rodd et al., *Brain Lang* 2015). Average BOLD time series extracted from each cortical seed were correlated with the time course from each other ROI, resulting in a 14 x 14 connectivity matrix. The correlations were transformed to z-scores and directly compared between groups. The results with uncorrected $p<0.05$ were adjusted using local false discovery rate (FDR) and correlated with measures of symptom severity.
Results: Direct group comparison revealed 11 pairs of correlations between language ROIs that survived FDR adjustment ($q< 0.18$). Of the 11 ROI pairs, 9 showed greater connectivity in the ASD group. The overconnectivity exhibited in the ASD group between left inferior frontal gyrus and left angular gyrus was negatively correlated with Autism Diagnostic Observational Schedule (ADOS-2) index of Social Affect ($r= -0.38$, $p=0.01$) and the ADOS-2 Total score ($r= -0.31$, $p=0.04$).
Conclusion: Our results support previous findings of aberrant within-network connectivity in individuals with ASD. However, contrary to some previous reports (e.g., Just et al., *Brain* 2004) the language network in ASD was predominantly characterized by overconnectivity. The link between overconnectivity of two left-hemisphere perisylvian regions (Broca's area and angular gyrus) and reduced symptom severity in ASD may suggest compensatory mechanisms, possibly related to a history of co-activation during effortful processing.

Disclosures: Y. Gao: None. **M. Berkebile:** None. **A. Jahedi:** None. **S. Punyamurthula:** None. **W. Zhao:** None. **C.H. Fong:** None. **R. Müller:** None. **I. Fishman:** None.

Nanosymposium

572. Autism: Physiology and Behavior

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Presentation Number: 572.02

Topic: A.07. Developmental Disorders

Support: NIH Grant R01- MH081023

NIH Grant K01-MH097972

Title: Aberrant patterns of cortical gyrification in children and adolescents with autism spectrum disorder

Authors: *J. S. KOHLI, R. A. CARPER, C. H. FONG, R.-A. MÜLLER;
Psychology, Brain Develop. Imaging Lab, San Diego State Uni, San Diego, CA

Abstract: Background: A large body of evidence supports a trajectory of early brain overgrowth in Autism Spectrum Disorder (ASD) followed by abnormally slow growth. The process of gyrification allows this growing cortical surface to be enclosed within a limited cranial volume. Gyrification begins prenatally but modifications continue through childhood and adolescence. The few studies of local gyrification index (lGI) in ASD are inconsistent, reporting atypically increased lGI in children (4-12y.o., Yang et al., 2016) but atypically decreased lGI in a small adolescent sample (9-18y.o., Schaer et al., 2013). Given the divergent findings, the current study sought to investigate developmental effects on gyrification in a large ASD sample ranging in age from 7 to 18 years.
Methods: T1 weighted MRI sequences ($1mm^3$) were collected on 157 participants aged 7–18 years (91 ASD, 66 TD). Following quality assurance, 60 ASD and 45 typically developing (TD) controls, matched on age, non-verbal IQ, and total brain volume, were included. LGI, the ratio of cortical surface area buried within sulcal folds to the outer brain surface area, was calculated using FreeSurfer v.5.3.0. Statistical analyses used a general linear model including age as a covariate and Social Responsiveness Scale (SRS) scores as a correlate. Results corrected for multiple comparisons using Monte Carlo null-z simulations at $p<0.05$.
Results: lGI was significantly greater in the ASD group in left superior parietal lobule and lingual gyrus and in right inferior parietal lobule and parahippocampal gyrus. A significant group by age interaction was found in left precentral gyrus: Whereas lGI was relatively stable across age in the TD group, the ASD group exhibited a significantly more negative slope, showing decreasing lGI with age. Correlation analyses in the ASD group revealed significant associations between higher lGI and greater social impairment (higher total SRS) in right hemisphere superior frontal and lateral orbitofrontal regions.
Conclusions: Clusters of increased lGI in ASD were consistent with the larger of two earlier studies (Yang et al., 2016), although localization of effects only partially overlapped. Declining

lGI with age is often reported in typical adolescence, and here we find regions of steeper age-related decrease in ASD compared to TD participants. This may echo the complex atypical growth trajectories of other neuroanatomical measures in children with ASD. Correlations between lGI and SRS support functional relevance of this morphometric measure. Further comparisons will be presented to assess associations between lGI and other morphometric indices (surface area, cortical thickness).

Disclosures: **J.S. Kohli:** None. **R.A. Carper:** None. **C.H. Fong:** None. **R. Müller:** None.

Nanosymposium

572. Autism: Physiology and Behavior

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Presentation Number: 572.03

Topic: A.07. Developmental Disorders

Support: Wellcome Trust grant

European Commission grant

Title: Atypical balance of grey matter volumes between cortical brain networks in autism.

Authors: ***T. WATANABE**[1], G. REES[1,2];
[1]Inst. of Cognitive Neuroscience, Univ. Col. London, London, United Kingdom; [2]Wellcome Trust Ctr. for Neuroimaging, Univ. Col. London, London, United Kingdom

Abstract: Core symptoms of autism spectrum disorder (ASD) consist of socio-communicational deficits and repetitive, restricted behaviours. Although the neuroanatomy underlying these two symptoms has been studied, the biological mechanisms that allow these two seemingly irrelevant behavioural characteristics to coexist in a single developmental disorder remains unclear. In this study using open anatomical neuroimaging data of high-functioning males with ASD and age-/sex-/IQ-matched controls, we found that atypical balance of grey matter volumes (GMV) between three large-scale cortical brain networks can be a key biological mechanism underlying such integration and segregation of these distinct symptoms of ASD. We identified age-related atypical increases in relative GMVs of the regions constituting auditory and visual networks, and an age-associated aberrant decrease in relative GMV of fronto-parietal network (FPN) regions in ASD children. In addition, the atypically enlarged relative GMV of the auditory network in ASD adults was associated with the severity of their socio-communicational deficits, and that of visual network was correlated with cognitive inflexibility. Moreover, the atypical decrease in relative GMV of FPN was related to both of the two core symptoms. These observations suggest that

disproportionate undergrowth of FPN may be a common anatomical basis for the two dissociable and seemingly heterogeneous core symptoms of ASD, and relative overgrowth of the two different sensory networks may selectively underlie the different autistic symptoms.

Disclosures: **T. Watanabe:** None. **G. Rees:** None.

Nanosymposium

572. Autism: Physiology and Behavior

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Topic: A.07. Developmental Disorders

Support: NIH Grant R01-MH081023

NIH Grant K01-MH097972

Title: Reduced network differentiation in children with autism spectrum disorder is associated with symptom severity

Authors: ***C. H. FONG**, W. ZHAO, S. NAIR, M. BERKEBILE, R.-A. MÜLLER, I. FISHMAN;
Psychology, SDSU, San Diego, CA

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder estimated to affect 1 in 45 children in the US. It is characterized by impaired social communication and interaction, and repetitive and stereotyped behaviors. By consensus, ASD is a disorder involving distributed brain networks, although the precise patterns of the network abnormalities in ASD are still under debate. Given recent reports of atypical network segregation in ASD, this study examined whether functional connectivity outside neurotypical brain networks is greater (i.e., networks are less differentiated) in children and adolescents with ASD, compared to typically developing (TD) peers.
Functional connectivity (FC) analysis was performed on resting state fMRI data acquired in 54 children and adolescents with ASD (7-17 years old) and 50 TD participants matched for age, non-verbal IQ, and in-scanner head motion. Standard preprocessing, including motion and field map correction, spatial and temporal filtering, nuisance regression (motion, white matter, CSF), and removal of time points with motion >0.5mm and two subsequent volumes, was applied. Five networks of interest previously implicated in ASD were identified using seed-based FC: default mode network (DMN), central executive network (CEN), salience network (SN), visual network (VN), and the mirror neuron system (MNS). For each participant, outside network (ON)

connectivity maps were created for each network, by excluding dilated network nodes from the whole-brain FC maps. The number of ON significant voxels, weighted by z', was then compared between the groups.
Of the five networks examined, a significant difference in the extent of ON connectivity (ASD > TD) was found for the SN ($p = 0.04$), and marginally for CEN and VN ($p = 0.08$ in both). When analyses were repeated for the ASD subset with higher symptom severity (excluding 14 children with Total Autism Diagnostic Observation Scale (ADOS) score ≤ 10), greater ON connectivity was observed in 3/5 networks: SN ($p = 0.01$), CEN ($p = 0.03$) and VN ($p = 0.04$). Within the ASD group, the degree of ON overconnectivity (averaged across 5 networks) was significantly correlated with ASD symptomatology, such that participants with greater ASD symptoms had more excessive ON connections ($r = 0.32$, $p = 0.01$).
Overconnectivity with regions outside neurotypical networks in children with ASD may reflect impaired functional network differentiation of the brain systems involved in reward processing (SN), executive control (CEN) and visual processing (VN). The observed links with increased behavioral symptoms suggest that ASD symptomatology may be tied to excessive connections between brain networks.

Disclosures: C.H. Fong: None. **W. Zhao:** None. **S. Nair:** None. **M. Berkebile:** None. **R. Müller:** None. **I. Fishman:** None.

Nanosymposium

572. Autism: Physiology and Behavior

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Topic: A.07. Developmental Disorders

Support: NIH R01- MH081023

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Title: Domain specificity of altered cortico-striatal functional connectivity in children and adolescents with autism

Authors: *A. MACARI, M. BERKEBILE, A. C. LINKE, W. ZHAO, R.-A. MÜLLER; Psychology, Brain Develop. Imaging Lab, San Diego State Uni, San Diego, CA

Abstract: Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental disorder marked by sociocommunicative impairment as well as restrictive and repetitive behaviors (American Psychiatric Association [APA], 2013). Several studies have shown increased

functional corticostriatal connectivity in ASD relative to typically developing (TD) groups. Additionally, two recent studies of thalamocortical and cerebro-cerebellar connectivity in ASD (Nair et al., 2015; Khan et al., 2015) revealed distinct patterns of atypically increased sensorimotor/limbic connectivity, but reduced connectivity with supramodal cerebral cortical regions, suggesting that such a dichotomy may generally characterize subcortico-cortical connectivity in ASD. The current study tested this question by examining domain-specific patterns of cortico-striatal functional connectivity in ASD.
Functional T2*-weighted eyes-open resting state scans from 52 ASD and 47 TD participants, ages 7-17, were included. Groups were matched on age, motion, and nonverbal IQ. Cortical and striatal ROIs were obtained from Harvard-Oxford and Jülich-Histological atlases. The average time series from each cortical ROI was correlated with each voxel within the ipsilateral striatum. The correlation coefficients were Fisher transformed (r-to-z), entered into a one- and two-sample two-tailed t-test for within-group and between-group comparisons. Results were adjusted for multiple comparisons using Monte Carlo simulation to a corrected $p < 0.05$.
ROIs in both the sensorimotor/limbic domain (A1, S1, S2, and M1) and the supramodal domain (PFC, OFC, VMPFC, precuneus, and paracingulate cortex) displayed significantly higher iFC with the striatum in the ASD group relative to the TD group. Thus, sensorimotor/limbic and supramodal cortico-striatal circuits were equally characterized by overconnectivity in ASD.
Our finding of broad cortico-striatal overconnectivity in ASD is consistent with previous findings (e.g., Di Martino et al., 2013). Expected differential patterns (relative overconnectivity for sensorimotor, relative underconnectivity for supramodal circuits) were not detected. In combination with previous studies, findings may suggest predominant overconnectivity in ASD for deep structures with inhibitory efferents (cerebellar cortex, striatum), but underconnectivity for those with excitatory efferents (thalamic nuclei), possibly implying reduced levels of both normal inhibition and excitation in subcortico-cortical connectivity.

Disclosures: **A. Macari:** None. **M. Berkebile:** None. **A.C. Linke:** None. **W. Zhao:** None. **R. Müller:** None.

Nanosymposium

572. Autism: Physiology and Behavior

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Presentation Number: 572.06

Topic: A.07. Developmental Disorders

Support: NIH R01-MH081023

K01-MH097972

Title: Amplitude of resting state low frequency BOLD fluctuations in autism.

Authors: ***A. HARIKUMAR**[1], A. LINKE[2], M. BERKEBILE[2], C. H. FONG[2], W. ZHAO[2], I. FISHMAN[2], R.-A. MÜLLER[2];
[1]Psychology, Brain Develop. Imaging Lab., San Diego, CA; [2]San Diego State Univ., San Diego, CA

Abstract: Atypical functional connectivity (both distal and local), including partial overconnectivity, has been reported in autism spectrum disorder (ASD) (Vissers et al., 2012). A few studies have indicated a relationship between low frequency oscillations in resting state fMRI and increased functional connectivity. Specifically, compared to typical controls, children with ASD showed increased functional connectivity as well as increased mean amplitude of low frequency fluctuations (ALFF) (Supekar et al., 2013). However, the regional patterns of atypical ALFF are not well understood. Identifying regions of increased ALFF will be a first step to an improved understanding of low frequency oscillations relate to the autistic brain. We hypothesized that children with ASD would show regional specific increases in ALFF compared to typically developing children.
Participants were selected from a larger cohort, and then subsequently matched for age, gender, and in scanner head motion. Only datasets that did not require censoring at a movement threshold of .5 mm were included . We performed a voxel wise analysis for mean ALFF (averaged within 0.01-0.1 Hz frequency) and fALFF(fractional amplitude of low frequency fluctuations) as the 2 main indices of interest in 35 children and adolescents with ASD(ages 10-17.8), and 33 typically developing children and adolescents (ages 8-17.6). Direct group comparisons revealed that children with ASD had significantly lower mALFF values in bilateral precuneus, left middle cingulate, left postcentral , left superior medial gyrus, as well as the right thalamus and left inferior parietal lobule. mALFF values (ASD > TD) were found in the right middle orbital gyrus and the left inferior temporal gyrus. Additionally, ASD children had increased fALFF values in the left lingual , inferior temporal gyrus , and bilateral calcarine gyri, with decreased fALFF in right SMA, and left inferior parietal lobule.

Disclosures: **A. Harikumar:** None. **A. Linke:** None. **M. Berkebile:** None. **C.H. Fong:** None. **W. Zhao:** None. **I. Fishman:** None. **R. Müller:** None.

Nanosymposium

572. Autism: Physiology and Behavior

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Topic: A.07. Developmental Disorders

Support: The Nancy Lurie Marks Family Foundation

NJ Governor's Council for Research and Treatment of Autism

Title: Index of neuromotor and physical development marks early risk of neurodevelopmental derail in the newborn

Authors: *E. B. TORRES[1], S. MISTRY[2], B. SMITH[4], C. P. WHYATT[3];
[1]Psychology Dept, Rutgers Univ. Dept. of Psychology, Piscataway, NJ; [2]Mathematics, [3]Psychology, Rutgers Univ., Piscataway, NJ; [4]Physical Therapy and Biokinesiology, USC, Los Angeles, CA

Abstract: Neurodevelopmental disorders have reached prevalence levels of epidemic proportion in the US and worldwide. Yet at present there is no objective way to detect risk of neurodevelopmental derail in newborn babies, thus missing a window of opportunity for early intervention. Current diagnostics methods are based on subjective criteria, including clinical observation and parental questionnaires, among others. These tools, discrete in nature, are not administered with enough frequency to detect critical changes in the rapidly growing baby. Furthermore the growth charts in use today to track physical changes enforce normality in data that is inherently skewed and use linear additive models that do not reflect the true nature of the empirical data. As such, the accelerated rates of the babies' incremental growth and the variable fluctuations in parameters of physical growth present in the population at large are obscured and early detection of neurodevelopmental problems consequently challenged. In this work we used publicly available data from 13,623 girls, 13,362 boys including body weight, body length and head circumference used to build the growth charts of the CDC and the WHO (Kuczmarski et al 2002) to unveil the true statistical nature of the longitudinal physical growth data. Drawing from the true statistical nature of these data and combining those results with new analytics of neuromotor control, we further tracked 36 newborn babies longitudinally. These babies (24 at risk and 12 full-term) were studied for 6 months across three visits. During these visits similar measurements to those of the growth chart were obtained but examined incrementally under appropriate multiplicative statistics along with data from wearable sensors continuously worn by each baby for 8-13 hours in each visit. The latter included acceleration, angular velocity and temperature registered in tandem. The new analyses revealed a linear index relating neuromotor control and physical growth whereby stagnation in noise-to-signal transitions from visit to visit flagged risk for neurodevelopmental derail. We next aim to complete data collection from 100 babies in the NICU as well as from full term controls to ascertain the reliability and validity of the uncovered linear rule signaling typicality vs. risk in the newborn.

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Title: Auditory thalamocortical overconnectivity in autism is related to altered topographic organization of auditory cortex

Authors: *A. C. LINKE, R. JAO KEEHN, E. PUESCHEL, R.-A. MUELLER;
Psychology, San Diego State Univ., San Diego, CA

Abstract: Numerous fMRI studies have found atypical functional connectivity in the brain of individuals with autism spectrum disorders (ASD). The underlying causes for these alterations are active topics of investigation. One hypothesis suggests that impaired cortical differentiation accounts for the differences in functional connectivity observed in ASD. Given the important role thalamocortical projections play in the early development of cortical differentiation, and known alterations of thalamocortical connectivity in ASD, we assessed whether differences in auditory thalamocortical connectivity were related to the differentiation of auditory cortex. A cohort of 53 children and adolescents (*m*=13.76 yrs, 9f) diagnosed with ASD, and 50 matched typically developing controls (*m*=13.32 yrs, 10f) were assessed using 6 minutes of resting state fMRI. Data were motion and slice-timing corrected, despiked, censored (with a .5mm movement threshold), denoised (using motion, white matter, and CSF nuisance regressors and their derivatives), bandpass filtered (0.008-0.08Hz), smoothed (to 6mm), normalized to MNI space, and resampled to 2 mm isotropic resolution. Functional connectivity (Pearson correlations) between grey-matter masked auditory and thalamic regions of interest (ROIs) showed auditory thalamocortical overconnectivity in ASD, as reported previously. Cluster analysis was then carried out on voxels within the auditory cortical and thalamic ROIs based on the functional connectivity of each voxel. The resulting parcellations of auditory and thalamic ROIs were evaluated for their similarity across individuals using a mutual information metric (variation of information - *VI*). Parcellation of the auditory ROI differed significantly between the ASD and TD groups. This was due to larger cluster sizes and higher voxel homogeneity within the ASD group. This reduced differentiation in the organization of auditory regions correlated

significantly with higher ROI-to-ROI auditory thalamocortical connectivity. No group differences were observed for the parcellation of thalamus. Additionally, individual differences in auditory, but not thalamus, parcellation correlated with measures of ASD symptom severity (ADOS-2, SRS, Sensory Profile). These results suggest that decreased cortical differentiation may account for previous findings of atypical functional connectivity in ASD. Furthermore, the concurrent lack of differences in thalamic organization points toward a disruption of early brain development - particularly the processes involved in the development of cortical topographic organization - as a potential cause for autism.

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Nanosymposium

572. Autism: Physiology and Behavior

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Presentation Number: 572.09

Topic: A.07. Developmental Disorders

Title: Melanotan-II reverses autistic features in the environmental mouse model of autism

Authors: ***E. MINAKOVA**, J. MEDEL, D. SHIN, R. SANKAR, A. MAZARATI;
UCLA, Los Angeles, CA

Abstract: Background: Autism spectrum disorder is a complex neurodevelopmental disorder characterized by impaired social interactions and difficulty with communication. The etiology of autism involves an interplay between genetic and environmental variables resulting in dysregulation of neurotransmitter expression, aberrant synaptogenesis, neuronal apoptosis, and decreased levels of the pro-social neuropeptide, oxytocin. Central release of oxytocin has neuromodulatory effects on the limbic system and can promote social bonding. Recent studies have shown that Melanotan-II (MT-II), a melanocortin-receptor agonist with blood-brain-barrier permeability, can promote central oxytocin release in the brain. Objective: To test the hypothesis that MT-II treatment improves social deficits in a validated environmental mouse model of autism.
Methods: To generate the environmental mouse model, a pro-inflammatory state in the fetal mouse brain was produced through cytokine injection of IL-6 in pregnant C57BL/6 females. Male mice underwent social behavioral testing using a validated three-chamber apparatus prior to treatment and post-treatment. Baseline ultrasound vocalizations (USVs) were also recorded to assess communication. Mice were treated by surgical placement of a subcutaneous pump with an intraventricular catheter allowing for continuous MT-II infusion over 7 days. Control mice were implanted with isotonic saline-filled pumps. Immunohistochemistry was done to assess for

oxytocin and vasopressin expression in the paraventricular nucleus (PVN)
Results: Following MT-II administration, IL-6 autistic mice showed significant improvement in their sociability and social novelty index scores ($p<$ of 0.0001 and $p< 0.05$, respectively) with levels found comparable to wild-type C57BL/6 mice. Control saline-administered IL-6 autistic mice continued to exhibit low social behavioral scores. MT-II-treated IL-6 autistic mice also demonstrated increased USVs compared to saline-administered controls. PVN staining of untreated adult IL-6 mice showed a significant reduction of oxytocin and vasopressin expression compared to wild-type mice with an increase in both neuropeptides following MT-II administration.
Conclusions: MT-II administration to IL-6 autistic mice corrected social deficits with normalization of behavior metrics to levels found in wild-type mice. This change in behavior is likely due to central release of oxytocin elicited by MT-II. The affected autistic IL-6 mice exhibited abnormally low oxytocin and vasopressin level in the PVN suggesting a novel role for the cytokine to regulate expression patterns of both neuropeptides.

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Topic: A.07. Developmental Disorders

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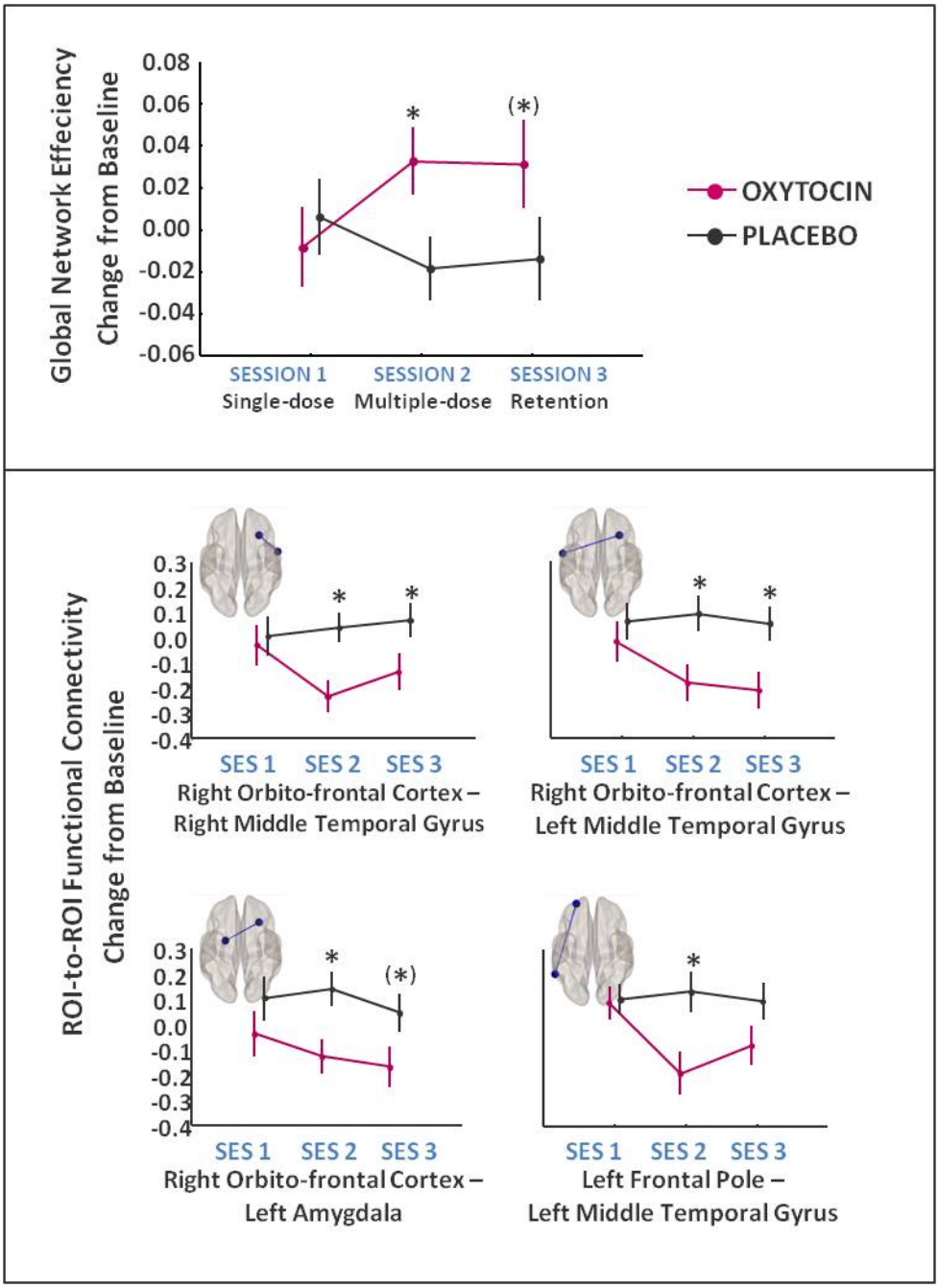
Title: Oxytocin therapy for Autism: Exploring neural and behavioral effects of single- and multiple dose treatment

Authors: *K. ALAERTS, J. PRINSEN, S. BERNAERTS, J. STEYAERT;
KU Leuven, Leuven, Belgium

Abstract: Autism spectrum disorders (ASD) are characterized by impairments in social communication and interaction. To date, no pharmacological treatments exists targeting the core symptoms of ASD. The past years, the use of the neuropeptide oxytocin (OT) has gained increasing interest to explore its potential for elevating the core social deficits in ASD. In the

brain, OT acts as a neurotransmitter where it is considered to form a mediator of prosocial behavior by increasing social salience, social motivation, and social awareness. A double-blind randomized placebo-controlled trial with nineteen young adult males with ASD was conducted to assess neural and behavioral effects (i) at baseline; (ii) after a single-dose (24 IU) of nasal spray administration (OT or placebo); (iii) after 4 weeks of daily nasal spray administration; and (iv) one month post-treatment to assess retention effects. Resting-state functional magnetic resonance imaging (fMRI) was used to assess therapy-induced changes in intrinsic functional connectivity (iFC) between regions of the 'social brain'. Region-to-region iFC and whole-network graph theoretical measures on global network efficiency were assessed. Analyses were performed using the Conn-toolbox within a network of 22 regions-of-interest (ROIs) based on the FSL Harvard-Oxford Atlas encompassing the social brain. Multiple-dose treatment with OT (one-month) induced increases in global efficiency of the social brain; and decreases in ROI-to-ROI iFC of the right orbito-frontal cortex, amygdala, middle temporal gyri and left frontal pole. Interestingly, reductions in iFC between these regions were predictive of increases in global efficiency of the entire social brain network. Furthermore, these neural changes outlasted the time of intervention (1-month post-treatment) and were related to one's ability to improve performance on a bodily emotion recognition task. Results provided indications that long-term treatment with OT (1-month) can induce neural changes in the social brain network of patients with ASD that outlast the time of intervention.



Disclosures: **K. Alaerts:** None. **J. Prinsen:** None. **S. Bernaerts:** None. **J. Steyaert:** None.

Nanosymposium

572. Autism: Physiology and Behavior

Location: SDCC 23A

Time: Tuesday, November 15, 2016, 1:00 PM - 4:45 PM

Presentation Number: 572.11

Topic: A.07. Developmental Disorders

Support: The Nancy Lurie Marks Foundation Early Career Award

Title: Micro-movement statistical signatures across multiple joints unveil direct connections with Autism

Authors: *D. WU[1], E. B. TORRES[2], J. NGUYEN[3], S. MISTRY[4], A. KOLEVZON[5], J. V. JOSÉ[1,6];
[1]Physics, Indiana Univ. Bloomington, Bloomington, IN; [2]Psychology, [3]Cell Biol. and Neurosci., [4]Biomath., Rutgers Univ., Newark, NJ; [5]Mount Sinai Hosp., New York, NY; [6]Integrative and Cell Physiol., Indiana UNIversity Med. Sch., Indianapolis, IN

Abstract: It is known that motor deficits are important core symptoms in Autism Spectrum Disorder (ASD). Being able to understand the nature of these deficits and identify their connection to ASD spoken abilities may provide insights into diagnostic and etiology studies. Because of the high heterogeneity in ASD motor behaviors, present results from traditional motor observational assessments are inconsistent. In our previous work, we introduced a quantitative movement biomarker (s-Peaks) based on a statistical analysis of motions at millisecond time scales. We used these biomarkers in ASD subgroups that had unknown genetic origins (Wu et al. 2016) and also in a group that developed ASD symptoms due to their Shank gene deletions (22q13 Deletion Syndrome, or Phelan-McDermid Syndrome, PMS) (Wu et al. 2015). We measured and characterized the s-Peaks' statistical signatures in the subjects' hands or feet movements in a basic pointing task or natural gait protocol. The signatures captured characterized the movement's continuities at millisecond time scales. Our results clearly separated both idiopathic ASD and the PMS from typical controls. Moreover, we obtained an automatic motion classification within the spectrum that surprisingly agrees with their spoken verbal abilities. Questions remain as where this s-Peaks' differences come from. To address them, we included in our study the upper level joints' motion analysis (like elbow, shoulder, knee, thigh, etc.). Pointing hand motion or feet locomotion are produced by integration of all these joints. Previous research has found that, for typical controls, there is more variability in the upper level joints than the end-point (hand or foot) (Latash, Scholz et al. 2007). Our results also found a decrease in the smoothness level from endpoint to upper level in typical controls. However, in PMDS and idiopathic ASD, there is no such difference between different joints. That is, the smoothness distinction among joints, as found in typical controls, is not present in ASD. Furthermore, the smoothness level in the upper level joints is not distinguishable among

controls and people with ASD/PMDS. We built models to understand the strategy that typical controls use to increase motion smoothness levels at the end
points and also its deficiency in ASD. Our results shed light on the foundation of motor deficits in ASD while further providing an insight into the etiology and treatments in ASD linking it to their spoken abilities.

Disclosures: D. Wu: None. **E.B. Torres:** None. **J. Nguyen:** None. **S. Mistry:** None. **A. Kolevzon:** None. **J.V. José:** None.

Nanosymposium

572. Autism: Physiology and Behavior

Location: SDCC 23A

Time: Tuesday, November 15, 2016, 1:00 PM - 4:45 PM

Presentation Number: 572.12

Topic: A.07. Developmental Disorders

Support: The Nancy Lurie Marks Family Foundation

NJ Governor's Council for Research and Treatment of Autism

Title: Intentionality in action from the brain to the heart during biofeedback training

Authors: *J. RYU, E. B. TORRES;
Psychology, Rutgers The State Univ. of New Jersey, Piscataway, NJ

Abstract: Evidence has shown that our motions exhibit different signatures of variability depending on the level of intent (Torres, 2011). Specifically, the stochastic signatures of speed variability from goal-directed movements differ from those of more spontaneous (supplemental) in nature. Moreover, when an individual becomes more intentional (e.g., waving a hand with the goal of triggering a video display), the stochastic trajectory of the continuous motion entails a wider range of changes in the statistics of the velocity-dependent parameters, than when one has less intent over those motions (e.g., waving randomly) (Torres, Yanovich, & Metaxas, 2013). As such, the level of intent can be characterized by the inherent variability of voluntary micro-motions and their stochastic signatures estimated from kinematic parameters. However, it remains to be seen if signatures of micro-motions in other systems are also revealing of levels of intent. Hence, we investigate the range of stochastic signatures of micro-motions in waveforms from the autonomic systems (heart rate variability) and those from cortical electroencephalography (EEG) as subjects perform a neurofeedback task.
Previous work revealed that differing levels of cognitive load were associated with changes in the statistics of heart rate variability (Ryu & Torres, 2015). Here we reasoned that the level of

intent may also entail changes in the statistics of the heart rate variability, as there may be an interaction between cognitive load and intent. Moreover, the stochastic trajectory of cortical-lead network nodes were shown to reveal the moment of discovery (i.e., a-ha moment) while mastering a brain-computer-interface control task (Tadimeti, Cole, & Torres, 2015), implying that the patterns of brain connectivity may also reveal the level of intent during such closed-loop neurofeedback tasks.
Hence, the current study explores the relationship between the level of intent and physiological signals coming from the brain, heart, and motion. This study examines the multiple signals obtained during actions involving varying levels of voluntary control. These signals will be parameterized for each individual, and the multiple signals will be integrated and analyzed under a new personalized statistical platform to examine the individualized influences of biofeedback in improving one's voluntary control.

Disclosures: J. Ryu: None. **E.B. Torres:** None.

Nanosymposium

572. Autism: Physiology and Behavior

Location: SDCC 23A

Time: Tuesday, November 15, 2016, 1:00 PM - 4:45 PM

Presentation Number: 572.13

Topic: A.07. Developmental Disorders

Title: Motor learning in children with autism spectrum disorder

Authors: *H. M. ROGERS[1,2], A. K. WEGRZYN[2], D. Q. BEVERSDORF[3,2,4], S. E. CHRIST[2,4]; [1]Radiology, Univ. of Missouri Columbia, Columbia, MO; [2]Psychological Sci., [3]Dept. of Neurology, Sch. of Med., Univ. of Missouri, Columbia, MO; [4]Thompson Ctr. for Autism and Neurodevelopmental Disorders, Columbia, MO

Abstract: In addition to social communication difficulties and stereotyped behaviors, individuals with an autism spectrum disorder (ASD) frequently experience problems in other domains such as motor control and learning. However, the nature of these difficulties is not very well understood. The goal of the current study was to further investigate differences in motor learning between individuals with and without ASD. In the present study, we isolated and examined the ballistic and corrective submovements associated with the learning of a rapid aimed limb movement (RALM) task in a sample of 23 individuals with ASD (mean age = 13.0 yrs) and an age and gender matched comparison group of 39 typically developing (TD) individuals without ASD (mean age = 13.4 yrs). All participants had a non-verbal IQ score of ≥ 75 (ASD group mean IQ = 96.6; control group mean IQ = 107.0). A 3D motion tracking system was used to record hand position while participants performed a RALM task (Abrams & Pratt, 1993).

Participants were instructed to move their right hand as quickly as possible from a starting position on the right to a target position on the left. Participants completed 5 blocks consisting of 20 trials per block (100 total movements). Analysis of total movement duration showed a clear trend for a block x diagnosis interaction [$F(4, 240) = 2.29$, $p = .06$, $\eta_P^2 = .04$]. The ASD and TD groups demonstrated similar movement duration for the first block (ASD = 767 ms; TD = 758 ms) [$t(60) < 0$, $p = .83$, $d = .06$]. However, by the fifth block, the TD group was significantly faster than the ASD group (ASD = 736 ms; TD = 674 ms) [$t(60) = 2.15$, $p = .04$, $d = .56$], suggesting a relatively steeper learning curve for the TD group compared to the ASD group. Examination of movement sub-components also revealed a significant block x diagnosis interaction [$F(4, 240) = 4.70$, $p = .001$, $\eta_P^2 = .07$]. As anticipated, with repeated practice, the TD group devoted a decreasingly smaller proportion of time to the corrective sub-component (compared to the ballistic sub-component) [$F(4,152) = 18.49$, $p < .001$, $\eta_P^2 = .33$]. A similar pattern was not observed for the ASD group [$F(4, 88) < 1$, $p = .50$, $\eta_P^2 = .04$]. The current results suggest intrinsic differences between individuals with and without ASD in the mechanisms underlying motor control. Importantly, these differences were only evident when examining motor control and its sub-components within the context of learning. Taken together, these findings support the hypothesis that individuals with ASD utilize different strategies/approaches to learning than individuals without ASD.

Disclosures: H.M. Rogers: None. **A.K. Wegrzyn:** None. **D.Q. Beversdorf:** None. **S.E. Christ:** None.

Nanosymposium

572. Autism: Physiology and Behavior

Location: SDCC 23A

Time: Tuesday, November 15, 2016, 1:00 PM - 4:45 PM

Presentation Number: 572.14

Topic: A.07. Developmental Disorders

Support: The Nancy Lurie Marks Family Foundation

NJ Governor's Council for Research and Treatment of Autism

Title: On mirrors, dancers, avatars: A platform to habilitate, rehabilitate and enhance voluntary control in autism spectrum disorders

Authors: *V. KALAMPRATSIDOU[1], E. B. TORRES[2];
[1]Computer Sci., Rutgers Univ., Piscataway, NJ; [2]Psychology Dept., Rutgers Univ., New Brunswick, NJ

Abstract: Since its discovery, the mirror has served as a tool to improve aesthetics and corporeal awareness. Dancers and athletes use mirrors to aid learning routines, improve their technique and enhance the beauty of their movements. However, the use of a mirror to guide training is limited to the real-time veridical feedback the person receives. What if we added a new layer of dynamic-variability to the image by slightly altering the person's reflection? In this work we use stochastic signatures of the person's veridical bodily rhythms to feedback an altered mirrored image of the person in the form of an avatar. We use the noise-to-signal transitions extracted from combinations of positional, velocity, acceleration and temperature data from wearables sensors guiding near real-time alterations in the persons avatar reflection so as to steer in parametric form the learning course of ballet routines.
To that end we follow several steps: 1) we map the real-time veridical motions of the subject to the avatar; 2) we find optimal segmentation of continuous streaming to insert the noise, perform online stochastic estimation of noise-to-signal ratios; 3) we apply three criteria for noise insertion to the person's reflection in the avatar: 3.1) the noise type to be used to distort the avatar's motions, 3.2) the body-parts to apply the noise to and 3.3) the time-segments within the routine, based on the intentionality/spontaneity levels of the routine segments. Finally, we evaluate the subject's levels of awareness about the noise insertion and determine the effectiveness or the interference of the noise type, body part and time of insertion within the routine as they impact the learning progression in relation to the learning progression with the veridical reflection only. We report our results in light of a main objective, namely to facilitate co-adaptive interactions between the subject and the avatar. In particular, we compare performance in intact nervous systems *vs.* nervous systems with noisy and random motor output. We evaluate the extent to which this tool can be used for sensory-motor-noise cancellation in autism spectrum disorders to boost systematic, well-structured-motor-noise as feedback signal to habilitate, rehabilitate and enhance voluntary control.

Disclosures: V. Kalampratsidou: None. **E.B. Torres:** None.

Nanosymposium

572. Autism: Physiology and Behavior

Location: SDCC 23A

Time: Tuesday, November 15, 2016, 1:00 PM - 4:45 PM

Presentation Number: 572.15

Topic: A.07. Developmental Disorders

Support: NJ Governor's Council for Research and Treatment of Autism

The Nancy Lurie Marks Family Foundation

Title: A change in stance on the social dance: a new framework to examine nonlinear, dynamic temporal interdependence across a social dyad.

Authors: *C. WHYATT, E. B. TORRES;
Rutgers Univ. - Busch Campus, Piscataway, NJ

Abstract: The 'social dance' is an implicit, yet vital, characteristic of dyadic interactions. Attempts to characterize this complex behavior have illustrated unconscious levels of *content* and *temporal* entrainment within artificial social contexts. Yet, when viewed in a naturalistic setting, this 'dark matter' of social neuroscience faces a number of methodological and theoretical challenges. Viewed from a computational perspective, inverse and forward models of control imply that members of the dyad extrapolate information for prediction of action and social intention using a common 'motor language'—facilitated by the mirror neuron system. This universal language results in smooth, shared *content* exchange—or a 'social dialogue'. However, despite ego- and allo-centric spatial coding within the parietal lobe, our ability to translate allocentric levels of complex *temporal* information to engage in a 'social dance' remains unaccounted for by this top-down content perspective. Indeed, within naturalistic social interactions, the dyad can be viewed as a nonlinear, dynamical system containing high levels of uncertainty, redundancy and time delay that must find a stable pattern for exchange, thus accurate prediction. With no *a priori* information, this setting initially renders inverse and forward models incomplete. Here, we present a theoretically grounded methodological framework to characterize unfolding, nonlinear temporal exchange and entrainment across a social dyad using precise kinematic recordings of sensorimotor control and peripheral noise. Building on foundational concepts, including kinesthetic reafference, we discuss the translation of allocentric temporal information to guide egocentric temporal control, while accounting for dimensional redundancy. Utilizing windowed correlational analyses and visualization techniques including network analysis, the model is presented to illustrate nonlinear refinement and adaptation across dyads within two environments in which social dynamics are crucial to higher-level outcomes; namely diagnostics and therapeutic exchange. First, exchange is profiled during the administration of the ADOS [17 children with ASD 8.8 ± 2.9 yrs; 11 controls 9.9 ± 2.7 yrs], a clinical tool that utilizes a structured, yet natural social environment to diagnose ASD. Second, we longitudinally profile physical therapy administration [5 sessions] within which the social dyad plays a core role in therapeutic outcomes. This re-conceptualization provides a framework of dyadic temporal interdependence and illustrates the role of sensorimotor control on higher-level behaviors.

Disclosures: C. Whyatt: None. **E.B. Torres:** None.

Nanosymposium

573. Neurodevelopmental Disorders: New Animal Models and Mechanisms

Location: SDCC 30B

Time: Tuesday, November 15, 2016, 1:00 PM - 4:30 PM

Presentation Number: 573.01

Topic: A.07. Developmental Disorders

Support: Brain Canada

Ontario Brain Institute

Title: Examining the effect of chronic intranasal oxytocin administration on the neuroanatomy and behaviour in three different autism-related mouse models

Authors: *Z. BUCHWALD[1], M. STUIVE[2], J. ELLEGOOD[1], E. ANAGNOSTOU[3], J. LERCH[1];
[1]Mouse Imaging Ctr., Toronto, ON, Canada; [2]Hosp. for Sick Children, Toronto, ON, Canada; [3]Holland Bloorview Res. Inst., Toronto, ON, Canada

Abstract: Introduction: Autism is a neurodevelopmental disorder characterized by social communication deficits and repetitive behaviors. Oxytocin is known for its ability to promote social behaviours and may be a promising therapeutic for autism. To determine what might contribute to response susceptibility, we treated three mouse models of autism with intranasal oxytocin: 16p11.2 deficiency model, Fmr1 knockout model, and the Shank3 knockout model.
Methods: Intranasal oxytocin was administered daily, for 28 days, starting at 5 weeks of age. During the third week of treatment, the behaviour of the mice was assessed in multiple domains: repetitive behaviours (as assessed by grooming), sociability (three chamber sociability task), anxiety and hyperactivity (open field), memory (novel object recognition), and learning and motor coordination (rotarod). The mice underwent three *in vivo* longitudinal MRI scans and a final *ex vivo* scan to assess the neuroanatomy in response to treatment.
Results: Oxytocin treatment did not seem to affect the mesoscopic neuroanatomy. However, it did increase social behaviours in the 16p11.2 mouse ($p<0.09$) and normalized a grooming deficits in the Fmr1 mouse ($p<0.005$). Experiments for the Shank3 line are ongoing.
Discussion: Untreated 16p11.2 mutant mice showed a trend towards deficits in social behaviours, that were corrected by oxytocin treatment. Mutant Fmr1 mice showed a significant decrease in grooming, which was corrected by treatment with oxytocin. No initial deficits in sociability were found in the Fmr1 mouse, and therefore no effect of oxytocin was observed either. This indicates that oxytocin does have an affect on sociability, but only when deficits are initially present. Future directions involve looking at the response of multiple strains of autism-related mouse models, including these three, to other promising therapeutics used in human patients with autism, yielding the ability to establish a translational paradigm for predicting responders from non-responders.

Disclosures: **Z. Buchwald:** None. **M. Stuive:** None. **J. Ellegood:** None. **E. Anagnostou:** None. **J. Lerch:** None.

Nanosymposium

573. Neurodevelopmental Disorders: New Animal Models and Mechanisms

Location: SDCC 30B

Time: Tuesday, November 15, 2016, 1:00 PM - 4:30 PM

Presentation Number: 573.02

Topic: A.07. Developmental Disorders

Support: Autism Science Foundation

Title: Understanding autism pathology in a 16p11.2 candidate-gene deletion mouse model

Authors: ***C. OCHOA**, I. FILONOVA, Z. XUAN, H. E. SPEED, C. M. POWELL; Neurol. & Neurotherapeutics, UT Southwestern, Dallas, TX

Abstract: Copy number variations (CNVs) are implicated in the etiology of Autism Spectrum Disorders (ASDs). Deletions/duplications in human chromosomal region 16p11.2 are frequently associated with ASD and other neurodevelopmental disorders. Specific genes within this region may be important for determining autism risk, though none have been individually linked to ASD. In an effort to understand how 16p11.2 genes genes might contribute to deletion pathology, we deleted a single candidate among the 16p11.2 genes to examine its function in mammalian brain. We report that our 16p11.2 candidate-gene deletion mouse model results in increased locomotor activity, a robust behavioral phenotype in 16p11.2 deletion mouse models. This KO mouse also displays decreased synaptic transmission (input/output curves and mEPSC frequency) in area CA1 of the hippocampus with normal paired-pulse ratio, suggesting a decreased in number of functional synapses. Consistent with this hypothesis, these changes were correlated with a decrease in dendritic length and spine density in area CA1 of hippocampus. We also observed altered protein levels in signaling pathways implicated in synaptic function as one potential molecular mechanism. These results suggest that our candidate gene is associated with regulating neuronal morphology and synapse number and likely acts through specific signaling pathways. Ultimately, the goal of these experiments is to identify novel targets for future preclinical therapeutic studies to directly benefit patients with autism.

Disclosures: **C. Ochoa:** None. **I. Filonova:** None. **Z. Xuan:** None. **H.E. Speed:** None. **C.M. Powell:** None.

Nanosymposium

573. Neurodevelopmental Disorders: New Animal Models and Mechanisms

Location: SDCC 30B

Time: Tuesday, November 15, 2016, 1:00 PM - 4:30 PM

Presentation Number: 573.03

Topic: A.07. Developmental Disorders

Support: Azusa Pacific University Faculty Research Council Grant

Title: Hypersocial behavior in mice associated with the heterozygous deletion of GTF2i, a gene deleted in Williams Beuren syndrome and duplicated in some cases of autism spectrum disorder

Authors: E. ICEBERG[1], G. ALLAF[1], A. AMACKER[1], J. AMACKER[1], K. HORTIZUELA[1], M. JAN[1], C. LIEW[1], *L. A. MARTIN[2];
[2]Psychology, [1]Azusa Pacific Univ., Azusa, CA

Abstract: Williams Beuren Syndrome (WBS) is a disorder caused by a deletion at human chromosome 7q11.23, with symptoms including mild to moderate intellectual disability and hypersocial behavior. Autism Spectrum Disorder (ASD) is a behaviorally-defined collection of syndromes of known and unknown etiology that share a common phenotype including impairments of social motivation. The hypersocial behavior associated with WBS appears opposite to the hyposocial behavior observed in ASD and, interestingly, duplications of 7q11.23 have been associated with ASD. The social phenotype of WBS has recently been linked to deletion of a single gene: *GTF2i*, or general transcription factor IIi (TFII-I). Duplication of *GTF2i* has also recently been associated with ASD, suggesting that it works in a dosage-type response in its effects on social behavior. In this study, we characterized the specific aspects of social behavior that are modulated by *GTF2i* by comparing mice having either a deletion ($GTF2i^{+/-}$) or duplication ($GTF2i^{+/Dup}$) of *GTF2i* to wildtype (WT) littermate controls in a series of social behavior tasks. Results from tests comparing $GTF2i^{+/-}$ mice to WT sibling controls have been completed but tests on $GTF2i^{+/Dup}$ mice are ongoing. In the social choice task, $GTF2i^{+/-}$ mice showed a significant preference for a stimulus mouse that was not observed in WT siblings. $GTF2i^{+/-}$ mice spent significantly more time in nose-to-nose contact compared to controls during social encounters and also demonstrated a significantly heightened preference for urine over water scents. To assess social motivation, test mice were trained to press a lever for a social reward in the form of 15s access to an unfamiliar stimulus mouse. The number of lever presses achieved in the final trial of a testing session was used as an index of social motivation (breakpoint). $GTF2i^{+/-}$ mice demonstrated significantly higher breakpoints than controls. The mice were then tested in an operant task involving a choice between food and social rewards. The percentage of total lever presses that were made for a social reward was significantly higher for the $GTF2i^{+/-}$ mice. Overall, $GTF2i^{+/-}$ mice consistently demonstrated increased social behavior across multiple testing paradigms supporting a role for this gene in the hypersocial

phenotype of WBS. However, the preliminary results from tests on *GTF2i* duplication mice do not support a role for this gene in the hyposocial phenotype of ASD.

Disclosures: **E. Iceberg:** None. **G. Allaf:** None. **A. Amacker:** None. **J. Amacker:** None. **K. Hortizuela:** None. **M. Jan:** None. **C. Liew:** None. **L.A. Martin:** None.

Nanosymposium

573. Neurodevelopmental Disorders: New Animal Models and Mechanisms

Location: SDCC 30B

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Presentation Number: 573.04

Topic: A.07. Developmental Disorders

Support: Hundred Talent Program of the Chinese Academy of Sciences (Technology)

Strategic Priority Research Program (B) of the Chinese Academy of Sciences (XDB02050000)

National Natural Science Foundation of China Grant (81571300)

Title: Increased gamma activity in transgenic monkeys overexpressing MeCP2 associated with autism-like behaviors

Authors: D.-C. CAI[1], Z.-W. WANG[1,2], M.-P. JIANG[1,2], Y.-S. DU[3], Z.-L. QIU[1], *Z. WANG[1]; [1]Inst. of Neurosci., Shanghai, China; [2]Univ. of Chinese Acad. of Sci., Shanghai, China; [3]Dept. Child & Adolescent Psychiatry, Shanghai Mental Hlth. Center, Shanghai Jiao Tong Univ. Sch. of Med., Shanghai, China

Abstract: Liu et al. recently described a macaque model with overexpression of Methyl-CpG binding protein 2 (MeCP2) in brain tissues. These transgenic monkeys exhibit autism-like behaviors, including higher frequency of repetitive circular locomotion, increased stress responses, and reduced social interactions (Liu et al., 2016, *Nature*). However, the relevant brain circuits underlying these observed abnormal behaviors remain unknown, as does the relationship between their neurophysiological activity and MeCP2 overexpression. Here we recorded simultaneous electroencephalogram (EEG) and functional magnetic resonance imaging (fMRI data will be reported separately) from five transgenic (TG: two males, mean age = 4.43 ± 0.29) and nine typically developing (TD: three males, mean age = 4.64 ± 0.36) macaques in the lightly anesthetized state. Eight to ten runs were collected from each subject. The raw EEG data from 22 channels were corrected for MR gradient and cardio-ballistic artifact, down-sampled to 1000 Hz, band-pass filtered at 1 to 100 Hz, and re-referenced to common average after removal of bad channels. Multi-taper time-frequency transformation (window length, 5 cycles of the frequency;

and number of tapers, 3) was then applied to each channel. The resulting power spectrum was divided into six bands, including delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz), lower gamma (30-60 Hz), and higher gamma (60-100 Hz). For each band in individual channel, the relative power was compared between 38 TG and 69 TD runs using a nonparametric permutation test. Relative band powers with significant group differences were further correlated with circular routing behavior in five TG and four TD subjects. We observed a significant EEG power increase in lower gamma band in middle frontal and right frontal channels (corrected $p <$ .00) in TG monkeys. These findings withstood both "leave one run out" and "leave one subject out" cross-validation. Furthermore, the higher gamma band power of right frontal channel was positively correlated with the time monkeys dwelt in circular routing ($r = 0.92$; corrected $p =$ 0.02). Our preliminary results are consistent with reports of increased gamma power observed in autistic children (Orekhova et al., 2007, *Biol Psychiat*), suggesting a potential link between abnormal neurophysiological activity and behavioral phenotype in MeCP2 overexpression monkeys.

Disclosures: **D. Cai:** None. **Z. Wang:** None. **M. Jiang:** None. **Y. Du:** None. **Z. Qiu:** None. **Z. Wang:** None.

Nanosymposium

573. Neurodevelopmental Disorders: New Animal Models and Mechanisms

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Time: Tuesday, November 15, 2016, 1:00 PM - 4:30 PM

Presentation Number: 573.05

Topic: A.07. Developmental Disorders

Support: Ontario Mental Health Foundation

Autism Research Training (ART) Program

Title: TrkB activation rescues Akt signaling and autistic-like behavior in the valproic acid-induced mouse model

Authors: ***M. FAHNESTOCK**[1], C. NICOLINI[1], V. AKSENOV[1], E. ROSA[1], B. MICHALSKI[1], C. D. ROLLO[1], J. A. FOSTER[1], F. M. LONGO[2];
[1]McMaster Univ., Hamilton, ON, Canada; [2]Stanford Univ., Stanford, CA

Abstract: The molecular mechanisms underlying autistic behavior remain to be elucidated. Genetic studies have focused attention on molecules such as brain-derived neurotrophic factor receptor TrkB and its downstream Akt/mTOR signaling pathway components that regulate dendritic spine formation, function and plasticity. In line with these findings, we previously

demonstrated reduced TrkB/Akt/mTOR protein and signaling in human idiopathic autism and in the valproic acid (VPA)-induced rodent model of autism. These results support the hypothesis that defective TrkB signaling is a molecular substrate of autistic behavior and a potential therapeutic target for autism. Here, we examined whether treatment with the partial TrkB agonist LM22A-4 would restore TrkB/Akt signaling and ameliorate autistic-like behavior in mice prenatally exposed to VPA. Pregnant females received a single intraperitoneal (i.p.) injection of 500 mg/kg VPA on gestational day 12.5, while controls were injected with saline. Pups were weaned on postnatal day (PD) 21 and received an i.p. injection of either saline or LM22A-4 (0.05 mg/g) once daily from PDs 21-35. Sociability and repetitive digging were evaluated on PDs 29-34 using the three-chambered social approach task and marble-burying test, respectively. Litters were killed and brain tissue harvested on PD 35. Akt protein and phosphorylation levels were measured by Western blotting. We found that VPA-exposed mice lacked sociability, showed increased repetitive digging behavior and had decreased phosphorylated Akt. In contrast, TrkB activation following treatment with the TrkB partial agonist LM22A-4 restored sociability, decreased repetitive behavior and normalized TrkB signaling through Akt. Our results support the hypothesis that reduced TrkB/Akt signaling contributes to autistic behavior and that this pathway might have a therapeutic role in treating idiopathic autism.

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Nanosymposium

573. Neurodevelopmental Disorders: New Animal Models and Mechanisms

Location: SDCC 30B

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Presentation Number: 573.06

Topic: A.07. Developmental Disorders

Support: John S. Dunn Foundation Grant

University of Texas Medical Branch Pilot Grant

Title: Human fetal brain neural stem cells for studying zika virus-associated neuropathology.

Authors: ***P. WU**[1], E. L. MCGRATH[1], S. L. ROSSI[2], J. GAO[1], T. J. DUNN[1], S. R. AZAR[2], S. R. AZAR[2], C. M. ROUNDY[2], S. C. WEAVER[2], N. VASILAKIS[2];
[1]Dept Neurosci/Cell Biol, [2]Inst. for Human Infections and Immunity and Dept. of Microbiology and Immunol., UTMB, Galveston, TX

Abstract: Zika virus (ZIKV) infection has recently been recognized as a major threat to human health. Particularly, the linkage between ZIKV infection and microcephaly raises a huge concern for a serious health problem worldwide. To date, little is known about the mechanism underlying ZIKV-associated neural damage, including microcephaly. Since a normal brain is developed from neural stem cells (NSCs) and their differentiated neural cells, abnormal brain development, such as microcephaly, is most likely associated with the abnormal function of these cells. Yet, it is unknown whether and how human fetal brain NSCs or their progenies are susceptible to ZIKV infection, whether different strains of ZIKV infect NSCs at the equal efficiency, and whether such infection affects the normal functions of NSCs that are important for the development of human brain. Recently we have established a human fetal brain NSCs-based *in vitro* system to study ZIKV infection in neural cells. We found that various strains of ZIKV, including a strain from the Mexican outbreak in spring of 2016 (Mex I-7), directly infect human NSCs *in vitro*. Additionally, we found that ZIKV infection alters NSC proliferation and differentiation. To the best of our knowledge, this is the first study examining clinically relevant ZIKV strains from recent outbreaks in an *in vitro* human fetal NSC culture to study the neuropathological and developmental effects of ZIKV. This system will facilitate further studies to characterize different strains or modified ZIKV, to understand ZIKV-induced pathological changes, or to develop therapeutic strategies and screen drugs to ameliorate ZIKV-mediated neural damages.

Disclosures: P. Wu: None. **E.L. McGrath:** None. **S.L. Rossi:** None. **J. Gao:** None. **T.J. Dunn:** None. **S.R. Azar:** None. **S.R. Azar:** None. **C.M. Roundy:** None. **S.C. Weaver:** None. **N. Vasilakis:** None.

Nanosymposium

573. Neurodevelopmental Disorders: New Animal Models and Mechanisms

Location: SDCC 30B

Time: Tuesday, November 15, 2016, 1:00 PM - 4:30 PM

Presentation Number: 573.07

Topic: A.07. Developmental Disorders

Support: 1R01 MH109706-01

Title: Functional dissection of the Zika genome reveals a coding component responsible for microcephaly

Authors: *E. OH;
Duke Univ., Durham, NC

Abstract: Infection by the Zika virus, a re-emerging flavivirus, has been associated with developmental abnormalities, including microcephaly. To determine whether a coding viral

element contributes to pathology, we expressed each structural and non-structural component in zebrafish embryos. Using light microscopy and whole-mount staining, we discovered a non-structural component that was sufficient to drive the microcephaly phenotype in 3 days post-fertilization embryos. The change in head size could be induced in a dose-dependent manner, and was accompanied by a decrease in phospho-histone H3 immunopositive neural progenitors. We have performed RNA-sequencing to examine transcriptional profiles and will show how a non-structural component can influence the mitotic index of human neural stem cells. Our studies will elucidate how the Zika coding genome interacts with the host and will inform novel therapeutic strategies aimed to ameliorate or at least prolong the onset of symptoms.

Disclosures: E. Oh: None.

Nanosymposium

573. Neurodevelopmental Disorders: New Animal Models and Mechanisms

Location: SDCC 30B

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Presentation Number: 573.08

Topic: A.07. Developmental Disorders

Support: AUTISMSPEAKS #7670

NIH 5P50MH086383-04

NSC 101-2917-I-564-039

MH100556

Title: The placental immune environment controls fetal brain development and behavior

Authors: *W.-L. WU, E. Y. HSIAO, Z. YAN, S. K. MAZMANIAN, P. H. PATTERSON; Div. of Biol. and Biol. Engin., Caltech, Pasadena, CA

Abstract: Epidemiological studies show that maternal infection during pregnancy is a risk factor for autism spectrum disorder (ASD). However, mechanisms for how maternal infection affects fetal brain development and behavioral deficits in offspring remain poorly described. Acute inflammatory responses were examined in the maternal immune activation (MIA) model, a validated preclinical model for an ASD risk factor. To determine whether placental IL-6 signaling is required for mediating MIA effects on the fetal brain, we generated mice with restricted deletion of the receptor for interleukin-6 (IL-6Rα) in placental trophoblasts (*Cyp19-Cre*$^{+}$*;Il6ra*$^{fl/fl}$), and tested offspring of *Cyp19-Cre*$^{+}$*;Il6ra*$^{fl/fl}$ mothers for immunological, pathological and behavioral abnormalities following induction of MIA. We reveal that MIA

results in acute inflammatory responses in the fetal brain, including increased IL-6 levels and activation of STAT3 signaling pathways. Lack of IL-6 signaling in trophoblasts effectively blocks inflammatory responses in the placenta and remarkably prevents immune activation in the fetal brain. Furthermore, ASD-related behavioral abnormalities and deficits in cerebellar Purkinje cells observed in MIA control offspring are prevented in *Cyp19-Cre^{+};Il6ra$^{fl/fl}$* offspring. Our results demonstrate that IL-6 activation in placental trophoblasts is required for relaying inflammatory signals to the fetal brain and impacting adult behaviors and neuropathologies. We propose that targeted inhibition of placental IL-6 signaling during the acute phase of maternal infection may be a potential prophylactic strategy during pregnancies at risk of resulting in births with an ASD diagnosis.

Disclosures: W. Wu: None. **E.Y. Hsiao:** None. **Z. Yan:** None. **S.K. Mazmanian:** None. **P.H. Patterson:** None.

Nanosymposium

573. Neurodevelopmental Disorders: New Animal Models and Mechanisms

Location: SDCC 30B

Time: Tuesday, November 15, 2016, 1:00 PM - 4:30 PM

Presentation Number: 573.09

Topic: A.07. Developmental Disorders

Support: Children's Research Institute

Title: Understanding late gestation maternal inflammation in establishment of cortical circuits

Authors: *H. LACAILLE[1], D. BAKALAR[1], A. PENN[1,2];
[1]Ctr. for Neurosci., Children's Natl. Hlth. Syst., Washington, DC; [2]Fetal Med. Institute, Neonatology, Washington, DC

Abstract: Preterm birth is a leading cause of neonatal mortality and morbidity that can induce long-term neurological impairment. More than 85% of pregnancies delivered at less than 28 weeks of gestation show evidence of placental inflammation. Prenatal immune activation and extreme prematurity have both been implicated in major neurodevelopmental disorders, including epilepsy and psychiatric disorders. A common feature of these disorders is an imbalance between neuronal excitation and inhibition, mediated by developmental alterations of GABAergic interneurons. We hypothesize that an inflammatory event during late rodent gestation will alter multiple steps in interneuron development, leading to long-term neurological effects that mimic those seen in extremely preterm survivors. To visualize interneurons, we used Gad65-GFP transgenic mice in which GABAergic interneurons express green fluorescent protein (GFP) under control of the Gad65 promoter. Pregnant dams were injected with

lipopolysaccharide (LPS), to mimic bacterial infection, at 150µg/kg (subclinical infectious dosing) or saline at embryonic day 15.5 (E15.5) and E16.5; fetal cortex was examined at E17.5. Comparing fetuses from LPS versus saline exposed gestations revealed a significant decrease in the number of GAD65-GFP^{+} cells in the 3 major subdivisions of developing cortex (marginal zone, cortical plate and subventricular/ventricular zone). Concurrently, alterations in cortical gene expression were measured using a panel of 20 RT-PCR primers targeting genes that regulate interneuron development. Maternal LPS exposure induced a significant increase in the expression of *nkx2.1* which is expressed in a specific subtype of interneurons (chandelier cells) and *ascl1*, an early marker of interneurons. We also observed increased expression of two migration-related genes (*dlx1* and *dlx5*), differentiation genes (*calb1* and *pvalb)*, and a decrease in *calb2* expression. Intriguingly, the decrease in the total number of interneurons is accompanied by an increase in interneuron-regulating transcripts, suggesting a compensatory mechanism against interneurogensis disruption. Further investigations are focused on determining the short- and long-term alterations induced by late gestation inflammation, including the mechanisms of interneuron loss and potential for recovery at later developmental stages. Understanding alteration of fetal interneuron populations after maternal inflammation is a critical step in developing targeted therapies to prevent the cortical dysfunction associated with extremely preterm birth.

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Nanosymposium

573. Neurodevelopmental Disorders: New Animal Models and Mechanisms

Location: SDCC 30B

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Presentation Number: 573.10

Topic: A.07. Developmental Disorders

Support: NIH DA036376

Title: Development of rapid and automated whole brain mapping in a mouse model of prenatal exposure to prescription pain medication

Authors: ***T. VAISSIÈRE**[1], S. BRIGGS[1], T. K. CRESON[2], I. TAKACS[1], G. RUMBAUGH[2], C. MILLER[1];
[1]Metabolism and Aging and Neurosci., [2]Neurosci., The Scripps Res. Institue, Jupiter, FL

Abstract: An estimated 6 million individuals in the United States abuse yearly prescription pain relievers. This has led to a five-fold increase in prescription drug abuse among expectant mothers over the last decade, giving rise to more than one baby born every hour addicted to prescription

pain medications, such as oxycodone. Upon birth, these babies suffer from neonatal abstinence syndrome. Long-term consequences of this prenatal exposure are unknown. We hypothesize that prenatal exposure to prescription pain medication affects neurodevelopment, altering brain structure and function required for lifelong normal behaviors. To address this hypothesis we: 1) developed a mouse model that mimics prenatal oxycodone exposure and 2) established new methodologies to investigate brain-wide cell type-specific structural and functional changes. We have found that maternal care was not affected by oxycodone. However, comprehensive behavioral analyses in their offspring suggests that prenatal exposure to oxycodone represents a neurodevelopmental insult that alters impulse control-like behaviors in males, indicated by a decrease in cliff avoidance in two independent mouse lines. Impulse-related behaviors are governed by a circuit whose primary players the PFC, the amygdala and the striatum. Therefore, we developed a study design that take into consideration litter effects and allowed us to combine multiple analyses of those brain regions. First, molecular and behavioral profiles were established in a subset of offspring exposed to oxycodone or vehicle in utero. Second, we used cell type-specific retrograde trans-synaptic tracing to investigate structural changes at the circuit and dendritic spine levels. Third, c-fos labeling was superimposed onto retrogradely labeled neurons to derive functionally active circuits. Data were acquired by repurposing a high-throughput robotic confocal plate reader commonly used for drug discovery studies to perform rapid whole-brain imaging. With this method we determined a 25% attrition rate, due to low topographic representation of the starter cell population in brain areas of interest and lack of brain alignment to a reference atlas. Our approach results in the acquisition of a whole brain in less than 15 min at cellular resolution of 1.6 pixel/μm. This, combined with universal bioinformatics tools allowed fast, versatile and automated generation of brain-wide connectivity and activity maps. These methodological developments led to insight into functional and structural brain changes due to prenatal exposure to oxycodone and can benefit the investigation of other animal models of brain disorders.

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Nanosymposium

573. Neurodevelopmental Disorders: New Animal Models and Mechanisms

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Topic: A.07. Developmental Disorders

Support: The Orphan Disease Center of University of Pennsylvania - grant number MDBR-16-122-PHP

European Union through the European Regional Development Fund - Project No. 2014-2020.4.01.15-0012

Title: Using *Drosophila* daughterless to study Pitt-Hopkins syndrome

Authors: *M. M. PALGI, M. JAAGO, K. GORONOVSKAJA, L. TAMBERG, M. SEPP, T. TIMMUSK;
Tallinn Univ. of Technol., Tallinn, Estonia

Abstract: Pitt-Hopkins syndrome (PTHS) is a rare intellectual disability syndrome characterized by specific facial features, severe developmental delay, lack of speech, and breathing problems. PTHS is caused by haploinsufficiency of *Transcription factor 4 (TCF4*). TCF4 is one of the three human class I basic helix-loop-helix transcription factors also called E-proteins. In Drosophila there is a sole E-protein called Daughterless (Da). In developing nervous system both TCF4 and Da are obligatory dimerization partners for proneural bHLH proteins. We have shown that Da is the functional homolog of TCF4 and that human TCF4 can replace Da in fly nervous system development. We have introduced several mutations of *TCF4* found in patients of PTHS to fruit fly *daughterless*. When we overexpressed mutated Da in developing eye tissue a specific rough eye phenotype was induced. Here we used the obtained eye phenotype in both gene silencing and overexpression approach to screen for potential targets and interaction partners. Our latest results will be presented.

Disclosures: M.M. Palgi: None. **M. Jaago:** None. **K. Goronovskaja:** None. **L. Tamberg:** None. **M. Sepp:** None. **T. Timmusk:** None.

Nanosymposium

573. Neurodevelopmental Disorders: New Animal Models and Mechanisms

Location: SDCC 30B

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Presentation Number: 573.12

Topic: A.07. Developmental Disorders

Support: Lois Pope LIFE Fellowship Award

Snyder-Robinson Foundation Predoctoral Fellowship

HHSN268201300038C

Title: Polyamine imbalance causes neuronal dysfunctions in a *Drosophila* model for Snyder-Robinson Syndrome

Authors: ***C. LI**[1], C. BELLO[1], J. BRAZILL[1], S. LIU[2], Y. ZHU[1], M. C. V. MALICDAN[3], R. PAULY[4], H. WANG[2], C. E. SCHWARTZ[5], W. A. GAHL[3], C. F. BOERKOEL[3], R. G. ZHAI[1]; [1]Mol. and Cell. Pharmacol., Univ. of Miami Miller Sch. of Med., Miami, FL; [2]Key Lab. of Mol. Pharmacol. and Drug Evaluation (Ministry of Educ. of China), Yantai Univ. Sch. of Pharm., Yantai, China; [3]Natl. Human Genome Res. Inst., Bethesda, MD; [4]Greenwood Genet. Ctr., JC Self Res. Inst., Greenwood, SC; [5]JC Self Res. Institute, Greenwood Genet. Ctr., Greenwood, SC

Abstract: Intracellular polyamines, including putrescine, spermidine, and spermine, are tightly regulated polycationic molecules that are essential for cell growth and differentiation. Loss-of-function mutations in human spermine synthase (SMS), an aminopropyl transferase that converts spermidine to spermine, were identified to cause Snyder-Robinson Syndrome (SRS). SRS is an X-linked recessive disease characterized by intellectual disability and developmental delay. The underlying pathogenesis, especially of the neurological phenotypes, is largely unknown. We used *Drosophila* as a model to study the neuronal function of SMS and found that loss of *Drosophila* Sms (dSms) recapitulates the polyamine imbalance of SRS patients and causes developmental and survival defects in *Drosophila*. We showed that abnormal polyamine oxidation resulted in oxidative stress, mitochondria dysfunction, and altered endosomal and autophagic membrane trafficking in both *Drosophila* nervous system and SRS patient fibroblasts. Importantly, we found that the elevated reactive oxygen species in the *Drosophila* model can by suppressed through enhanced antioxidant activity either genetically or pharmacologically. Our findings provide significant insights into possible therapeutic strategies for SRS and polyamine-associated neurological disorders.

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Nanosymposium

573. Neurodevelopmental Disorders: New Animal Models and Mechanisms

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Topic: A.07. Developmental Disorders

Support: National University of Ireland Galway (grant number RSU002 to S.S)

Science Foundation Ireland (grant number 09/SRC/B1794s1 and 13/IA/1787 to S.S)

Title: Ulk4 is essential for ciliogenesis and CSF flow

Authors: *M. LIU[1], Z. GUAN[5], Q. SHEN[6], P. LALOR[2], U. FITZGERALD[3], T. O'BRIEN[4], P. DOCKERY[2], S. SHEN[4];
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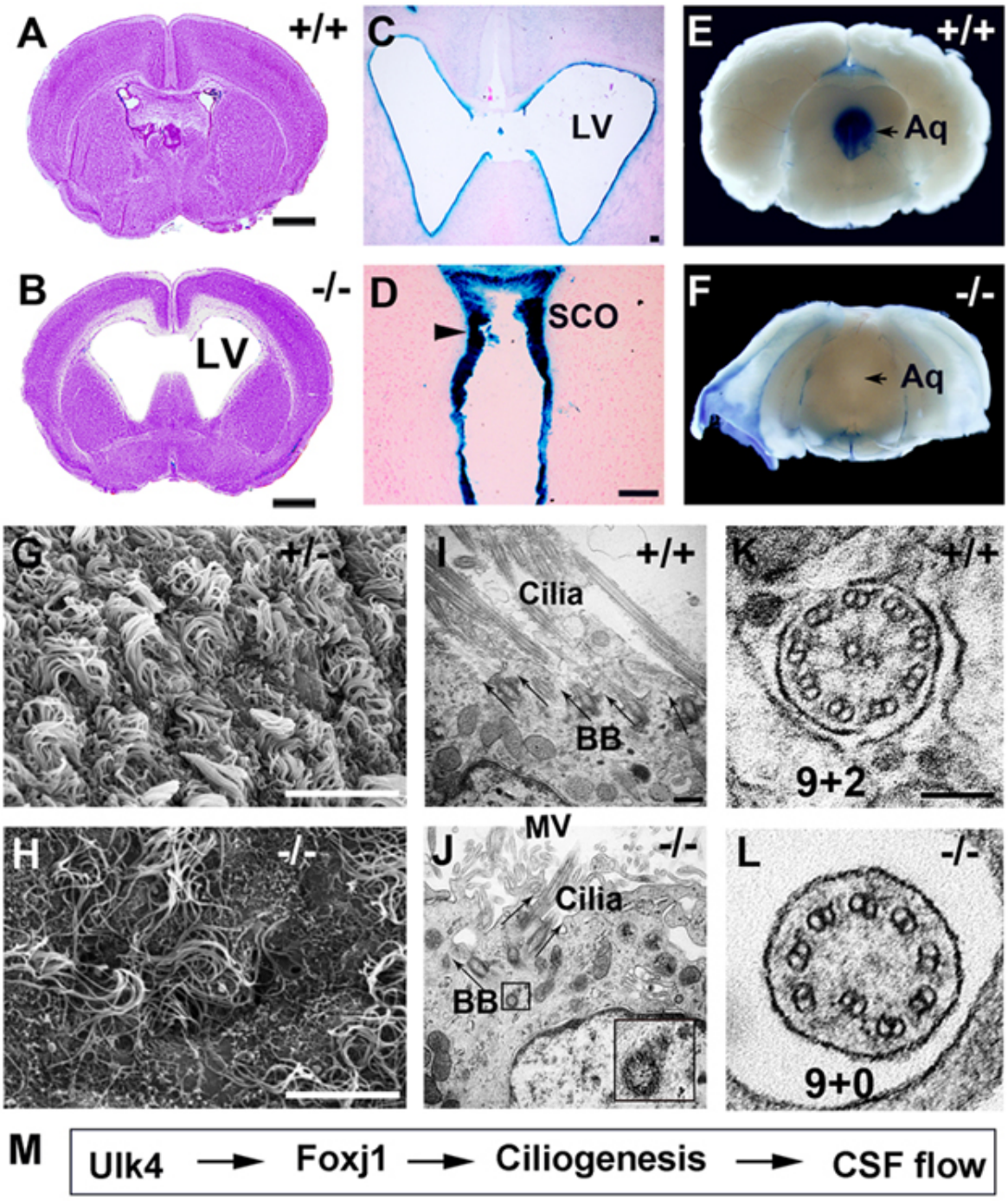
Abstract: Ciliopathies are an emerging class of devastating disorders with pleiotropic symptoms affecting both the central and peripheral systems, which are commonly associated with hydrocephalus. Despite ciliary components and three master transcriptional regulators identified, little is known about the signaling molecules involved. We previously identified a novel *Unc51-like-kinase 4* (*ULK4*) as a risk factor of neurodevelopmental disorders. Here we took multidisciplinary approaches and uncovered essential roles of *Ulk4* in ciliogenesis. We show that *Ulk4* is predominantly expressed in the ventricular system, and $Ulk4^{tm1a/tm1a}$ ependymal cells display reduced/disorganized cilia with abnormal axoneme. $Ulk4^{tm1a/tm1a}$ mice exhibit dysfunctional subcommissural organ, obstructive aqueduct and impaired cerebrospinal fluid flow. Mechanistically, we performed whole genome RNA sequencing and discovered that *Ulk4* regulates the *Foxj1* pathway specifically and an array of other ciliogenesis molecules. This is the first evidence demonstrating that *Ulk4* plays a vital role in ciliogenesis and deficiency of *Ulk4* can cause hydrocephalus and ciliopathy-related disorders.



Disclosures: **M. Liu:** A. Employment/Salary (full or part-time): National University of Ireland Galway, Galway, Ireland. **Z. Guan:** None. **Q. Shen:** None. **P. Lalor:** None. **U. Fitzgerald:** None. **T. O'Brien:** None. **P. Dockery:** None. **S. Shen:** None.

Nanosymposium

573. Neurodevelopmental Disorders: New Animal Models and Mechanisms

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Presentation Number: 573.14

Topic: A.07. Developmental Disorders

Support: NIH RO1 NS093016

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Title: A novel functional connection of the schizophrenia risk genes fez1 and qki in oligodendroglia development

Authors: X. CHEN[1,2], X. ZHAO[2], W. LI[2], L. KU[2], *L. XIAO[1], Y. FENG[2];
[1]Dept. of Histology and Embryology, Third Military Med. Univ., Chongqing, China; [2]Emory Univ., Atlanta, GA

Abstract: The FEZ1 gene that encodes the fasciculation and elongation protein ζ-1 is a recognized risk factor implicated in the pathogenesis of schizophrenia (SZ). In brain neurons, FEZ1 plays important roles in neuronal differentiation and neural network assembly. However, whether FEZ1 is also expressed in oligodendroglia (OL), the myelinating cells in the CNS that are also affected in SZ, remains elusive. Here we report that FEZ1 is also expressed in OLs through the OL lineage development in culture and in vivo. We observed robust up-regulation of FEZ1 during neuronal differentiation and myelination, suggesting an increased functional requirement of FEZ1 synergistically in both the neuronal and OL cell lineage. In developing OL progenitor cells (OPCs), FEZ1 is predominantly detected in the cytoplasm, deposited to the distal end of OL process and colocalized with F-actin, suggesting a role of FEZ1 in governing morphogenesis of OPC in early differentiation. Interestingly, FEZ1 3'UTR harbors consensus binding sequence for the selective RNA-binding protein QKI that is also implicated in SZ pathogenesis. We further show that FEZ1 mRNA is associated with the cytoplasmic QKI isoform in OLs and QKI deficiency results in significant reduction of FEZ1 expression in OLs of the quakingviable (qkv) mutant mice. Finally, we found that the QKI regulates FEZ1 expression in rodent and human OLs. Our data provide the first functional link betweentwo SZ risk factors in OLs, QKI and FEZ1, suggesting that the QKI-FEZ1 pathway may contribute to the OL pathology in SZ.

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Nanosymposium

574. Mechanisms of APOE-Mediated Pathology in Alzheimer's Disease

Location: SDCC 33C

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Presentation Number: 574.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NCCIH RO1 AT006816 (GMC, SAF)

Easton Drug Discovery Foundation (GMC, SAF)

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Title: Apolipoprotein E isotype-dependent modulation of microRNA-146a in plasma and brain and in response to dietary fatty acids

Authors: *B. TETER[1], P. M. SULLIVAN[3], M. LADU[4], S. A. FRAUTSCHY[2], G. M. COLE[2]; [1]Dept. Neurol. (UCLA) and GRECC (VA), [2]Dept. Neurology, Med. (UCLA) and GRECC (VA), Univ. Calif. Los Angeles, Veterans Admin. GLAHS, Los Angeles, CA; [3]Med. (Geriatrics)(Duke), GRECC (VA), Duke University,Durham Veterans Affairs Med. Ctr., Durham, NC; [4]Dept. of Anat. and Cell Biol., Univ. of Illinois at Chicago, Chicago, IL

Abstract: The apolipoprotein E (apoE) isotype apoE4 is a prevalent genetic risk factor for Alzheimer's disease (AD) that can modulate systemic and central inflammation, independent of amyloid accumulation. Although disruption of innate immune toll-like receptor (TLR) signaling is modulated by apoE isotype and observed in AD, these ApoE isotype specific effects remain poorly understood. Therefore, we examined the effect of apoE isotype on brain levels of major regulators of TLR signaling including miR146a, a microRNA enriched in the brain. We used 6 month-old apoE3 or apoE4 targeted replacement mice with and without mutant familial AD (FAD) transgenes. ApoE4 reduced levels of miR146a compared to apoE3, both in the brain (29%; $p < 0.0001$) and plasma (47%; $p < 0.05$), which correlated with each other ($r2=0.74$; $p < 0.05$). The presence of 5xFAD transgenes increased brain miR146a in both apoE3 (E3FAD) and apoE4 (E4FAD) mice; however, miR146a levels in E4FAD mice remained lower than in E3FAD mice (62%; $p < 0.05$), despite increased amyloid and inflammation. Supporting these observations, apoE4 brains showed increased expression of interleukin receptor associated kinase-1, IRAK1 (160%; $p < 0.05$) (normally downregulated by miR146) that inversely correlated with miR146a levels ($r2=0.637$; $p < 0.0001$). Reduced negative feedback of TLR signaling (by miRNA146a) can explain early-life hypersensitivity to innate immune stimuli (including Aβ) in apoE4 carriers. Thus, apoE4 causes early dysregulation of a central controller

of the innate immune system both centrally and systemically. This defect persists with FAD pathology and may be relevant to ApoE4 AD risk. To examine whether dietary fatty acids modulate miR146a in an apoE-dependent manner, E3FAD and E4FAD mice were fed either standard rodent breeder chow (5015), or a diet enriched in linoleic acid, an n-6 polyunsaturated fatty acid (PUFA), or the high n-6 diet supplemented with the n-3 fatty acid, DHA. The high n-6 diet increased brain miR146a levels only in the E4FAD mice eliminating the deficit compared to E3FAD while the DHA-supplemented diet reduced miR146a in both E3FAD and E4FAD mice. This may help explain the complex ApoE-dependent effects of n-6 PUFA that selectively reduced amyloid but increased the TLR pathway index cytokine IL-1ß only in the E4FAD mice. DHA opposed these changes. These results suggest that dietary fatty acid modulation of innate immune function and amyloid clearance may depend on ApoE isotype effects on immune function involving miR146a control of TLR signaling, a candidate nexus of interaction with other factors including exercise, age and Aß pathology.

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Nanosymposium

574. Mechanisms of APOE-Mediated Pathology in Alzheimer's Disease

Location: SDCC 33C

Time: Tuesday, November 15, 2016, 1:00 PM - 4:00 PM

Presentation Number: 574.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AT008916-01 (Cole)

VA Merit BX000542-01 (Cole)

NCCIH RO1 AT006816 (GMC, SAF)

Easton Drug Discovery Foundation (GMC, SAF)

VA Merit BX000542 (GMC)

Title: Hign Dietary n-6 fatty Acid Reduces Neuroinflammation and plaque burden while increasing Anti-Aβ autoantibody production in ApoE4 carrying EFAD mice

Authors: *Q.-L. MA[1,2], X. ZUO[1,2], B. TETER[1,2], K. GYLYS[1], M. R. JONES[1,2], J. AKERS[3], M. J. LADU[4], S. A. FRAUTSCHY[1,2], G. M. COLE[1,2];
[1]UCLA, Los Angeles, CA; [2]GRECC GLA VA, Los Angeles, CA; [3]UCSD, San Diego, CA; [4]Univ. of Illinois at Chicago, Chicago, IL

Abstract: Since Aβ accumulates decades prior to clinical onset, trials with Aβ vaccines are moving toward treating earlier but some people make endogenous anti-abeta antibodies. Relative to the more common Apolipoprotein E isoform (E3), ApoE4 (E4) is widely believed to increase Alzheimer Disease (AD) risk by accelerating onset of amyloid (Aβ42) deposition, but E4 also increases inflammation. Increased amyloid and Toll receptor signaling are observed in AD patients and AD animal models with human E3 or E4 including 5xFAD human ApoE targeted replacement mice (EFAD mice) expressing either E3 (E3FAD) or E4 (E4FAD) and familial Alzheimer's transgenes (5FAD) that cause amyloid plaques. Here we show that putting E4FAD mice on a high n-6 fat diet selectively reduces amyloid burden and inflammation but increases endogenous anti-Aβ antibodies. While IV-Ig treatment with endogenous anti-Aß antibodies failed in a recent clinical trial, subgroup analysis showed benefits to ApoE4-carriers. In epidemiological studies, high n-6 PUFA diet appeared to reduce risk for MCI in E4 carriers but how dietary -6 PUFA impacts CNS inflammation or amyloid regulated by ApoE4 is unknown. Because high n-6 diets supply more precursor to elevate arachidonic acid (the substrate for cyclooxygenases) and pro-inflammatory lipid mediators, n-6 is widely regarded as pro-inflammatory. However, surprisingly, we observed that high dietary n-6 diet markedly reduced pro-inflammatory IL-1ß, TNFα and Aß in Tg2576 AD mice. When the same high n-6 diet was fed to E4FAD mice from 4 to 8 months of age, MSD cytokine analysis showed that high n-6 diet reduced brain pro-inflammatory IL-1ß, IL-6 and IL-8 and increased anti-inflammatory IL-10 supporting an anti-inflammatory effect. High n-6 shifted E4 from pro-inflammatory TLR4>IRAK-1>Traf6>AP-1/ NFkB pathways regulating COX-2, inflammation and tolerance but increased plasma IFN-γ and Aβ auto-antibodies. Consistent with possible anti-Aβ antibody-mediated amyloid clearance, high n-6 PUFA increased vascular amyloid while reducing insoluble Aß and plaque burden- but only with E4. Since E4 is known to be ineffective relative to E3 in exerting inhibitory control over the innate immune system, we also examined anti-inflammatory feedback by miRNA. High n-6 and E4 interacted with the innate immune system and IL1β/TLR4 pathway mechanisms related to tolerance, anti-Aβ antibody production and Aβ clearance. This data suggests higher n-6 fatty acid consumption may reduce dementia risk in ApoE4-carriers by immunomodulatory activity providing early and prolonged exposure to higher levels of endogenous anti-Aß antibodies, a novel prevention approach.

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Nanosymposium

574. Mechanisms of APOE-Mediated Pathology in Alzheimer's Disease

Location: SDCC 33C

Time: Tuesday, November 15, 2016, 1:00 PM - 4:00 PM

Presentation Number: 574.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG034103

Title: Obesity accelerates Alzheimer-related pathology in APOE4 but not APOE3 male EFAD mice

Authors: *V. A. MOSER[1,2], C. J. PIKE[1];
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Abstract: A number of environmental and genetic risk factors for Alzheimer's disease (AD) have been identified. Importantly, the strength of environmental modifiers is affected by genetic factors, although how such interactive relationships affect AD risk is poorly understood and has typically not been addressed in experimental studies. One significant modifiable lifestyle factor that increases AD risk is obesity in midlife. Prior work in rodent AD models demonstrated that obesity can accelerate the development of AD-related pathology. The strongest genetic risk factor for late-onset AD is the E4 allele of apolipoprotein E (apoE), increasing risk by as much as 15 times in homozygous carriers. Several epidemiological studies indicate that obesity and apoE4 interact, suggesting that presence of both obesity and apoE4 may be especially harmful in increasing AD risk. However, the potential interaction between obesity and apoE4 in promoting AD pathogenesis has not been rigorously investigated. The goal of the present study was to determine whether the AD-promoting effects of obesity interact with apoE genotype. To accomplish this, we compared measures of AD-related pathology and metabolic function using a paradigm of diet-induced obesity in the apoE-familial Alzheimer's disease (EFAD) mouse model. Male EFAD mice, which have AD transgenes as well as humanized *APOE3* or *APOE4*, were maintained on either a normal diet (10% fat, 7% sucrose) or a western diet (45% fat, 17% sucrose) for a period of 3 months. Data outcomes included indices of obesity and metabolic function (e.g., body weight, fat mass, glucose tolerance), β-amyloid (Aβ) accumulation (e.g., plaque load, cerebrovascular amyloid), and both systemic and neural inflammation. In the context of normal diet, E4FAD mice have similar metabolic measures but significantly higher Aβ plaque load than E3FAD mice. In response to western diet, E4FAD but not E3FAD mice exhibited significantly increased Aβ accumulation compared to their normal diet-fed controls. These findings demonstrate that environmental risk factors for AD such as obesity, may differentially impact risk of developing the disease based on presence of genetic factors. Thus, this study demonstrates a gene-environment interaction, and points to the importance of lifestyle factors in modifying the risks associated with genetic factors like *APOE4* in Alzheimer's disease. EFAD mice were generously provided by Dr. Mary Jo LaDu, University of Illinois at Chicago.

Disclosures: V.A. Moser: None. **C.J. Pike:** None.

Nanosymposium

574. Mechanisms of APOE-Mediated Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

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R21 AG044682

Title: The effect of aging on APOE-modulated AD pathology in EFAD mice.

Authors: *A. C. VALENCIA, N. COLLINS, C. SMITH, P. YAMCHUEN, R. SALZMAN, S. GHURA, M. LADU;
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Abstract: The *APOE* ε4 allele of apolipoprotein E (apoE) is the greatest genetic risk factor for Alzheimer's disease (AD) compared to *APOE3*, with *APOE2* providing a protective effect. *APOE4* is associated with accelerated amyloid-β peptide (Aβ) accumulation, both as amyloid and soluble oligomeric forms of Aβ (oAβ), the latter considered a proximal neurotoxin. Using EFAD mice, a novel, tractable human-*APOE*/familial AD-transgenic (FAD-Tg) preclinical mouse model, we have developed the mechanistic hypothesis that risk factors associated with AD pathology, *APOE4,* and females cause a reduction in apoE lipidation, resulting in inefficient clearance of soluble Ab synaptic loss, and memory deficits. By 6-months, E4FAD mice have greater cognitive impairment, AD pathology and lower apoE lipidation levels compared to E3FAD. This response is mirrored by the comparison of E4FAD females to E4FAD males. However, the effect of aging on EFAD mice has not been analyzed beyond 6-months. In this study, at 8-, 10-, 14- and 18-months of age, we measured apoE lipidation, soluble Aβ42, oAβ and apoE/Aβ complex levels, as well as amyloid deposition, neuroinflamamation, tau pathology and neuronal cell counts. There is a continued development of AD pathology with age (*APOE4* > *APOE3,* female > male*)* with pathology spreading from the subiculum and the deep layer of the frontal cortex to the entire length of the cortex and spreading to the thalamus and the amyloid accumulation and neuroinflammation. The failure of AD clinical trials questions the predictive validity of preclinical FAD-Tg mouse models that lack h-*APOE,* the major genetic risk factor for AD. However, the greatest risk factor for AD is age; aged EFAD mice address both these critical risk factors.

Disclosures: **A.C. Valencia:** None. **N. Collins:** None. **C. Smith:** None. **P. Yamchuen:** None. **R. Salzman:** None. **S. Ghura:** None. **M. LaDu:** None.

Nanosymposium

574. Mechanisms of APOE-Mediated Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Toll-like receptor 4 antagonism leads to protective effects in the E4FAD transgenic mouse model of Alzheimer's disease.

Authors: *M. LADU[1], J. YORK[1], S. GHURA[1], V. CALABRESE[2], F. PERI[2], G. R. J. THATCHER[3], F. NEUMAN[4];
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Abstract: Alzheimer's disease (AD) is the most common form of dementia, characterized by amyloid-β (Aβ) plaques, neurofibrillary tangles and a chronic neuroinflammatory phenotype. Recent evidence demonstrates that soluble Aβ, particularly soluble oligomeric Aβ (oAβ), is neurotoxic and activates receptor-mediated neuroinflammatory pathways. *APOE4* is the greatest genetic risk factor for AD, increasing risk 4- or 15-fold with one or two alleles, respectively, compared to *APOE3*. One pathway through which *APOE4* may impart risk is via modulation of neuroinflammatory pathways, including toll-like receptor 4 (TLR4)-dependent signaling. Indeed, our published data in EFAD mice (which overexpress human Aβ42 and expres shuman *APOE*) demonstrate that microgliosis and astrogliosis are greater in E4FAD than E3FAD (expressing *APOE4* and *APOE3*, respectively) mice. In addition, inflammatory mediators, as a result of TLR4 activation, are increased in E4FAD mice relative to E3FAD mice. Complementing these *in vivo* findings, our *in vitro* data reveal that stimulation of TLR4 in mixed glial cultures with lipopolysaccharide (LPS; endotoxin) or oAβ induces a similar neuroinflammatory response, as characterized by secretion of tumor necrosis factor (TNF)-α, an effect more pronounced with *APOE4* than *APOE3*. Importantly, both LPS- and oAβ-induced responses are inhibited by LPS from *Rhodobacter sphaeroides* (LPS-RS), a natural TLR4 antagonist. Recent *in vitro* data also demonstrate potent TLR4 antagonism of oAβ-induced responses using the synthetic small molecule IAXO (Innaxon), developed at the University of Milano-Bicocca, and shown to inhibit innate or auto-inflammatory processes. Thus, our working hypothesis is that treatment of EFAD mice with IAXO will improve Aβ pathology and cognitive behavior in an isoform-specific manner. Pharmacokinetic (PK) analysis in wild type mice indicates that IAXO can be nano-formulated to be brain-penetrant, and approaches the *in vitro* EC50 concentration. To test the hypothesis, EFAD mice (E3FAD vs E4FAD; male vs female) were treated with IAXO or

vehicle, from 6-7-months of age in a treatment paradigm. An array of behavior tests, IHC, and biochemical measures demonstrated the beneficial effects of the treatment. To expand on these findings, EFAD mice are currently being treated from 4 to 6 months of age (prevention paradigm) to evaluate the effect of the drug as a preventative therapeutic option. Together, these findings highlight the important role of *APOE4*-modulated TLR4 activation by endogenous ligands in AD, and the therapeutic potential of its antagonism.

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Nanosymposium

574. Mechanisms of APOE-Mediated Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: The effect of sex on APOE-modulated AD pathology in EFAD mice

Authors: ***D. BALU**, M. LADU, N. COLLINS, S. GHURA, A. VALENCIA;
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Abstract: *APO*E4 allele of apolipoprotein E (apoE) is the greatest genetic risk factor for Alzheimer's disease (AD) and is associated with accelerated accumulation of both amyloid-β peptide (Aβ) and soluble oligomeric forms of Aβ (oAβ), likely a proximal neurotixin. Importantly, female *APOE4* carriers have a greater lifetime risk for developing AD, an increased rate of cognitive decline and accelerated accumulation of Aβ compared to male *APOE*4 carriers. *In vivo* progress has been limited by the lack of a tractable familial AD-transgenic (FAD-Tg) mouse model expressing human (h)- rather than mouse (m)-*APOE.* To study the interactions among sex, h-*APOE* and AD pathology, we developed the EFAD-Tg mice by introducing the h-*APOE* genotypes into the 5xFAD-Tg mice. Based on the EFAD mice, and confirmed in human control and AD samples of CSF and brain, the consistent observation has been that apoE lipidation is lower with *APOE4* vs. *APOE3*. Importantly, apoE lipidation negatively correlates with soluble Aβ levels. Thus, we have developed *the mechanistic hypothesis that AD pathology and APOE4 cause a reduction in apoE lipidation, resulting in inefficient clearance of soluble Aβ,*

synaptic loss, memory/cognitive deficits, and dementia. Novel preliminary data demonstrate that differences in female vs. male EFAD mice mimic established *APOE4* vs. *APOE3* differences in Aβ aggregation/accumulation, and apoE levels/lipidation. In addition, amyloid deposition, neuroinflammation and tau pathology is significantly greater in the EFAD females compared to males. These data suggest that sex profoundly influences *APOE* genotype-specific effects on AD pathology, with apoE lipidation state possibly at the intersection between these two AD risk factors. Together, these data will help to address the critical need for treatment options for women at high risk for AD from the *APOE4* allele.

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Nanosymposium

574. Mechanisms of APOE-Mediated Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: ApoE genotype regulates brain exosome production

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Abstract: The E4 variant of ApoE is the major gene controlling Alzheimer (AD) risk, but ApoE is also known for binding receptors to carry lipid cargo into early endosomes and onto sorting endosomes. These can bud intraluminal vesicles in multi-vesicular bodies (MVB), which can be exported as 30-150 nm extracellular vesicles called exosomes. MVB are the earliest site of intraneuronal Abeta in AD. Before trafficking to higher affinity lipoprotein receptors, ApoE binds heparan sulfate proteoglycan/ syndecans at the cell surface. Recently, a pathway involving syndecan ligand binding was shown to recruit syntenin-1, Alix and CD63 to stimulate exosome

biogenesis in MCF7 cells. As one of the more abundant syndecan binding proteins, we hypothesized ApoE might regulate exosome production. In vitro, ApoE3, reconstituted with ApoE-depleted HDL or DMPC added to MCF7 in serum free media, stimulated exosome production. We confirmed this observation using a hippocampal neuronal cell line HT22, showing that ApoE increased syntenin-1 and CD81 exosome release. To look for in vivo ApoE effects on exosomes, we prepared exosome-enriched fractions from brain containing abundant exosome markers (CD63, ALIX, HSP70). To confirm that exosome markers in these fractions were from exosomes, we treated ApoE3 and ApoE4 mice carrying mutant 5xFAD genes (EFAD) with the sphingomyelinase-2 inhibitor, GW4869, from 4.5 to 6 months of age, which reduces exosome production. GW4869 reduced levels of exosome markers by Western blot, but only in the E3FAD fractions. In order to determine whether ApoE genotype regulated exosome production in aging brains, we prepared exosome fractions from 13 month old ApoE3 and ApoE4 targeted replacement mice. Nanosight analysis showed fewer typical exosome sized particles in 13 month ApoE4 than ApoE3 brains. Yields of exosome markers including CD63, Alix, Flotillin-1 and Rab5 were markedly lower from cortex of ApoE4 compared with ApoE3 mice consistent with lower levels of lipidated ApoE4 stimulating fewer exosomes. Exosomes are believed to export pathological proteins including Abeta42 from neurons. Because EFAD mice accumulate intraneuronal Abeta, we examined intraneuronal Abeta in EFAD mice treated with GW4869. E3FAD mice treated with GW4869 had significantly more intraneuronal abeta and fewer exosomes but GW4869 had no effect on E4FAD intraneuronal abeta which had low baseline exosomes, consistent with defective Abeta export. Because MVB have been shown to be an initial site of intraneuronal Abeta accumulation that precedes plaques, defective Abeta clearance via exosomes from MVB may contribute to the ApoE4 acceleration of Abeta deposition and AD risk.

Disclosures: **G.M. Cole:** A. Employment/Salary (full or part-time): University of California, Los Angeles, Veterans Greater Los Angeles Healthcare System. 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; NIH R01 AT008916, VA Merit BX003485. **X. Zuo:** A. Employment/Salary (full or part-time): University of California, Los Angeles, Veterans Greater Los Angeles Healthcare System. **B. Teter:** A. Employment/Salary (full or part-time): University of California, Los Angeles, Veterans Greater Los Angeles Healthcare System. **K.H. Gylys:** A. Employment/Salary (full or part-time): University of California, Los Angeles. **Q. Ma:** A. Employment/Salary (full or part-time): University of California, Los Angeles. **M.R. Jones:** A. Employment/Salary (full or part-time): University of California, Los Angeles, Veterans Greater Los Angeles Healthcare System. **J. Akers:** A. Employment/Salary (full or part-time): University of California, San Diego. **B.S. Carter:** A. Employment/Salary (full or part-time): University of California, San Diego. **M. LaDu:** A. Employment/Salary (full or part-time): University of Illinois at Chicago. **S.A. Frautschy:** A. Employment/Salary (full or part-time): University of California, Los Angeles, Veterans Greater Los Angeles Healthcare System.

Nanosymposium

574. Mechanisms of APOE-Mediated Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: UIC Start-up Fund

Title: The interactive effects of peripheral inflammation and apoe4 on ad pathology *In vivo*

Authors: *F. MAROTTOLI, K. P. KOSTER, R. THOMAS, L. M. TAI;
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Abstract: The lack of effective therapeutic and life-style treatments for Alzheimer's disease (AD) patients highlights the critical need to identify novel pathways of AD progression. Dysfunction of blood vessels in the brain, the cerebrovasculature (CV), is re-emerging as a major contributor to cognitive decline in AD. However, whether CV dysfunction is a cause of, coincident with, or a consequence of AD pathology remains to be elucidated. As the CV represents a physical and metabolic interface between the CNS and periphery, it seems negligent to ignore the effects of AD risk factors on CV dysfunction, particularly APOE genotype and peripheral inflammation. APOE4 is the greatest genetic risk factor for AD, increasing risk up to 12-fold compared to APOE3. Peripheral inflammation serves as a cohesive link for other known AD risk factors including diabetes, hypertension, hypercholesterolemia, and atherosclerosis. Importantly, in humans, peripheral risk factor-induced cognitive decline is greater in APOE4 carriers, and both APOE4 and peripheral inflammation can individually induce CV dysfunction. Therefore, this study addresses the hypothesis that peripheral inflammation causes CV dysfunction and cognitive deficits in APOE4 carriers. Chronic, low-level peripheral inflammation was induced in EFAD mice, a novel, AD-relevant, tractable model of APOE-modulated Aβ pathology. EFAD mice express human APOE3 or APOE4 and overproduce human Aβ via the expression of 5 Familial Alzheimer's disease mutations (5xFAD). EFAD carriers are $5xFAD^{+/-}/APOE^{+/+}$ (EFAD+) and non-carrier, littermate controls are $5xFAD^{-/-}/APOE^{+/+}$ (EFAD-). Thus, EFAD+ and EFAD- mice enable a comparison of the effects of inflammation in the absence and presence of high Aβ levels. Peripheral inflammation induced cognitive decline and CV dysfunction, as well as modulated neuroinflammation only in E4FAD+ mice. Soluble Aβ42 and apoE levels, however, remained unchanged in both APOE3 and APOE4 carriers. These data imply that APOE4 and Aβ prime the CV to damage by peripheral inflammation, resulting in cognitive dysfunction. Therefore, preventing peripheral inflammation and improving vascular health are attractive treatments for AD.

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Nanosymposium

574. Mechanisms of APOE-Mediated Pathology in Alzheimer's Disease

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Presentation Number: 574.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIHR01AG027465

Title: Synaptic localization of LRP1 and LDLR indicate functions in endocytosis and post-synaptic signaling

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Abstract: Background: *APOE* genotype is the major contributor to late onset Alzheimer's disease (AD) and has been associated with increased amyloid beta (Aβ) aggregation and deposition; *APOE* is also strongly linked to Aβ clearance. We have previously shown that Aβ is concentrated in apoE-positive synapses, along with *APOE2*-mediated synaptic clearance of Aβ, and that both apoE and Aβ demonstrate strong accumulations in LDLR and LRP1-positve synaptosomes. **Methods:** In the present experiments to examine localization, synaptosomes from AD cases (n=4) were immunolabeled for the apoE receptors LDLR-receptor related protein (LRP1) and low density lipoprotein receptor (LDLR), along with the presynaptic marker synaptophysin and the postsynaptic marker PSD-95. **Results:** Of the total population of synaptosomes examined (5,000/sample), 86% were positive for synaptophysin, consistent with previous results demonstrating focus of the analysis on a relatively pure synaptosome population, and 21% of synaptosomes of the total population were positive for PSD-95. The apoE receptors LDLR and LRP1 labeled 48 and 57% of synaptosomes respectively. However, when the analysis was restricted to PSD-95-positive synaptosomes, the fraction positive for LDLR increased to 74% ($p<0.003$), and the fraction positive for LRP1 increased to 94% ($p<5.6E-07$), confirming previous results that these receptors coprecipitate with NMDA and PSD-95. When analysis was restricted to LDLR and LRP1-positives, the PSD-95-positive fraction was 41% for LDLR-positive and 24% for LRP1-positives. **Conclusions:** Taken together, these results indicate that a majority of LDLR receptors are not associated with PSD-95 and are present on presynaptic terminals, likely for endocytosis of apoE and other ligands. A smaller fraction of LDLR and LRP1 receptors are tightly associated with PSD-95, consistent with the previously demonstrated nontraditional signaling mechanism of lipoprotein receptors.

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Nanosymposium

574. Mechanisms of APOE-Mediated Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Alzheimer Association NIRP14-304720

Title: Dissecting molecular mechanisms underlying apoe4 pathologic functions in alzheimer's disease

Authors: *D. CAI[1,5], F. EL GAAMOUCH[2], L. ZHU[2], J. CAO[1,6], M. OHLMEYER[3], G. ELDER[5], C. CARDOZO[5], V. HAROUTUNIAN[5,7], B. ZHANG[4];
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Abstract: **Background:** The Apolipoprotein E4 (ApoE4) is the strongest risk factor for sporadic Alzheimer's Disease (AD). Our recent findings suggest that ApoE proteins are critical determinants of brain phospholipid homeostasis and that the ApoE4 isoform is dysfunctional in this process. We have found that the levels of phosphoinositol biphosphate (PIP_2) are reduced in human and mouse brain tissues of ApoE4 carriers. These changes are secondary to increased expression of the phosphoinositol phosphatase synaptojanin 1 (synj1) in ApoE4 carriers. **Results:** We have found that like ApoE null conditions, ApoE4 fails to stimulate efficient degradation of synj1 mRNA, in contrast to ApoE3. mRNA stability is often regulated by microRNA binding to 3'-UTR regions. We identified changes in two microRNAs (miR195 and miR374) in ApoE4 mouse brain tissues, as well as in human brain samples of ApoE4 carriers (with diagnosis of mild cognitive impairment - MCI or early AD), as compared to non-ApoE4 counterparts. Over-expression in ApoE4 neurons of miR195 but not miR374 reduced synj1 protein levels. These results implicate a role for miR195 in ApoE4/synj1/PIP_2 pathways. We then investigated the effects of small vessel cerebrovascular disease (CVD) on brain phospholipid homeostasis. We found that ApoE4-induced PIP_2 reduction is exacerbated by the presence of small vessel CVD in human brains, as well as by chronic high fat diet exposure in ApoE4 KI mouse brains. In cultured adult mouse endothelial cells, exposure to ApoE4-conditioned media increases synj1 expression with a subsequent reduction of PIP_2 levels, and a decreased expression of phosphoinositol-binding clathrin assembly protein (PICALM) that is regulated by PIP_2 levels. Finally, we investigated the effects of synj1 reduction on tau hyper-

phosphorylation. In N2a cells over-expressing human wild-type tau, reduction of synj1 by shRNA leads to reduced *p*-tau levels without changes in total tau. These results in combination with our prior findings suggest multiple beneficial effects of synj1 reduction on AD-related pathologies (ApoE4, amyloid and tau). **Conclusions:** Our findings suggest the involvement of miR195 in the molecular pathways underlying ApoE4-induced PIP_2 dysregulation. Small vessel CVD exacerbates ApoE4-induced PIP_2 changes which could contribute to an increased injury and/or impaired repair within the neurovascular unit leading to vascular dysfunction in association with other pathological effects of ApoE4. Reduction of synj1 has potential therapeutic benefits for AD. Together, our studies could guide future development of novel therapeutic strategies targeted at brain phospholipid dysregulation.

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Nanosymposium

574. Mechanisms of APOE-Mediated Pathology in Alzheimer's Disease

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EMBO ATLF-815-2014

Title: Conformation of secreted ApoE is cell type specific and depends on its lipidation status

Authors: *E. KARA[1], E. HUDRY[1], S. WEGMANN[2], Z. FAN[2], A. ROE[2], K. KROGH[2], C. LUO[2], R. MYLVAGANAM[2], E. SAPP[2], M. MAESAKO[2], O. BEREZOVSKA[2], B. HYMAN[2]; [1]Massachusetts Alzheimer Dis. Res. Ctr., Boston, MA; [2]Massachusetts Alzheimer's Dis. Res. Ctr., Boston, MA

Abstract: Introduction: Apolipoprotein E (ApoE) is a lipoprotein regulating lipid homeostasis within the brain and the periphery. ApoE has three isoforms, E2, E3 and E4, with E4 being an

important risk factor for the development of Alzheimer's disease (AD). It has been suggested that E4 adopts a more closed conformation than E3 because of a salt bridge forming between residues R61 and G255. In addition, it is thought that lipidation has an important role in the function of ApoE, with E4 being less lipidated than E3 and E2. However, neither the conformation of the fully intact protein, nor the role of lipidation in the conformation of ApoE has been studied in physiological systems.
Methods: We cloned a series of constructs encoding each of the ApoE isoforms, with RFP and GFP fused to the N- and C-termini respectively, which we used on a Förster resonance energy transfer (FRET) flow cytometry assay after transient transfection of HEK cells and immortalized astrocytes with ApoE knockout. Conformation of intracellular ApoE was assessed through single cell analysis, whereas of secreted ApoE after attachment of ApoE particles to magnetic beads through antibodies.
Results: Intracellularly, E4 adopted a more closed conformation than E2 and E3 within HEK cells and astrocytes, with FRET efficiency being E2<E3<E4. The R61T E4 mutant had a more open conformation than wild type (WT) E4, with FRET efficiency levels similar to WT E3, consistent with the hypothesis that the salt bridge is important for ApoE conformation. The conformation of secreted ApoE differed between HEK cells and astrocytes grown in nutrient-poor medium, with FRET efficiency being E2>E3>E4 for HEK cells, and E2<E3<E4 for astrocytes. We hypothesised that this was caused by differences in the lipidation status of secreted ApoE. Treatment with 3uM of cholesterol modified the conformation pattern of ApoE secreted from HEK cells to resemble astrocyte-secreted ApoE. Analysis of secreted ApoE particles using the generic lipid dye DiD (ThermoFisher) showed that ApoE secreted from both cell types is lipidated, indicating that cholesterol is the lipid determining ApoE conformation.
Conclusions: Our findings suggest that both isoform and lipidation status are directly relevant to the conformation of ApoE. Future studies are needed to understand the role of conformation and lipidation status in the aggregation process of amyloid.

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Nanosymposium

574. Mechanisms of APOE-Mediated Pathology in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Sortilin impacts APOE isoform-specific actions in brain lipid homeostasis

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Abstract: Sortilin is a member of the VPS10P domain receptor gene family, a class of sorting receptors with central roles in control of neuronal viability and function. Previously, we showed that sortilin acts as a neuronal receptor for apolipoprotein E (APOE), the main apolipoprotein to deliver lipids to neurons and major risk factor for sporadic AD. Sortilin-mediated uptake regulates levels of APOE in mice and represents a major endocytic pathway for clearance of APOE-bound amyloid beta (Aβ) peptides. Consequently, loss of sortilin in gene-targeted mice results in accumulation of murine APOE and of Aβ in the brain, and promotes senile plaque formation (Carlo et al., J Neurosci 2013). While our earlier work elucidated a role for sortilin in control of murine APOE metabolism, its relevance for the (patho) physiological functions of the human APOE3 and APOE4 variants remained unclear. Thus, we introduced the sortilin gene defect into mice carrying a targeted replacement (TR) of the murine *Apoe* locus with the human *APOE3* or *APOE4* genes. Both (APOE3 TR; *Sort1*$^{-/-}$) and (APOE4 TR; *Sort1*$^{-/-}$) mice exhibit increased levels of human APOE and Aβ compared to wild-type mice, recapitulating previous findings on the murine APOE background. To investigate the APOE isoform–specific contribution of sortilin to brain lipid homeostasis, we also performed global lipidomics analyses in the brain of APOE3 and APOE4 TR animals either wild-type or deficient for sortilin. Major differences in the brain lipidome were detected comparing APOE3 and APOE4 TR mice wild-type for sortilin, substantiating distinct functions for APOE3 and APOE4 in brain lipid metabolism. Interestingly, we also noted profound lipid changes in the brain of sortilin-deficient compared to wild-types on a APOE3 TR background, but only minor differences between the two genotype groups in APOE4 TR mice. These findings suggest distinct functions for APOE3 in the brain that are dependent on sortilin. In contrast, no sortilin-dependent activities in neuronal lipid metabolism can be assigned to APOE4. Current studies aim at further elucidating the molecular mechanisms whereby sortilin impacts isoform-specific functions of APOE in the brain and potentially impacts the risk of AD associated with APOE4.

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Nanosymposium

575. Phenotype Characterization of New Genetic and Non-Genetic Models of Parkinson's Disease

Location: SDCC 24A

Time: Tuesday, November 15, 2016, 1:00 PM - 4:15 PM

Presentation Number: 575.01

Topic: C.03. Parkinson’s Disease

Support: Parkinson Canada

CIHR

MJFF

Title: Neurotransmission in cortical neuronal cultures from LRRK2 and VPS35 mutant mice and rescue through acute LRRK2 knock-down.

Authors: *I. TATARNIKOV, D. BECCANO-KELLY, C. KADGIEN, M. FARRER, A. MILNERWOOD;
Neurosci., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Mutations in the gene encoding Leucine-Rich Repeat Kinase 2 (LRRK2) are the most common cause of familial PD. The p.G2019S mutation lies in the kinase domain and alone accounts for ~2% of PD in Caucasians, rising to 20-40% in Ashkenazi Jewish and North African Berber Arab populations. The p.R1441C is another autosomal dominant mutation located in the ROC domain of LRRK2. Despite many advances, our current understanding of the physiological function of LRRK2, and subsequently the effects of LRRK2 dysfunction produced by PD mutations, is disappointingly inadequate. A greater understanding of LRRK2 biology is essential to the development of therapeutic interventions aiming to prevent or delay progression of this devastating disease. We recently reported that neurons cultured from LRRK2 p.G2019S knock-in mice (GKI) have altered synaptic connectivity: glutamatergic and GABAergic transmission was increased without alterations in synapse numbers, and Synapsin-1 phosphorylation was reduced (Beccano-Kelly 2014). Here, we confirmed these findings and found that cortical neurons cultured to 21 days from a second LRRK2 p.R1441C knock-in mouse model (RKI) also exhibited similar increases in synaptic transmission. Recently a mutation in the VPS35 gene has also been linked to late onset familial PD (Vilarino-Guell 2013), therefore we went on to examine neurons cultured from VPS35 p.D620N knock-in mice (VKI) and found similar increases in glutamatergic transmission with no alterations in synapse number. In culture LRRK2 antisense oligonucleotides produce an acute knock down of LRRK2 (>80%) within 5 days and reverse increases in synaptic transmission in all 3 knock-in mutant models.

Disclosures: I. Tatarnikov: None. **D. Beccano-Kelly:** None. **C. Kadgien:** None. **M. Farrer:** None. **A. Milnerwood:** None.

Nanosymposium

575. Phenotype Characterization of New Genetic and Non-Genetic Models of Parkinson's Disease

Location: SDCC 24A

Time: Tuesday, November 15, 2016, 1:00 PM - 4:15 PM

Presentation Number: 575.02

Topic: C.03. Parkinson's Disease

Support: Canada Excellence Research Chair program

Michael J. Fox Foundation

Parkinson Society Canada

Title: Characterization of the VPS35 p.D620N knock-in mouse model of Parkinson's disease.

Authors: *S. CATALDI[1,2], I. TATARNIKOV[2], C. KADGIEN[2], J. KHINDA[2], J. FOX[2], B. SMAILA[2], A. J. MILNERWOOD[3], M. J. FARRER[2];
[1]Grad. program in Neurosci., [2]Dept. of Med. genetics, [3]Dept. of Neurol., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: VPS35p.D620N is genetically linked to autosomal-dominant parkinsonism (Vilariño-Güell, 2011). VPS35 is a core component of the retromer system which regulates sorting of proteins from endosomes to lysosomes, the trans-Golgi network, or the plasma membrane. Cargos include CIM6PR, β2 adrenergic and AMPA receptors (Seaman, 2012; Munsie, 2015). In addition VPS35p.D620N disrupts lysosomal delivery of cathepsin D, required for α-synuclein degradation (Follett, 2014). In transfected primary cortical neurons we have shown VPS35 wild type and p.D620N mutant overexpression alter glutamate synapse number and mEPSC, whereas mutant overexpression specifically effects AMPA receptor trafficking and mEPSC amplitudes (Munsie, 2015). To study the physiological consequences of VPS35p.D620N we engineered the mouse genome to constitutively express this missense mutation. Characterization of gene expression and retromer components compared to their wild type littermates shows the expression and stoichiometry of retromer core subunits is unperturbed. However, VPS35p.D620N binding to FAM21 and the WASH complex is decreased. In cultured neurons the knock-in mutation also confers increases in glutamatergic mEPSC frequency and amplitude. Mice were tested in standardized behavioural tests at 3 and 18 months. Motor activity (open-field, rotarod) and anxiety (open-field and elevated plus maze) test were conducted. At 3 months mutant mice exhibited increased anxiety-like behavior compared to wild type littermates, but do not show significant locomotion alterations. In contrast, at 18 months behavioural alterations are reversed, with mutant animals exhibiting anxiolytic-like behaviour. At 3 months ex vivo fast scan cyclic voltammetry was conducted to assess nigrostriatal dopamine (DA) function. Electrically stimulated DA release was significantly elevated in brain slices prepared from young

mutant mice, and pharmacological responses to DA agonist were also altered. Elevated DA release may relate to increased anxiety at 3 months. Further evaluation at 18 months is in progress, as well as complementary evaluation of DA and other neurotransmitters by in vivo microdialysis. Immunohistochemical markers of nigrostriatal function (tyrosine hydroxylase, DAT) will be assessed, in addition to proteins implicated in human neurodegenerative pathology in young and old animals. The differential synaptic-endosomal, DAergic, and motor phenotypes observed at early and late stages in this knock-in model of VPS35 parkinsonism provide insight into the underlying pathophysiology of prodromal PD.

Disclosures: **S. Cataldi:** None. **I. Tatarnikov:** None. **C. Kadgien:** None. **J. Khinda:** None. **J. Fox:** None. **B. Smaila:** None. **A.J. Milnerwood:** None. **M.J. Farrer:** None.

Nanosymposium

575. Phenotype Characterization of New Genetic and Non-Genetic Models of Parkinson's Disease

Location: SDCC 24A

Time: Tuesday, November 15, 2016, 1:00 PM - 4:15 PM

Presentation Number: 575.03

Topic: C.03. Parkinson’s Disease

Support: Canadian Institutes of Health Research

Parkinson Society Canada

Michael J. Fox Foundation

Title: Neurotransmitter receptor trafficking and glutamatergic synapse maintenance in a novel VPS35 p.D620N knock-in mouse model of Parkinson’s disease

Authors: *C. KADGIEN, L. MUNSIE, J. KHINDA, L. CAO, I. TATARNIKOV, A. MILNERWOOD, M. J. FARRER;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The pathogenic D620N (DN) mutation in vacuolar protein sorting 35 (VPS35) is linked to late-onset, autosomal-dominant Parkinson’s disease (PD). VPS35 is a core component of the retromer complex, involved in endosomal recycling and intracellular trafficking. Data from our overexpression model suggests that the mutation confers a loss of function in AMPA-type glutamate receptor (AMPAR) recycling, resulting in aberrant synaptic connectivity. Here we explore differences in binding of known and novel retromer cargoes and early synaptic dysfunction in a novel D620N knock-in mouse model of PD. Western blot and co-immunoprecipitation were performed in tissue from wild-type and mutant mice to explore

differences in binding of neurotransmitter receptors and known VPS35 interactors. We found alterations in VPS35 binding to FAM21, a component of the WASH complex. Fluorescence recovery after photobleaching was used to assay AMPAR surface recycling. Whole-cell patch clamp and immunocytochemistry were used to explore differences in synapse number and response amplitude in cultured cortical cells. Cultured cortical cells showed alterations in spontaneous glutamate release, and synaptic strength. Here we conclude that the D620N mutation alters glutamatergic synapse maintenance in cultured cortical cells. Many genes linked to PD appear are involved in synaptic transmission; thus, understanding the role of VPS35 is important for uncovering how disruptions of neurotransmission lead to neurodegeneration in PD.

Disclosures: C. Kadgien: None. **L. Munsie:** None. **J. Khinda:** None. **L. Cao:** None. **I. Tatarnikov:** None. **A. Milnerwood:** None. **M.J. Farrer:** None.

Nanosymposium

575. Phenotype Characterization of New Genetic and Non-Genetic Models of Parkinson's Disease

Location: SDCC 24A

Time: Tuesday, November 15, 2016, 1:00 PM - 4:15 PM

Presentation Number: 575.04

Topic: C.03. Parkinson's Disease

Support: The Michael J Fox Foundation

Parkinson Canada

CIHR

Title: Early hyperactivity, increased glutamate and dopamine release precede cognitive impairment, hypoactivity and nigrostriatal dysfunction in LRRK2 G2019S knock-in mice.

Authors: M. VOLTA[1], D. BECCANO-KELLY[2], S. PASCHALL[2], S. CATALDI[2], S. MACISAAC[2], J. FOX[2], E. MITCHELL[2], S. BERGERON[2], H. MELROSE[3], M. J. FARRER[2], *A. J. MILNERWOOD[2];
[2]Neurology, Ctr. for Applied Neurogenetics & Brain Res. Ctr., [1]Univ. of British Columbia, Vancouver, BC, Canada; [3]Mayo Clin. Florida, Jacksonville, FL

Abstract: Genetic variation and mutations in LRRK2 contribute the highest genetic risk factor for Parkinson's disease (PD). Prior to clinical diagnosis of PD by motor symptoms, LRRK2 mutation carriers exhibit cognitive alterations (Thaler 2012). Other evidence suggests LRRK2 mutant carriers exhibit increased striatal dopamine turnover during a prodromal/premotor phase (Sossi 2010), prior to classical reductions in nigrostriatal dopamine and resultant motor deficits.

While the physiology and pathophysiology of LRRK2 remain largely unclear, we recently reported that LRRK2 G2019S knock-in (GKI) mice develop latent reductions in striatal dopamine tonus (Yue 2015), and that glutamate transmission is elevated in GKI cortical neuron cultures (Beccano-Kelly 2014). Here we longitudinally assessed glutamate and dopamine in GKI mice with parallel motor and cognitive testing. Young GKI mice perform well in several cognitive tasks, but are hyperactive. Acute brain slice physiology in young mice demonstrated increased spontaneous release at glutamatergic synapses onto striatal projection neurons (SPNs) and augmented nigrostriatal dopamine release. This occurs in the absence of alterations to synapse numbers or markers of VGluT1,VGLuT2, DAT and TH. As GKI mice age, they become cognitively impaired in visiospatial paradigms, hypoactive and exhibit progressive reductions in glutamate and dopamine release. Thus, at physiological expression levels, the GKI point mutation produces age-dependent, progressive, behavioural phenotypes that correlate with specific neurophysiological alterations. The natural history of biological alterations in knock-in mice provides evidence for early pathophysiological effects of LRRK2 mutations and later parkinsonian-like deficits. These insights inform design of novel therapeutic strategies that may provide neuroprotection for this, and potentially other, forms of parkinsonism.

Disclosures: **M. Volta:** None. **D. Beccano-Kelly:** None. **S. Paschall:** None. **S. Cataldi:** None. **S. MacIsaac:** None. **J. Fox:** None. **E. Mitchell:** None. **S. Bergeron:** None. **H. Melrose:** None. **M.J. Farrer:** None. **A.J. Milnerwood:** None.

Nanosymposium

575. Phenotype Characterization of New Genetic and Non-Genetic Models of Parkinson's Disease

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Marianne and Marcus Wallenberg Foundation

Swedish Brain Foundation

Parkinson Research Foundation

Swedish Parkinson Foundation

Swedish Alzheimer Foundation

Swedish Society of Medicine

Title: Early fine motor impairment and behavioral disturbances in transgenic mice, expressing human alpha-synuclein with the A30P mutation.

Authors: *S. EKMARK LEWÉN[1,2], V. LINDSTRÖM[2], A. GUMUCIO[2], A. ERLANDSSON[2], P. KAHLE[3], E. NORDSTRÖM[4], M. ERIKSSON[4], L. LANNFELT[2], J. BERGSTRÖM[2], M. INGELSSON[2];
[1]Uppsala Univ., Uppsala, Sweden; [2]Dept. of Publ. Hlth. and Caring Sciences, Mol. Geriatrics, Uppsala Univ., Uppsala, Sweden; [3]Dept. of Neurodegeneration, Hertie Inst. for Clin. Brain Res. and German Ctr. for Neurodegenerative Dis., Tübingen, Germany; [4]BioArctic Neurosci. AB, Stockholml, Sweden

Abstract: Parkinson's disease (PD) and dementia with Lewy bodies are common neurological disorders among the aging population. Transgenic (tg) mouse models of these disorders have become a valuable tool to study disease mechanisms and evaluate potential treatments. The (Thy-1)-h[A30P] alpha-synuclein (α-syn) tg mice show elevated α-syn protofibril levels and develop severe age-dependent motor impairments, corresponding to symptoms in PD patients. However, fine motor impairments developed at an early age have not previously been evaluated in this mouse model. We have analyzed fine locomotion and gait deficits as well as behavioral profiles in (Thy-1)-h[A30P] α-syn tg mice and age-matched wild type control animals at 2, 4.5, 8 and 11 months of age. In the tg mice, we observed significant fine motor impairments in the challenging beam test already at 2 months of age with a progressive deterioration until 11 months of age, as compared to control mice. Moreover, at 4.5 and 8 months of age, the tg mice showed a decreased general activity (significant reduction in distance moved, velocity and entries into zones) in the multivariate concentric square field test for behavioral profiling. However, increased exploratory (more rearing) and high risk taking behaviors (more entries on the illuminated bridge and lower shelter/risk index) compared to controls were observed. Further, the tg mice did not show any impairment in muscle strength and coordination in the hanging wire test. In previous studies on (Thy-1)-h[A30P] α-syn tg mice we have shown a correlation between α-syn protofibril levels in spinal cord and severe motor symptoms, in aged animals. However, in this study analyses of the spinal cord and brain stem in 8 and 11 months old mice showed relatively low α-syn protofibril levels with a high variability (from 5 up to 1200pM) that did not correlate with the outcome on the motor and behavioral tests, indicating another mechanistic explanation for the changes observed in young animals. In conclusion, these results show that the (Thy-1)-h[A30P] α-syn tg mouse model is a useful tool to study early PD related symptoms. Further, such subtle motor and behavioral changes offer novel phenotypic readouts to assess effects of therapeutic intervention in this mouse model of α-synucleinopathy.

Disclosures: **S. Ekmark Lewén:** None. **V. Lindström:** None. **A. Gumucio:** None. **A. Erlandsson:** None. **P. Kahle:** None. **E. Nordström:** Other; Employed at Bioarctic Neuroscience AB. **M. Eriksson:** Other; Employed at Bioarctic Neuroscience AB. **L. Lannfelt:** Other; Lars Lannfelt is a founder of Bioarctic Neuroscience. **J. Bergström:** None. **M. Ingelsson:** None.

Nanosymposium

575. Phenotype Characterization of New Genetic and Non-Genetic Models of Parkinson's Disease

Location: SDCC 24A

Time: Tuesday, November 15, 2016, 1:00 PM - 4:15 PM

Presentation Number: 575.06

Topic: C.03. Parkinson’s Disease

Support: Interdisciplinary Center for Clinical Research (IZKF Erlangen, TP E18)

Bavarian State Ministry of Education and Culture, Science and Arts in the framework of the Bavarian Research Network Induced Pluripotent Stem Cells (ForIPS)

Title: α-synuclein impairs myelin formation in multiple system atrophy

Authors: *J. WINKLER;
Univ. Hosp. Erlangen, Erlangen, Germany

Abstract: Introduction: In multiple system atrophy (MSA), myelin loss and neurodegeneration are associated with α-synuclein accumulation in oligodendrocytes, but underlying pathomechanisms are poorly understood. Here, we analyzed the impact of oligodendroglial α-synuclein on the formation of myelin sheaths in order to define a potential interventional target for MSA. **Methods:** Post-mortem analyses of MSA patients and controls were performed to quantify myelin and oligodendrocyte numbers. As pre-clinical models, we used transgenic MSA mice, a mouse embryonic stem cell-derived oligodendrocyte-neuron co-culture, and primary rat oligodendrocytes to determine functional consequences of oligodendroglial α-synuclein overexpression on myelination. **Results:** Myelin loss was accompanied by preserved or even increased numbers of oligodendrocytes in MSA and transgenic mouse forebrains, indicating an oligodendroglial dysfunction in myelin formation. Corroborating this observation, overexpression of α-synuclein in primary and stem cell-derived oligodendrocytes severely impaired myelin formation, defining a novel α-synuclein-linked pathomechanism in MSA. We used the pro-myelinating activity of benztropine to analyze the reversibility of the myelination deficit. Transcriptome profiling of primary oligodendroglia demonstrated that benztropine readjusts myelination-related processes, including cholesterol and membrane biogenesis, being compromised by oligodendroglial α-synuclein. Additionally, benztropine restored the α-synuclein-induced myelination deficit of stem cell-derived oligodendrocytes and ameliorated myelin loss in transgenic MSA mice. **Key Conclusions:** This study defines the α-synuclein-induced myelination deficit as a novel and crucial pathomechanism in MSA. Importantly, the reversible nature of this oligodendroglial dysfunction opens a novel avenue for an early intervention in MSA.

Disclosures: **J. Winkler:** A. Employment/Salary (full or part-time): University Hospital Erlangen, Molecular Neurology, Schwabachanlage 6, 91054 Erlangen.

Nanosymposium

575. Phenotype Characterization of New Genetic and Non-Genetic Models of Parkinson's Disease

Location: SDCC 24A

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Presentation Number: 575.07

Topic: C.03. Parkinson's Disease

Support: Parkinson Canada

Brain Canada/Krembil Foundation

Title: Increasing axonal arborization size of dopamine neurons to produce a better animal model of Parkinson's disease

Authors: ***P. CASSIDY**, W. TANGUAY, L.-É. TRUDEAU;
Univ. de Montréal, Montreal, QC, Canada

Abstract: INTRODUCTION: In Parkinson's disease, dopamine (DA) neurons of the *substantia nigra* (SNc) are a key subset of neurons particularly vulnerable to degeneration. Recent work in our laboratory showed that in cultured DA neurons, energetic metabolism, levels of oxidative stress, and vulnerability to toxins are a function of axonal arborization size (Pacelli et al., 2015). It has been theorized that the arborization size of a single DA neuron in humans is much larger than in rodents, which could account for the apparently higher resilience of rodent DA neurons. **HYPOTHESIS:** Partial lesions of DA neurons in the SNc have been shown to induce a compensatory axonal sprouting mechanism in surviving neurons in the rat. Our hypothesis is that a partial lesion in the neonate mouse SNc will result in adult mice that have a population of DA neurons with a much larger axonal arborization, elevated energetic needs, and increased basal vulnerability. This compensating DA neuron population would thus exhibit increased vulnerability to toxins and would potentially undergo age-dependent PD-like neurodegeneration. **METHODS:** We will induce a lesion of approximately 50% of SNc DA neurons by a unilateral injection of the neurotoxin 6-hydroxydopamine (6-OHDA) in neonatal (P5) transgenic DAT::IRES-Cre mice. These mice will then be evaluated at 3 months of age. In a first step, we will quantify the axonal arborization size of surviving DA neurons by infecting a sub-population of these neurons, via intranigral injection, with a virus allowing conditional expression of green fluorescent protein (GFP). In a second step, the vulnerability of compensating DA neurons at adult age will be compared to control DA neurons by injecting these mice systemically with the

DA-selective toxin MPTP. **RESULTS:** Our initial results have identified doses of 6-OHDA required to create a partial lesion of SNc DA neurons. For optimization, a volume of 0.5ul 6-OHDA at concentrations ranging from 0.05ug/ul to 6ug/ul was tested in mice of three different genetic backgrounds: C57/Bl6, DAT-Cre +/- knock-in (50% lower DAT levels), and DAT::IRES-Cre +/- (17% lower DAT levels). Our results show that vulnerability to a given dose of 6-OHDA varies greatly depending on the genetic background and levels of DAT, and confirm the axonal sprouting of surviving SNc DA neurons at early time points (10 days) post-lesion. Further experiments are now planned to quantify sprouting at later time points and to establish whether the vulnerability of surviving adult SNc DA neurons in these mice is increased, which could represent a novel mouse model of Parkinson's disease more representative of the high vulnerability of DA neurons in humans.

Disclosures: P. Cassidy: None. **W. Tanguay:** None. **L. Trudeau:** None.

Nanosymposium

575. Phenotype Characterization of New Genetic and Non-Genetic Models of Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NIH NIA 5T32 AG000057-37 to Peter Rabinovitch

Title: Alterations in lipid metabolism modify GBA1-mediated neurodegeneration in a *Drosophila* model of Parkinson's disease.

Authors: *M. DAVIS[1], R. E. THOMAS[2], S. YU[2], A. GERMANOS[2], B. N. WHITLEY[2], K. JIANG[2], L. J. PALLANCK[2];
[1]Neurol., [2]Univ. of Washington, Seattle, WA

Abstract: Objective: To understand how glucocerebrosidase (*GBA1*) mutations increase susceptibility to Parkinson's disease (PD) through genetic modifiers.
Background: PD is a common neurodegenerative disorder characterized by progressive motor and cognitive decline. Our understanding of its pathogenesis is limited, and currently no disease-modifying therapies exist. Mutations in *GBA1* are the strongest genetic risk factor for PD, and *GBA1* encodes glucocerebrosidase, an important enzyme in lipid metabolism. However, most *GBA1* carriers do not develop PD, suggesting the presence of modifiers. To investigate how *GBA1* influences PD pathogenesis, we created a *Drosophila* model of *GBA1* deficiency ($GBA1^{del}$) that has age-dependent phenotypes due to neurodegeneration and impaired lysosomal

protein degradation (Davis, et al. PLoS genetics 2016).
Methods: A pilot screen using our *GBA1*^{del} model was conducted to identify genetic modifiers. Chromosomal deletions, each containing 30-40 genes, were screened in heterozygous state for suppression or enhancement of the 5 day old climbing deficit present in *GBA1*^{del} homozygotes compared to controls. The modifier locus within a deletion was identified by narrowing candidate regions using publicly available smaller overlapping deletions and mutated alleles. Modifiers were further characterized by enhancement/suppression of other *GBA1*^{del} phenotypes, including impairment in autophagy.
Results: Two candidate genes were identified: 1) Glucosylceramide transferase (*GlcT-1*) and 2) *Brainwashing* (*bwa*). A publicly available mutation of *GlcT-1* suppressed accelerated insoluble protein aggregation in *GBA1*^{del} homozygotes. Ectopic expression of *GlcT-1* enhanced the climbing deficit, increased insoluble ubiquitinated protein aggregation, and shortened lifespan of *GBA1*^{del} homozygotes.
A mutation in the putative alkaline ceramidase *bwa* also modified the *GBA1* mutant climbing phenotype and partially rescued the accelerated ubiquitinated protein aggregation in *GBA1* mutant flies.
Conclusions: *GlcT-1* and *bwa* are modifiers of *GBA1*-mediated pathogenesis in *Drosophila*. Since mutations in both of these genes suppressed *GBA1* mutant phenotypes, and loss of function in both enzymes are predicted to increase ceramide levels, we hypothesize that decreased levels of ceramide may impair autophagy flux, leading to neurodegeneration in *GBA1*^{del} homozygotes. Further studies characterizing these modifiers, including targeted lipidomics, will elucidate the pathogenic mechanisms causing PD and may reveal new targets for disease-modifying therapies in the most common neurodegenerative movement disorder.

Disclosures: M. Davis: None. **R.E. Thomas:** None. **S. Yu:** None. **A. Germanos:** None. **B.N. Whitley:** None. **K. Jiang:** None. **L.J. Pallanck:** None.

Nanosymposium

575. Phenotype Characterization of New Genetic and Non-Genetic Models of Parkinson's Disease

Location: SDCC 24A

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Presentation Number: 575.09

Topic: C.03. Parkinson’s Disease

Support: NRC-IRAP

ACOA

Title: Induction of a parkinsonian phenotype following central administration of stigmasterol-β-d-glucoside in the rat brain

Authors: *J. M. VAN KAMPEN[1], C. A. SHAW[2], H. A. ROBERTSON[3], D. G. KAY[1]; [1]Neurodyn Inc., Charlottetown, PE, Canada; [2]Univ. of British Columbia, Vancouver, BC, Canada; [3]Pharmacol., Dalhousie Univ., Halifax, NS, Canada

Abstract: The development of effective neuroprotective treatments to slow or stop the progression of Parkinson's disease (PD) will require animal models of the disease with proper face and construct validity, something the field is currently lacking. Indeed, though preclinical studies have offered a plethora of neuroprotective candidates, translation to the clinic has been disappointing. This may be due to the paucity of appropriate animal models for preclinical screening. We have been working to develop a novel animal model of PD that more closely approximates the progressive nature of the disease and recapitulates more of its characteristic features. Consumption of dietary neurotoxins derived from the seed of the cycad plant has been linked to the Guamanian neurological disease cluster ALS-parkinsonism dementia complex (ALS-PDC) in humans. When fed to rodents, cycad flour triggers the progressive development of neurological deficits, with behavioural and cellular features that closely approximate those observed in patients. Clinical signs and histopathological changes continue to develop for several months following cessation of exposure to the neurotoxic insult. *In vitro* studies using isolated cycad compounds have identified stigmasterol-β-D-glucoside as a key neurotoxic component. Preliminary studies have shown consumption of stigmasterol glucoside to be neurotoxic in rodents, triggering oxidative stress and excitotoxicity, leading to apoptosis of sensitive populations of neurons. Here, we demonstrate the *in vivo* neurotoxicity of stigmasterol-β-D-glucoside delivered directly into the lateral ventricle of the adult rat brain. Central delivery for 4 weeks resulted in a significant loss of nigrostriatal dopaminergic neurons, along with an elevation in markers of inflammation and apoptosis. Intracellular accumulation of proteinase-K-resistant synuclein aggregates was also observed. Histopathological changes were associated with significant locomotor deficits in these animals. This work represents the next step in the development of a novel environmental model of PD that will serve as an effective screening tool for neuroprotective therapies.

Disclosures: **J.M. Van Kampen:** A. Employment/Salary (full or part-time): Neurodyn Life Sciences. **C.A. Shaw:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurodyn Life Sciences. **H.A. Robertson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurodyn Life Sciences. **D.G. Kay:** A. Employment/Salary (full or part-time): Neurodyn Life Sciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurodyn Life Sciences.

Nanosymposium

575. Phenotype Characterization of New Genetic and Non-Genetic Models of Parkinson's Disease

Location: SDCC 24A

Time: Tuesday, November 15, 2016, 1:00 PM - 4:15 PM

Presentation Number: 575.10

Topic: C.03. Parkinson's Disease

Title: Long-term inoculations of a-synuclein strains cause distinct synucleinopathies *In vivo*

Authors: W. PEELAERTS[1], L. BOUSSET[2], R. MELKI[2], *V. BAEKELANDT[1];
[1]KU Leuven, Lab. for Neurobio. and Gene Therapy, Leuven, Belgium; [2]Paris-Saclay Inst. of Neuroscience, CNRS, Gif-sur-Yvette, France

Abstract: α-Synuclein aggregation is considered to play a central role in several neurodegenerative diseases, such as Parkinson's disease (PD), Multiple System Atrophy (MSA) and Dementia with Lewy Bodies (DLB), but the exact relationship between α-SYN aggregation and pathogenesis remains unclear. Synucleinopathies are determined by the deposition of α-synuclein aggregates but segregate in distinct pathological phenotypes and diagnostic criteria. α-Synuclein is recently shown to aggregate into different polymorphs or 'strains'. This has led to the hypothesis that strains might account for the distinct clinico-pathological traits within synucleinopathies. Intracerebral injection of distinct α-synuclein assemblies, with distinct conformational properties, resulted in specific neuropathological hallmarks via seeding and strain-specific effects. We assessed long-term effects of intracerebral inoculations in rat and performed histochemical and biochemical analysis. We observed distinctive pathological prion-like effects of α-synuclein strains after long-term inoculations, which could provide a basis for the heterogeneity observed in synucleinopathies and open new therapeutic opportunities such as targeting the degradation of α-synuclein higher molecular weight species.

Disclosures: W. Peelaerts: None. **L. Bousset:** None. **R. Melki:** None. **V. Baekelandt:** None.

Nanosymposium

575. Phenotype Characterization of New Genetic and Non-Genetic Models of Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Vetenskapsrådet Grant (Sweden): C0573901 and 2015-02275

Title: Intracerebral injections of human α-synuclein preformed fibrils exacerbate the pathology caused by AAV-mediated over expression of α-synuclein in rats

Authors: *L. S. BREGER[1], P. THAKUR[1], O. W. WAN[1], B. MATTSSON[1], K. C. LUK[2], V. M. Y. LEE[2], J. Q. TROJANOWSKI[2], A. BJÖRKLUND[1];
[1]Wallenberg Neurosci. Ctr., Lund, Sweden; [2]Dept. of Pathology and Lab. Med., Univ. of Pennsylvania, Philadelphia, PA

Abstract: The aim of this study is to produce a rat model of Parkinson's disease (PD), which faithfully reproduces progressive α-synuclein (α-syn) pathology and profound neuronal loss. Here, we aimed to determine whether sonicated preformed α-syn fibrils (PFFs), injected into either the substantia nigra (SN) or the striatum, can exacerbate nigral α-syn pathology caused by a low dose of an adeno-associated virus (AAV) α-syn vector injected into the SN.
AAV-mediated over expression of α-syn has been used for over a decade to model PD. Although synucleinopathy was observed in animals over expressing α-syn, neuronal degeneration and protein aggregation is generally slow and highly variable.
In this study, rats were injected with a low dose of an AAV6 vector coding for expression of human α-syn protein in SN and ventral tegmental area (VTA). They subsequently received 10 μg of human PFFs, either into the SN-VTA or in the striatum and nucleus accumbens. Motor functions of the animals were followed over time. Brains were perfused 3-24 weeks post-PFFs injection and processed for immunochemistry to assess dopaminergic cell survival, immune response and protein aggregation.
Rat brains collected 3 weeks after PFFs showed markedly increased α-syn pathology in comparison to animals injected with AAV-α-syn or fibrils alone. Aggregates, containing phosphorylated α-syn, were found in the nucleus and cytoplasm, as well as in the projections of the SN dopaminergic neurons, regardless of the site of fibril injection. Injections of PFFs in SN-VTA led to a more profound loss of nigral dopaminergic neurons than injection in striatum-accumbens (22% higher), seen already 3 weeks post-injection. In contrast, dopaminergic neurons survival in the VTA were not affected at that stage by the added injection of α-syn PFFs. In both groups, significant motor impairments were observed in animals injected with both AAV-α-syn and PFFs in comparison with animals that received AAV-α-syn or PFFs alone.
We showed that α-syn PFFs could act as seeds to potentiate pathological changes and aggravate nigral cell loss induced by AAV- mediated over expression of α-syn. The combination of intra-nigral injections of AAV-α-syn and human α-syn PFFs provides a potentially useful an interesting new approach to model progressive PD-like α-syn pathology in rats.

Disclosures: **L.S. Breger:** None. **P. Thakur:** None. **O.W. Wan:** None. **B. Mattsson:** None. **K.C. Luk:** None. **V.M.Y. Lee:** None. **J.Q. Trojanowski:** None. **A. Björklund:** None.

Nanosymposium

575. Phenotype Characterization of New Genetic and Non-Genetic Models of Parkinson's Disease

Location: SDCC 24A

Time: Tuesday, November 15, 2016, 1:00 PM - 4:15 PM

Presentation Number: 575.12

Topic: C.03. Parkinson's Disease

Support: Munich Cluster for Systems Neurology (SyNergy)

Title: Seeded and transgene dependant cortical accumulation of alpha synuclein triggers dendritic spine pathology.

Authors: *S. BLUMENSTOCK[1,2], E. RODRIGUES[1], F. SCHMIDT[3], A. GIESE[3], J. HERMS[1,2,3];
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Abstract: OBJECTIVES:
α-synuclein is considered to be a crucial player in the disease progression of synucleinopathies like Parkinson's disease (PD) or Lewy body dementia (LBD) and also has been reported in modulating brain plasticity. Alterations in distal neuronal compartments like dendritic spines during the course of neurodegenerative disease progression could hold important implications for the functioning of neural networks. In fact, cognitive decline in PD and LBD is a common symptom and could be attributed to impairments in cortical circuitries and synaptic plasticity.
METHODS:
We investigate how α-synuclein accumulation affects the dynamics of dendritic spines in the mouse somatosensory cortex. Long-term *in vivo* imaging of layer V apical dendrites through a chronic cranial window was performed in PDGF-h-α-syn x GFP-M mice overexpressing wild type human α-synuclein, at three different age groups. A second approach involved the intracranial injection of preformed α-synuclein fibrils into wild type mice and the study of the resulting structural consequences on cortical dendrites and spines.
RESULTS:
We find that overexpression of α-synuclein profoundly affects spine dynamics as early as at 3 months of age. Compared to controls, α-synuclein overexpressing mice show decreased spine density and abnormalities in spine dynamics in an age-dependant matter. We also provide the first evidence for the detrimental effects of seeded α-synuclein aggregates on dendritic architecture. We observed spine loss as well as dystrophic deformation of dendritic shafts in layer V pyramidal neurons. Our results might not only provide a link to the pathophysiology

underlying dementia associated with PD and LBD, but also permits to investigate the effects of putative PD drugs *in vivo* on dendritic spine pathology.

Disclosures: S. Blumenstock: None. **E. Rodrigues:** None. **F. Schmidt:** None. **A. Giese:** None. **J. Herms:** None.

Nanosymposium

575. Phenotype Characterization of New Genetic and Non-Genetic Models of Parkinson's Disease

Location: SDCC 24A

Time: Tuesday, November 15, 2016, 1:00 PM - 4:15 PM

Presentation Number: 575.13

Topic: C.03. Parkinson's Disease

Support: Jerry T. and Glenda G. Jackson Fellowship for Parkinson's Disease Research to the University of Arizona

NSCS Summer Research Program

Undergraduate Biology Research Program

Title: AAV-mediated over-expression of vegf-b in pink1 gene knockout rats: a behavioral evaluation

Authors: *M. J. BARTLETT[1,2], B. D. SILASHKI[1], D. C. Y. MULLER[1], C. T. TRAN[1], S. J. SHERMAN[1], T. FALK[1,2];
[1]Dept. of Neurol., The Univ. of Arizona Col. of Med., Tucson, AZ; [2]Dept. of Pharmacol., Univ. of Arizona Col. of Med., Tucson, AZ

Abstract: L-DOPA is the current gold-standard treatment for Parkinson's disease (PD). However, while L-DOPA treats PD symptoms, it does not slow the progressive degeneration of the dopaminergic neurons in the substantia nigra pars compacta. Therefore, recent PD research has emphasized the protection of these neurons, rather than treatment of symptoms. One such neuroprotective agent of interest is vascular endothelial growth factor B (VEGF-B). Previous studies in our lab have shown that VEGF-B is naturally upregulated in rodent dopaminergic cells treated *in vitro* with rotenone, a PD-inducing toxin. In addition, exogenously added VEGF-B has a neuroprotective effect *in vitro*. In an *in vivo* rat 6-hydroxydopamine (6-OHDA) model, injection of VEGF-B into the striatum protected motor function and reduced loss of dopaminergic neurons in the substantia nigra (SN) and terminals in the striatum. In this study, we further assess the neuroprotective effects of VEGF-B using a PTEN-induced putative kinase 1 (PINK1) knockout (KO) rat model, a novel genetic model of PD. Mutations in the PINK1 gene

have been shown to be a cause of some human familial PD cases. PINK1 KO rats gradually develop motor impairment, evident at 8 month of age, and dopaminergic cell loss, which better reflect the slow and progressive degeneration that occurs in human PD.
In a preliminary study, at 5 months of age PINK1 KO (n = 6) rats were injected unilaterally with an adeno-associated virus expressing human VEGF-B (AAV2/1-VEGF-B) into two sites in the striatum (AP +1.0 mm, ML +3.0 mm, DV −5.0 mm; and AP −0.6 mm, ML +3.5 mm, DV −5.0 mm) and one in the substantia nigra (AP −5.0 mm, ML −2.0 mm, DV −7.2). VEGF-B expression is driven with a CAG promoter. Behavioral analyses are compared to PINK1 KO rats (n = 6) and wild-type Long-Evans controls (n = 6). Changes in motor function are tracked monthly using an array of behavioral tests: cylinder test, vibrissae-stimulated forelimb placement (VSFP) test, and forelimb adjusting steps to evaluate forepaw function, as well as the tapered balance beam to evaluate hindlimb function. Presented are the results of monthly testing up to 3 months post-injection of AAV2/1-VEGF-B, compared with baseline testing. At this point there are no differences apparent in forelimb function between the 3 groups. An increase in bilateral hindlimb slips is evident in PINK1 KO rats, and has not been corrected by unilateral VEGF-B over-expression. The study will continue until 9 month post AAV2/1-VEGF-B injection. After euthanasia of the animals we will quantify striatal dopamine content and test for the integrity of the dopaminergic system, by analyzing tyrosine hydroxylase (TH) content in the SN and striatum.

Disclosures: **M.J. Bartlett:** None. **B.D. Silashki:** None. **D.C.Y. Muller:** None. **C.T. Tran:** None. **S.J. Sherman:** None. **T. Falk:** None.

Nanosymposium

576. Exploring Treatment Strategies in Experimental Spinal Cord Injury Models

Location: SDCC 32B

Time: Tuesday, November 15, 2016, 1:00 PM - 4:00 PM

Presentation Number: 576.01

Topic: C.09. Brain Injury and Trauma

Support: Support by NSFC Grant 31371004

Support by NSFC Grant 31570999

Title: Physiological electric field induces neurogenesis in adult isolated filum terminale multipotent progenitor differentiation

Authors: Z.-Y. DONG[1], *Z. PEI[2], X.-T. MENG[1];
[1]Dept. of Histology and Embryology, Col. of Basic Med. Sciences,Jilin Univ., Changchun, China; [2]CCNY- Jilin Univ., Flushing, NY

Abstract: Currently, adult neural stem cell transplantation is a potential therapeutics to treat spinal cord injury and relevant paraplegia or quadriplegia. Adult filum terminale (FT) is an atypical region where multi-potent neural progenitors can be isolated and exhibit tripotent differentiation into neurons, astrocytes and oligodendrocytes. Whereas the low neuronal differentiation efficient blocks the potential of transplantation of FT cells directly into neural injury regions. We applied a small, physiological electric field to neural progenitors from adult rats' FT , and demonstrate the neuronal differentiation rate significantly increases. The following signaling pathway analysis and fluorescence imaging studies uncovered potential mechanisms underlying this electrical field induced neuronal differentiation. In vivo data also suggests an enhanced neural integration rate with functional recovery in FT neuron transplanted animal models. Generally, our work provides a potential therapeutic intervention after spinal cord injury by using FT source of adult neural progenitors for autoplastic transplantation.

Disclosures: **Z. Dong:** None. **Z. Pei:** None. **X. Meng:** None.

Nanosymposium

576. Exploring Treatment Strategies in Experimental Spinal Cord Injury Models

Location: SDCC 32B

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Presentation Number: 576.02

Topic: C.09. Brain Injury and Trauma

Support: NIH 1 R01 NS074882-01A1

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Foundation for Physical Therapy Promotion of Doctoral Studies Level I Scholarship

Title: Region-specific inflammatory responses within remote central pattern generator networks early after spinal cord injury

Authors: *T. D. FAW[1,2,3,4], D. M. NORDEN[2,3], R. J. DEIBERT[2,3], L. C. FISHER[2,3], J. F. SHERIDAN[5,6], J. P. GODBOUT[2,4,6], D. M. BASSO[2,3];
[1]Ohio State Univ., Columbus, OH; [2]Ctr. for Brain and Spinal Cord Repair, [3]Hlth. and Rehabil. Sci., [4]Neurosci. Grad. Program, [5]Col. of Dent., [6]Inst. for Behavioral Med. Res., The Ohio State Univ., Columbus, OH

Abstract: Spinal cord injury (SCI) produces a toxic inflammatory microenvironment that negatively affects plasticity and recovery. Recently, we showed microglial activation, infiltration

of bone marrow-derived myeloid cells, and increased cytokine production that extended into the remote lumbar cord within the first 24 hours after thoracic SCI (T9). At the same time, cervical regions were protected from this robust central and peripheral inflammatory response. Importantly, inflammation and myeloid cell infiltration may impede exercise-based recovery. The purpose of this study was to characterize the regional specificity of inflammatory responses in remote spinal cord regions important for motor function after thoracic SCI. Mice received a 75kdyn contusion at T9 using the Infinite Horizons device and inflammation was measured at cervical and lumbar sites 1-14 days later. Within 24 hours, myeloid cells increased in circulation ($p<.05$). Circulating myeloid cells peaked in number at 7 days and returned to baseline by 14 days post SCI. CD11b+/CD45^{high} myeloid cells infiltrated into the lumbar but not cervical parenchyma by 24 hours and persisted at least 7 days ($p<.05$). GFP+ bone marrow-chimeric mice revealed the continued presence of bone marrow-derived myeloid cells in the lumbar but not cervical cord at 14 days despite their decreased CD45 expression. In addition to peripheral immune cell infiltration, central signs of inflammation and gliosis (Iba-1, GFAP) were evident within 24 hours and remained elevated at 14 days only in the lumbar cord ($p<.05$ at all time points). Further, real-time (RT)-PCR identified a pro-inflammatory profile specific to the lumbar microenvironment from 1-7 days ($p<.05$) with increased expression of inflammatory cytokines (TNFα), chemokines (CCL2), and adhesion molecules (ICAM). Interestingly, while inflammatory mediators were decreasing in the lumbar cord by 14 days, ICAM expression gradually increased in the cervical cord and was highest at 14 days. These data suggest that a toxic microenvironment driven by central and peripheral immune responses develops in the remote lumbar cord after SCI. Remarkably, the cervical cord is protected from inflammation which may render it uniquely capable of adaptive plasticity early after SCI when the lumbar microenvironment renders rehabilitation ineffective.

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Nanosymposium

576. Exploring Treatment Strategies in Experimental Spinal Cord Injury Models

Location: SDCC 32B

Time: Tuesday, November 15, 2016, 1:00 PM - 4:00 PM

Presentation Number: 576.03

Topic: E.09. Spinal Cord Injury and Plasticity

Support: Travis Roy Foundation (JBC and AM)

R21-EB020318-01A1 (JBC)

Title: Timing and location of spinal cord stimulation are critical to augment motor cortex stimulation responses, suggesting convergence of motor and sensory inputs onto common targets in the spinal cord of rats

Authors: *A. M. MISHRA[1], A. PAL[1], J. B. CARMEL[1,2,3];
[1]Motor Recovery Laboratory, Burke Med. Res., White Plains, NY; [2]Neurol. and Pediatrics, [3]Brain and Mind Res. Inst., Weill Cornell Med. of Cornell Univ., New York, NY

Abstract: The goal of this study was to determine the parameters of spinal cord stimulation that could most augment motor cortex-evoked muscle responses. We hypothesized that spinal cord stimulation given below the threshold for provoking a muscle response would be effective in modulating supra-threshold corticospinal response and that the timing and side of the spinal cord stimulation would be critical. Experiments were conducted in intact, anesthetized adult rats. We stimulated the forelimb area of motor cortex and recorded electromyogram (EMG) in the contralateral biceps muscle. To modulate the motor cortex responses, we placed silver ball electrodes on the dorsum of the spinal cord and delivered electrical stimulation below the threshold for provoking an EMG response. In the first experiment, we altered the timing between brain and spinal cord stimulation. We found that stimulating the brain 10ms before the spinal cord was optimal for augmenting EMG responses. There was a striking concordance between the timing of the opimal augmentation and the latency of a cord dorsum potential recorded in the spinal cord, which averaged ~9ms. In the second experiment, we altered the site of spinal cord stimulation, both the laterality of stimulation and the spinal cord level. The laterality was a crucial determinant of the strength of augmentation of cortically-evoked EMG by spinal cord stimulation. Specifically, stimulating the dorsal root entry zone on the side the EMG was being recorded was more effective that stimulating at the midline or on the other side of the spinal cord. In contrast, changing the spinal cord level where stimulation was applied within the cervical enlargement did not change the augmenting response of spinal cord stimulation. Finally, to determine if the stimulation of the spinal cord recruits afferents, we performed repetitive spinal cord stimulation and altered the rate of stimulation. In this case, we stimulated the spinal cord at a suprathreshold intensity. Repetitive stimulation (15 times) caused a rate-dependent decrease in EMG response, suggesting that the large diameter afferents are recruited with spinal cord stimulation. Together, these experiments suggest brain and spinal cord stimulation interact by convergent input to common targets in the spinal cord. This convergent input model could be used to strengthen neural connections in a circuit-specific manner.

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Nanosymposium

576. Exploring Treatment Strategies in Experimental Spinal Cord Injury Models

Location: SDCC 32B

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Presentation Number: 576.04

Topic: C.09. Brain Injury and Trauma

Support: Ontario Institute of Regenerative Medicine

Krembil Foundation

Wings for Life

Title: Human iPSC derived neural progenitor cells engineered to secrete GDNF show enhanced survival, neuronal differentiation and improve functional recovery after spinal cord injury

Authors: *M. KHAZAEI[1], N. NAGOSHI[1], H. NAKASHIMA[1], D. SELIGMAN[1], L. LI[1], A. BADNER[1,2], J. CHIO[1,2], M. G. FEHLINGS[1,2,3];
[1]Toronto Western Res. Inst., TORONTO, ON, Canada; [2]Inst. of Med. Sciences, Univ. of Toronto, Toronto, ON, Canada; [3]Dept. of Surgery, Univ. of Toronto, Toronto, ON, Canada

Abstract: Transplantation of induced pluripotent stem cell derived neural precursor cells (iPSC-NPCs) is a promising therapeutic strategy for spinal cord injury (SCI), but significant challenges remain regarding neuronal integration, survival and functional connectivity. GDNF is a potent neurotrophic factor that promotes survival and integration of graft cells. To increase the survival and integration of graft cells, we have engineered hiPSC-NPCs using piggyback vectors to express GDNF. GDNF-expressing hiPSC-NPCs were transplanted into the spinal cords of a rodent model of moderate contusion SCI at the cervical level 6/7 two weeks after injury while GFP-expressing hiPSC-NPCs transplanted as control. Cell grafts in both groups were localized rostrocaudally surrounding the lesion through white and grey matter with significantly better survival and integration of GDNF expressing cells compared to control (21.3±2% vs. 12.23±3%) at 8 weeks after transplantation. Although a considerable subset of transplanted cells in both groups remained undifferentiated ($Pax6^+$ and $Nestin^+$; 36% in GFP group vs 27% in GDNF group) the majority of cells differentiated to all three neuro-glial lineages. In GDNF group, transplanted cells formed longer $NF\text{-}H^+$ fibers than in GFP-hiPSC-NPCs. Axonal tracing showed a significant increase of biotin dextran amine positive corticospinal tract fibers in GDNG-cell transplanted animals caudally to the lesion site. Interestingly, secretion of GDNF also enhanced the preservation of key endogenous motor-neurons pools (up to 50%) and resulted in overall neural tissue sparing and reduction in cavity size (up to 28% reduction compared to non GDNF expressing cell). Cell transplantation resulted in functional recovery across both GDNF and GFP groups in both forelimb grip strength and CatWalk gait analysis. By 8 weeks, animals receiving GDNF-secreting cells demonstrated grips strengths of 553g versus 394g for control GFP cells.

These results mark an important step forward to improve hiPSC-NPC transplantation outcomes by optimizing transplanted cell survival and cell fate.

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Nanosymposium

576. Exploring Treatment Strategies in Experimental Spinal Cord Injury Models

Location: SDCC 32B

Time: Tuesday, November 15, 2016, 1:00 PM - 4:00 PM

Presentation Number: 576.05

Topic: E.09. Spinal Cord Injury and Plasticity

Support: Natural Sciences and Engineering Research Council of Canada, RGPIN-2014-06318

SensoriMotor Rehabilitation Research Team (SMRRT), RMF111622

Postdoctoral Fellowship, Canadian Institutes of Health Research (CIHR), SV

Title: Inhibitory mechanisms of the human spinal cord associated with motor sequence learning

Authors: ***S. VAHDAT**[1], C. SAYOUR[1], K. BLACK[2], O. LUNGU[1], H. BENALI[3], V. MARCHAND-PAUVERT[4], J. DOYON[1];
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Abstract: Despite ample evidence of brain plasticity in motor skill learning, the contribution of human spinal cord in this learning process has been largely overlooked. Recent electrophysiological evidence has revealed, however, a modulation of the Hoffman reflex (H-reflex) elicited in forearm muscles following motor skill learning [1]. Moreover, through simultaneous functional magnetic resonance imaging of the brain and spinal cord, we provided the first neuroimaging evidence for local learning-induced plasticity in the human spinal cord [2]. Yet, the neurophysiological mechanisms underlying spinal plasticity in early stages of motor learning remain elusive.
Twenty-four subjects performed a serial reaction time task in two different conditions: one involved learning a specific sequence of wrist movements (learning), and the other movements toward random targets (control) [1]. We measured their unconditioned and conditioned (through radial nerve stimulation) FCR H-reflex in a baseline condition as well as during rest periods between task blocks, early and late in both learning and control conditions. We examined the ascending part of the H-reflex recruitment curve (HRC) and four synaptic mechanisms that can

modulate the motoneuron excitability including homosynaptic depression (HD), reciprocal inhibition (RI), presynaptic inhibition of group Ia terminals, and recurrent inhibition (RCI). Subjects were divided in two groups: in one group, we measured HRC and HD (N=14), while RI, PI, and RCI were measured in the other (N=10). All measurements were done in an alternating fashion and randomized order.
Consistent with our previous study [1], the results showed a significant reduction of H-max amplitude estimated from the HRC fitting (using a sigmoid function) in the learning compared to the control and baseline conditions. There was no significant difference in H-max amplitude between the early and late learning phases. Furthermore, we did not find any significant change in HD and RCI across experimental conditions. Yet significant increases in RI and PI during learning were found compared to baseline and control conditions. Specifically, PI increased only late in learning as compared to early learning phase, while RI significantly increased in both early and late learning phases compared to the control condition. Our results suggest that both reciprocal and presynaptic inhibitory mechanisms are involved in spinal plasticity leading to H-reflex inhibition during learning, supporting selective enhancement of local inhibitory sensorimotor pathways in the human cervical cord during motor sequence learning.
[1] Lungu et al., 2010. [2] Vahdat et al., 2015

Disclosures: **S. Vahdat:** None. **C. Sayour:** None. **K. Black:** None. **O. Lungu:** None. **H. Benali:** None. **V. Marchand-Pauvert:** None. **J. Doyon:** None.

Nanosymposium

576. Exploring Treatment Strategies in Experimental Spinal Cord Injury Models

Location: SDCC 32B

Time: Tuesday, November 15, 2016, 1:00 PM - 4:00 PM

Presentation Number: 576.06

Topic: E.09. Spinal Cord Injury and Plasticity

Support: Wings for Life WFL-CA011/15

CIHR MOP-130528

Title: Optogenetic stimulation of diencephalic dopaminergic A13 neurons control locomotion in mice

Authors: *S. SHARMA, K. A. MAYR, P. J. WHELAN;
Univ. of Calgary, Hotchkiss Brain Inst., Calgary, AB, Canada

Abstract: Dopamine (DA) is well studied modulator of locomotor rhythmicity in a variety of species. The diencephalic locomotor region (DLR) is known to be involved in goal directed

locomotion in the vertebrate and invertebrate species. However the precise role of DAergic cells in the DLR in locomotion and their connectivity pattern remains poorly understudied. The DAergic A13 cell group is part of the DLR and one of the least studied dopaminergic centers within the brain. We have previously shown DAergic phenotype of A13 cells, their projections to key brainstem locomotor regions like mesencephalic locomotor region (MLR), medullary reticular formation (MRF) using retrograde chemical tracers and role in locomotion via chemogenetic activation. The goal of current study was to gain insight on the synaptic connectivity pattern of DAergic A13 to MLR, MRF and lumbar spinal cord using transgenic mice and viral tracing tools and to improve spatial and temporal resolution of the activation of DAergic A13 cells. We used a Cre-driver mouse line that expresses Cre-recombinase in neurons expressing tyrosine hydroxylase (TH-IRES-Cre mouse). Using dual injection strategies for mapping connectivity we used microinjections of AAV-DIO-eYFP in A13 of TH-IRES-Cre mice and a fluorogold injection at the level of target brainstem locomotor region or lumbar spinal cord. We show that DAergic A13 neurons project to key locomotor regions such as the mesencephalic locomotor region, medullary reticular formation and lumbar spinal cord. We next examined the role of DAergic A13 photostimulation. We injected adeno-associated viral vector constructs containing AAV-DIO- hChR2(H134R)-eYFP or control AAV-DIO- eYFP virus into the A13 of TH-IRES-Cre mice littermates. Our preliminary data indicate that photostimulation of A13 with 470nm light in awake behaving mice can initiate and modulate locomotion. Viral expression and localization were confirmed using immunohistochemistry. In summary A13 neurons contain the full complement of enzymes to produce and release dopamine and these cells have descending projections MLR, MRF and lumbar spinal cord. Our findings indicate that A13 dopaminergic cells represent a novel target for regulating locomotion that may lead to new treatments for locomotor deficits.

Disclosures: **S. Sharma:** None. **K.A. Mayr:** None. **P.J. Whelan:** None.

Nanosymposium

576. Exploring Treatment Strategies in Experimental Spinal Cord Injury Models

Location: SDCC 32B

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Presentation Number: 576.07

Topic: E.09. Spinal Cord Injury and Plasticity

Support: Travis Roy Foundation (JBC and AM)

R21-EB020318-01A1 (JBC and WV)

Title: Softening spinal cord electrode arrays for safe and effective neuromodulation

Authors: *A. PAL[1], A. M. MISHRA[1], A. GARCIA-SANDOVAL[2], W. VOIT[2], J. B. CARMEL[1,3,4];
[1]Burke Med. Res. Inst., White Plains, NY; [2]Departments of Mechanical Engin. and Materials Sci. and Bioengineering, Univ. of Texas at Dallas, Richardson, TX; [3]Departments of Neurol. and Pediatrics, [4]Brain Mind Res. Inst., Weill Cornell Med., New York, NY

Abstract: The goal of this project is to develop a safe and effective electrode array for stimulation of the cervical spinal cord in the rat. Spinal epidural electrical stimulation is particularly challenging in the cervical spinal cord due to the thin epidural space and large degree of movement of the neck. Stimulating electrode arrays must be supple to avoid injury to the underlying spinal cord. However, the electrode arrays also need to be stiff, in order to place it into the thin epidural space. Current arrays, designed for the lumbar spinal cord, are made of Parylene-C, which is stiff unless made very thin, or silicone rubber, which is supple but difficult to place. To overcome these limitations, we have developed an array using shape memory polymer (SMP) that is stiff at room temperature and becomes as supple as silicone rubber when implanted into the warm aqueous environment of the body. Despite the polymer changing its stiffness, it can withstand the high temperatures required by photolithography, which allows precise patterning of electrodes on the SMP surface. We compared the durability, safety, and efficacy for neuromodulation of SMP-arrays against those made with Parylene-C. All the experiments were conducted in uninjured, awake behaving adult rats. To deliver spinal epidural stimulation, electrode arrays were implanted with the stimulating electrodes over the dorsal C5-C6 spinal cord. To test the physiology of the motor system, screw electrodes were implanted over the primary motor cortex for stimulation and wire electrodes were implanted in the biceps muscle for recording EMG. We tested electrode impedance and the spinal stimulation intensity required to provoke an EMG response from the day of implantation to device failure over days to weeks. We also tested the effects of neuromodulation by pairing brain and spinal cord stimulation. SMP arrays provoked responses to spinal cord stimulation at lower stimulus intensity and showed less change in impedance from before to after implantation as compared to Parylene-C arrays. However, Parylene-C based arrays were more durable after implantation compared to SMP arrays. Both types of arrays produced effective neuromodulation, both at the time of pairing brain and spinal cord stimulation and after. In addition, there was no behavioral or histological evidence for injury to the spinal cord for either array. Thus, SMP is a good electrode substrate for safe and effective epidural stimulation of the cervical spinal cord.

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Nanosymposium

576. Exploring Treatment Strategies in Experimental Spinal Cord Injury Models

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Presentation Number: 576.11

Topic: E.09. Spinal Cord Injury and Plasticity

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Wings for Life Foundation

CIHR Foundation Grant

Djavad Mowafaghian Collaborative Grant

Title: Re-establishment of cortical motor output maps and spontaneous functional recovery via spared dorsolaterally projecting corticospinal neurons after dorsal column spinal cord injury in adult mice

Authors: *B. J. HILTON[1,2], E. ANENBERG[2], T. HARRISON[2], J. BOYD[2], T. MURPHY[2], W. TETZLAFF[2,1];
[1]Zoology, ICORD, Vancouver, BC, Canada; [2]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Motor cortical plasticity contributes to spontaneous recovery after incomplete spinal cord injury (SCI), but the pathways underlying this remain poorly understood. We performed optogenetic mapping of motor cortex in channelrhodopsin-2 expressing mice to assess the capacity of the cortex to re-establish motor output longitudinally after a C3/C4 dorsal column SCI that bilaterally ablated the dorsal corticospinal tract (CST) containing ~ 96% of corticospinal fibers but spared ~ 3% of CST fibers that project via the dorsolateral funiculus. Optogenetic mapping revealed extensive early deficits, but eventual reestablishment of motor cortical output maps to the limbs at the same latency as preoperatively by 4 weeks after injury. Analysis of skilled locomotion on the horizontal ladder revealed early deficits followed by partial spontaneous recovery by 6 weeks after injury. To dissociate between the contributions of injured dorsal projecting versus spared dorsolateral projecting corticospinal neurons, we established a transient silencing approach to inactivate spared dorsolaterally projecting corticospinal neurons specifically by injecting adeno-associated virus (AAV)-expressing Cre-dependent DREADD (designer receptor exclusively activated by designer drug) receptor hM4Di in sensorimotor cortex and AAV-expressing Cre in C7/C8 dorsolateral funiculus. Transient silencing uninjured dorsolaterally projecting corticospinal neurons via activation of the inhibitory DREADD receptor hM4Di abrogated spontaneous recovery and resulted in a greater change in skilled locomotion than in control uninjured mice using the same silencing approach. These data demonstrate the

pivotal role of a minor dorsolateral corticospinal pathway in mediating spontaneous recovery after SCI and support a focus on spared corticospinal neurons as a target for therapy.

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Nanosymposium

576. Exploring Treatment Strategies in Experimental Spinal Cord Injury Models

Location: SDCC 32B

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Presentation Number: 576.12

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH Grant 055976

Title: Intrathecal delivery of BDNF to the lumbar cord via implanted mini-pump restores stepping in a large animal model of spinal cord injury

Authors: ***M. A. LEMAY**, F. MARCHIONNE, 19140;
Bioengineering, Temple Univ. Col. of Engin., Philadelphia, PA

Abstract: Delivery of neurotrophins to the injury site promotes recovery of locomotor behavior in the absence of locomotor training. The present study evaluated if brain derived neurotrophic factor (BDNF) delivered to the lumbar locomotor centers using a clinically translational delivery method restores stepping in a large animal model of spinal cord injury. Fourteen female adult cats were used for this study. Animals were acclimated on a treadmill at different velocities (0.3-0.8 m/s) prior to spinal transection at the T11/T12 level. In eight cats, a 50 ng/day dose of BDNF was delivered intrathecally to the lumbar spinal cord for 48 days post-transection through a programmable mini-pump implanted subdermally. The catheter was inserted between the L7/S1 vertebrae. In two cats, it was tunneled subdurally until reaching approximately the L3 spinal segment, and in the other six cats it was inserted until about the L7 spinal segment. The remaining six animals underwent spinal transection and pump/catheter implant as well, but the pump was filled with 0.9% NaCl and those animals served as controls. Kinematic evaluation was conducted before, 3 and 5 weeks after injury/pump implant. Results show that treated cats are able to bipedally step on a treadmill at all velocities tested (0.3-0.8 m/s), while control cats do not recover stepping ability, especially at higher velocities. Controls tend to drag their hind paws on the treadmill and show dorsal foot placement during stance. Gross examination revealed no damage to the cord or the roots with minimal encapsulation of the catheter/pump. Although inserted on the dorsal aspect, the catheter tips were found on the ventral aspect of the cord. Immunohistochemistry (IHC) of the lumbar segments shows higher concentration of BDNF in

the dorsal root ganglions, with BDNF IHC extending from L3 to L7 in all treated cats. BDNF was also found within multiple cells of the grey matter, although BDNF presence did not extend as far rostrocaudally in the cord. Terminal experiments evaluating lumbar interneuronal firing during a locomotor behavior showed longer bouts of air-stepping in BDNF treated animals. Neuronal spikes are being processed to evaluate activity patterns in BDNF treated and control cats. Those results demonstrate the therapeutic potential of intrathecal lumbar BDNF delivery in spinalized animals. Constant infusion of BDNF to the locomotor centers promotes locomotor recovery similar to training or delivery via cellular transplants after SCI. Intrathecal delivery by an implantable/programmable pump is a safe and effective method for delivery of a controlled BNDF dosage; it poses minimal risks to the cord and is clinically usable.

Disclosures: **M.A. Lemay:** None. **F. Marchionne:** None.

Nanosymposium

576. Exploring Treatment Strategies in Experimental Spinal Cord Injury Models

Location: SDCC 32B

Time: Tuesday, November 15, 2016, 1:00 PM - 4:00 PM

Presentation Number: 576.08

Topic: C.09. Brain Injury and Trauma

Support: 70BF-01. Uniformed Services University Exploratory Grant.

Title: Altered glucose uptake profiles in the aged rat spinal cord with and without injury: A PET study

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Abstract: Aging brain and spinal cord tissue shows increases in oxidative stress and reactive oxygen species, which may increase the activation of glial cells, reduce neuronal viability and worsen outcome following a spinal cord injury (SCI). Our previous work demonstrated that positron emission tomography (PET)-based measurements of glucose uptake after SCI in 3 month old rats show decreased glucose uptake at 6 hours post injury, reflecting a reduction in neuronal viability, with a return to baseline levels by 14 days post injury (dpi), reflecting elevated glial activity. The purpose of this study was to determine how age alters this glucose uptake profile. Briefly, young adult (3 months old) and aged (12 months old) male Sprague-Dawley rats were subjected to a moderate contusion SCI and PET imaging with [18F]Fluorodeoxyglucose (18F-FDG), which was performed prior to injury and at 6 and 24 hours, and 15 dpi. Using region of interest (ROI - T9 spinal cord) analysis with reference region

(cerebellum) normalization, 18F-FDG PET imaging reveals that 12 month old aged rats show a significant decrease in glucose uptake ($p<0.0001$) compared to 3 month old rats at baseline. At 6 hours post injury, 12 month old rats show significantly increased glucose uptake ($p=0.0158$) in comparison to 3 month old rats. No difference in uptake was observed at 24 hours. At 14 dpi, 12 month old injured rats demonstrate significantly increased glucose ($p=0.0038$) uptake compared to 3 month old injured rats. Further, 12 month old sham rats demonstrate significantly increased glucose uptake ($p=0.0174$) compared to 3 month old sham rats, suggesting an influence of surgery alone. However, 12 month old injured rats show significantly higher glucose uptake than 12 month old sham rats ($p=0.0172$), demonstrating that injury results in greater alteration in uptake pattern than surgery alone. These results show that glucose uptake in 12 month aged rats is altered compared to 3 month old rats, suggesting age related alterations to both neuronal and glial activity directly influence glucose uptake both with and without injury. The decreased baseline glucose uptake in aged rats suggests decreased neuronal functionality with age, and the increased uptake at 6 hours and 14 dpi suggests elevated glial activity above that seen in 3 month old rats, both of which could affect functional outcomes. These findings match our previous work in 12 month old rats, which demonstrated significant motor function changes in both naïve and injured rats in comparison to 3 month old rats. Further, this study demonstrates that 18F-FDG PET imaging can be used as a non-invasive measurement of differences in functional outcome from injury based on age.

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Nanosymposium

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Title: Volitional myoelectric activity in lower extremity of human subjects with chronic motor complete spinal cord injury

Authors: *E. HEALD[1], R. HART[2], K. KILGORE[1], H. PECKHAM[1];
[1]Biomed. Engin., Case Western Reserve Univ., Cleveland, OH; [2]FES Ctr., Louis Stokes Veterans Affairs Med. Ctr., Cleveland, OH

Abstract: **Introduction**: Clinically, human spinal cord injuries (SCIs) are classified, based on the presence or absence of visible or palpable muscle contraction in key muscles below the injury, into motor incomplete or complete injuries. While this classification can be useful, the clinical exam does not include electrophysiological testing, and thus it lacks sensitivity to differentiate small levels of residual ability, as it is quite possible for motor unit activity to occur without producing measurable joint movements. Previous studies have demonstrated the presence of intact neuronal axons across the spinal cord lesion, even in those clinically diagnosed with complete SCI. Though in the past such low-level below-injury activity may have had limited utility, today's rehabilitative and restorative technologies might be able to use this activity to assist or restore function to a person with SCI. Thus, quantifying residual volitional motor activity in chronic, clinically complete SCI is an important task, and a first step toward using this activity in a useful manner. **Methods:** Twenty-four subjects with chronic (>9 months post-injury) cervical SCI (levels C4-C7) who had been classified as motor complete, AIS A (n=16) or B (n=8), were tested for the presence of volitional below-injury EMG activity. Bipolar surface electrodes recorded EMG from 8-12 locations of each lower limb. In each trial, participants were asked to attempt a specific movement of the lower extremity in response to visual and audio cues. Trials were repeated 2-3 times for each movement type, for a total of approximately 30 trials per limb. Recorded EMG was processed using an amplitude threshold algorithm to identify motor unit activity. Trials with sufficient detected activity were then analyzed for correlation of activity with the movement cues. A muscle was said to have significant volitional control if at least 75% of total detected activity was within the movement cues in at least three trials. **Results**: Of the 24 subjects tested, 20 (83%) had at least one lower extremity muscle with sufficient volitional EMG activity. 12 subjects (50%) had at least one muscle on each side that met this criterion. **Conclusion**: The surface EMG protocol utilized here is relatively simple and non-invasive, ideal for a clinical screening tool. The majority of subjects tested were able to produce a volitional signal that met the designated criteria. The presence of this volitional, recordable, myoelectric activity in the lower extremity could provide an innovative new command signal source. Our future work will explore the effects of biofeedback training on these signals with the goal of further improving the signal quality.

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Nanosymposium

576. Exploring Treatment Strategies in Experimental Spinal Cord Injury Models

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Presentation Number: 576.10

Topic: E.09. Spinal Cord Injury and Plasticity

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Title: Changes in motor function and reflex circuits with repetitive transspinal stimulation after spinal cord injury

Authors: *L. M. MURRAY[1,2], B. TAHAYORI[1,2], M. CHIACCHIERO[1,2], M. KNIKOU[1,2,3]; [1]Dept. of Physical Therapy, CUNY Col. of Staten Island, Staten Island, NY; [2]Grad. Center, City Univ. of New York, New York, NY; [3]Biol. PhD Program, City Univ. of New York, New York, NY

Abstract: Spinal cord injury (SCI) results in maladaptive plasticity of spinal neuronal circuits, impaired muscle tone, and poor movement performance. However, the injured human spinal cord possesses the ability to reorganize in a functional manner in response to internal and external stimuli. In this study, we delivered noninvasive transcutaneous stimulation to the thoracolumbar region (transspinal stimulation) in two people with chronic SCI (complete and incomplete) and in two healthy control subjects. Transspinal stimulation was delivered for approximately 15 days (1h/day, 5 days/week) with the cathode electrode placed over the thoracic 10 to L1-2 vertebrae level. Two anode electrodes, connected to function as a single electrode, were placed bilaterally on the abdominal muscles. Changes in spinal reflex excitability and recovery of motor function were assessed before and after stimulation. In complete SCI, transspinal stimulation decreased spinal reflex excitability (as much as 50%) and excitation threshold of motor axons. In incomplete SCI, transspinal stimulation increased spinal reflex excitability without altering the excitation threshold of motor axons. Clinical evaluations indicated improvements in the strength of muscles innervated by nerves in segmental levels above and below the stimulation site. The person with motor incomplete SCI also reported better sensation following transspinal stimulation. In healthy control subjects, transspinal stimulation altered the excitation threshold of Ia afferents and motor axons. Despite being an ongoing project, the current findings clearly indicate that transspinal stimulation produces spinal excitability changes which may promote recovery of motor function in people with motor complete and incomplete SCI.

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Nanosymposium

577. Pain Imaging

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Presentation Number: 577.01

Topic: D.02. Somatosensation: Pain

Support: Wellcome Trust and Royal Society (Henry Dale) 104128/Z/14/Z

Title: Non-invasive brain stimulation relieves phantom pain in amputees; an fMRI study

Authors: ***S. KIKKERT**[1], M. MEZUE[1], J. O'SHEA[1], C. F. BECKMANN[2], D. HENDERSON-SLATER[3], H. JOHANSEN-BERG[1], T. MAKIN[1];
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Abstract: Following arm amputation individuals frequently report experiencing vivid, and often painful sensations of the missing limb (phantom limb pain, PLP). PLP is an intractable chronic neuropathic pain syndrome. We recently showed that chronic PLP associates with functional disconnection of the cortical territory of the missing hand (contralateral to the amputation, hereafter deprived cortex) from the sensorimotor system. The current study was aimed at alleviating PLP using non-invasive brain stimulation, in a within-subject, double blind, counterbalanced and sham-controlled design. We hypothesized that excitatory stimulation over deprived sensorimotor cortex during phantom movement would relieve PLP, by reinstating the deprived cortex into the brain's global sensorimotor network.
Fifteen unilateral upper-limb amputees suffering from chronic PLP underwent twenty minutes of excitatory (anodal, 1mA) brain stimulation (transcranial direct current stimulation; tDCS) over deprived sensorimotor cortex while performing PLP-inducing phantom hand movements. Two additional brain stimulation conditions were included to control for active neuromodulation (i.e. sham tDCS) and neuromodulation site (excitatory tDCS to intact hand sensorimotor cortex). Subjective pain ratings, as well as task-based BOLD and quantitative cerebral blood flow functional scans to indirectly assess neural activity were obtained prior to, during, and post brain stimulation. Subjective pain ratings were also obtained in the week following each stimulation session.
While PLP was significantly increased in both control conditions immediately after brain stimulation - a common occurrence during phantom movement, excitatory stimulation over the deprived sensorimotor cortex averted this pain increase. Further, excitatory stimulation resulted in a significant decrease in PLP ratings at ninety minutes following stimulation. This pain relief lasted at least one week, while no long-term change in pain ratings was observed in the two control conditions. Functional imaging analysis using converging measurements revealed modulated ipsilateral posterior insula activity, a region recently identified as neurally active

during ongoing pain, as a key neural correlate underpinning the lasting pain relief.
Our results suggest non-invasive brain stimulation as a promising tool for inducing lasting relief of PLP. Given the established role of the insula cortex in the coding of persistent pain, changed activity in this region may reflect modulated nociceptive readout, induced by excitatory tDCS.

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Nanosymposium

577. Pain Imaging

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Topic: D.02. Somatosensation: Pain

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EFIC grant to FM

Title: Functional organisation of cortical somatotopic maps in neuropathic pain

Authors: ***F. MANCINI**[1], A. WANG[3], J. MCAULEY[3], M. SCHIRA[4], C. RAE[4], G. D. IANNETTI[1], M. I. SERENO[2], L. MOSELEY[5];
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Abstract: Previous studies have suggested that the functional organization of somatotopic maps of the body in primary somatosensory cortex (SI) is different in people with chronic pain relative to healthy controls. For instance, neuroimaging studies have shown that the SI representation of the painful hand is smaller than that of the unaffected hand, in people with Complex Regional Pain Syndrome (CRPS). However, such findings have been criticized for their vulnerability to bias and incomplete comparisons to control data. Therefore, we used a data-driven, blinded approach (based on phase-encoded mapping) to study the somatotopic representation of the digits in SI, in 18 patients with unilateral CRPS and 18 controls. We used 3T fMRI to image the

SI response to periodic stimulation of the digits of each hand. We studied whether the area, location, and functional organization of the hand map differed across hemispheres and groups. Strikingly, we found no coarse changes in the somatotopic representation of the affected hand relative to the unaffected hand, in CRPS patients. We also found that the coarse functional organization of the hand map in the left and right hemispheres was comparable between CRPS patients and controls. Fine-grained changes will be discussed, and related to the sensory profile of CRPS patients.

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Nanosymposium

577. Pain Imaging

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Topic: D.02. Somatosensation: Pain

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CIHR

Title: The psychological and neurophysiological mechanisms of clinical placebo response in chronic back pain

Authors: *E. VACHON-PRESSEAU, S. E. BERGER, T. ABDULLAH, B. PETRE, T. J. SCHNITZER, V. APKARIAN;
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Abstract: Most of the literature regarding placebo analgesia has been investigated in healthy participants in contexts involving acute pain and manipulations of expectation. The translation of these findings to clinical settings remains speculative. This study uses the setting of a randomized clinical trial that included 43 chronic back pain (CBP) patients exposed to repeated 2-week-long placebo treatment periods, as well as 20 CBP patients allocated to a no treatment arm to control for the natural history of pain and other potential confounds. Data was collected from numerous questionnaires and multimodal brain imaging before the administration of treatment.
The 43 patients in the placebo treatment group were stratified into responders (n = 24) and non-responders (n = 19) based on their pain response measured daily with a smartphone application. From the 29 initial questionnaires items, a combination of 5 items explained an important

amount of variance in who would be stratified as placebo responders or non-responders (pseudo $R^2 = 0.54$, $p < 0.001$): Pain Catastrophizing scale (PCS) rumination and PCS helplessness, Emotional Regulation Questionnaire (ERQ) suppression, and Multidimensional Assessment of Interoceptive Awareness (MAIA) emotional awareness and not-worrying.
We next used resting state functional connectivity to construct brain networks from Pearson correlations of the average time course between brain parcels. Permutation tests on the weighted connections indicated stronger connections between nodes of the lateral frontal and sensorimotor communities in placebo responders compared to both non-responders and the no treatment group ($F_{(2,60)} = 11.67$; $p < 0.001$). Stronger connections were further observed between the dorsolateral prefrontal cortex (DLPFC) and the periaqueductal grey (PAG) in placebo non-responders compared to both placebo responders and the no treatment group ($F_{(2,60)} = 10.04$; $p < 0.001$). Moreover, placebo responders showed smaller grey matter density in post-central gyrus ($F_{(2,60)} = 6.95$; $p = 0.002$) and subcortical leftward asymmetry ($F_{(2,60)} = 4.14$; $p = 0.02$).
Backwards-stepwise logistic regressions demonstrated that higher emotional awareness, less emotional suppression, stronger frontal-to-sensorimotor functional connectivity, and weaker DLPFC-to-PAG functional connectivity represented independent contributors to the placebo response (pseudo $R^2 = 0.71$; $p < 0.001$). These findings demonstrate that placebo response depends on inverse coupling between DLPFC
with sensorimotor system and the PAG, which is consistent with the mechanisms previously describe in osteoarthritis patients and healthy individuals.

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Nanosymposium

577. Pain Imaging

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Presentation Number: 577.04

Topic: D.02. Somatosensation: Pain

Title: Generalizable representations of pain and negative affect in medial prefrontal cortex

Authors: P. A. KRAGEL[1], H. LY[2], L. VAN OUDENHOVE[2], M. KANO[3], P. GIANAROS[4], S. MANUCK[4], *T. D. WAGER[1];
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Abstract: Neural activity in the anterior mid-cingulate cortex (aMCC) has been linked to numerous mental functions, including cognitive control, pain, and emotion, among others. However, researchers disagree on whether its function is reducible to one underlying process (e.g., 'conflict detection'), or whether it contains distinct brain representations for different domains. To address this question, we applied hierarchical modeling to multi-voxel pattern similarity measures across 180 participants drawn from 12 neuroimaging studies. The studies covered three domains: Cognitive control, pain, and emotion, with 4 studies and two distinct tasks in each. Across the medial prefrontal cortex (mPFC) and within specific subregions, we decomposed pattern similarity into components (a) specific to a single study; (b) generalizable across studies but specific to a particular task; or (c) generalizable across the entire domain. The analysis revealed a domain-general representation of somatic pain (generalizable across cutaneous and visceral pain) in the aMCC, and a domain-general representation of negative emotion (across rejection-related and negative pictures) in the vmPFC. Representations of cognitive control were study-specific and did not generalize. In sum, our findings identify distinct representations of pain and negative affect in mPFC that generalize across studies and tasks, and are not reducible to a single, common underlying process. More broadly, they identify the utility of multi-study, multi-domain approaches to identify generalizable brain representations underlying psychological constructs.

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Nanosymposium

577. Pain Imaging

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Title: Cerebral mechanisms of pain avoidance learning

Authors: *M. ROY[1], T. D. WAGER[2], V. TAYLOR[3];
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Abstract: Pain signals the presence of an urgent threat to our corporal integrity; it calls for our complete attention and commands immediate action. However, pain also marks an error in the long chain of decisions that ultimately led us to expose ourselves to danger. Pain therefore also compels us to reassess our future action plans. The effects of pain thus resonate far beyond the immediate painful experience: anticipation of future pain based on past painful experiences influences our current decisions, mood, and pain sensitivity. Alas, we are still lacking basic knowledge of the neurophysiological mechanisms underlying pain's behavioral effects, as research in humans has so far predominantly focused on the immediate experiential properties of painful stimulations (i.e., how pain feels). In a first experiment we show that the aversive prediction error (PE) signals that are crucial for learning to avoid pain are generated in the periaqueductal gray matter (PAG). Moreover, we demonstrate that avoidance of the actions that have caused pain are determined by two behavioral controllers: 1) a fronto-striatal instrumental controller that considers all avaible actions before making its decision, and 2) a Pavlovian controller causing an immediate avoidance of the action that was just punished, even when the action remains the most advantageous option compared to alternatives. Interestingly, Pavlovian influences over avoidant behavior correlated with subjective pain perception, as well as with pain-predictive patterns of cerebral activity, suggesting that subjectively perceived pain may drive direct avoidance of the actions that caused it. Finally, in a second experiment we show that learned associations between pain and pain-predictive cues, as well as uncertainty related to the predictive value of cues, both increase pain perception and spinal nociceptive reflexes. Altogether, these results suggest a strong bi-directional relationship between pain-related prediction and choices, and subjective pain perception.

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Nanosymposium

577. Pain Imaging

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National Institute of Neurological Disorders and Stroke (NS035115)

National Center for Complementary and Integrative Health (AT007987) of U.S. National Institutes of Health

Canadian Institute of Health Research (Postdoctoral fellowship MFE 135472)

Title: Differential analgesia related functional and structural plasticiy following duloxetine or placebo treatment in chronic knee osteoarthritis pain

Authors: *P. TÉTREAULT[1], M. N. BALIKI[1], E. VACHON-PRESSEAU[1], T. J. SCHNITZER[2], V. APKARIAN[1,3];
[1]Physiol., Northwestern Univ. Dept. of Physiol., Chicago, IL; [2]Rheumatology, [3]Anaesthesia and Surgery, Northwestern Univ., Chicago, IL

Abstract: Purpose: Structural and functional brain changes have been shown to be associated with the development and progression of chronic pain. This study was undertaken to determine the brain structural and functional characteristics related with responses to duloxetine (DLX) and placebo (P) treatment in patients with knee osteoarthritis (OA). **Methods:** 39 participants were entered into a randomized trial of DLX 60 mg qd vs matching P. All participants met ACR radiographic and clinical criteria for knee OA, had pain ≥ 4 on 11 point VAS scale, met all inclusion and exclusion criteria, and were treated for 3 months. Pain measurements were assessed at weeks 0, 2, 3, 6 and 16. Anatomic and resting state MRI scans were done at the baseline and at the end of treatment. Cortical and subcortical gray matter density (GMD) changes were evaluated using a voxel based morphometry approach (FSL-VBM). Functional plasticity was assessed with the change in nodal degree count (DC). To evaluate those brain properties, longitudinal contrast was made by using a two-way RM-ANOVA. **Results:** Although there was no overall difference in the mean pain response between treatment groups, both the DLX and P groups could be divided to responders (≥ 20% pain decrease from baseline, hence classified as "responders or +") and non-responders ("non-responders or -"). We generated the reorganization maps for both GMD and DC and the conjunction maps to analyze regions with shared structural and functional plasticity. Our design was made to observe changes due to treatment (all DLX vs all P), response (all responders vs all non-responders), and the interaction of treatment and response. We identified that both functional and structural reorganization in the left middle frontal and precentral gyrus are linked to analgesia outcome for the DLX group, while structural plasticity in the right anterior cingulate cortex is associated with analgesia for the placebo group. We also showed that some cortical regional GMD or DC reorganization were shared among all 4 subgroups, identifying brain modifications naturally occurring in time independent of the treatment received. **Conclusions:** Treatment in participants with OA pain affects cortical reorganization as a function of the type of treatment, the treatment response and the interaction of both. Understanding how placebo and pharmacological treatment affect structural and functional brain properties is important to allow more personalized approaches to therapy in chronic pain conditions.

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Nanosymposium

577. Pain Imaging

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Topic: D.02. Somatosensation: Pain

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Title: Dynamic but not static functional connectivity differs across frequency bands between hubs of the salience and executive control networks

Authors: J. CHENG[1,2], R. BOSMA[1], K. HEMINGTON[1,2], A. KUCYI[1,4], M. LINDQUIST[5], *K. D. DAVIS[1,2,3];
[1]Krembil Res. Inst, Univ. Hlth. Network, Toronto, ON, Canada; [2]Inst. of Med. Sci., [3]Surgery, Univ. of Toronto, Toronto, ON, Canada; [4]Stanford Univ. Sch. of Med., Stanford, CA; [5]Johns Hopkins Univ., Baltimore, MD

Abstract: Objective: Regional variation in specific frequency bands in the resting-state fMRI (rs-fMRI) signal has been linked to bipolar depression, mania (Martino et al., 2016), and chronic pain (Baliki et al., 2011; Alshelh et al., 2016). However, how dynamic communication between regions (i.e., dynamic functional connectivity (dFC)) varies across specific frequency bands and how dFC in each band relates to brain health, behaviour, and disease is currently unknown. Our research focuses on brain mechanisms of pain and attention. As a first step to understand dFC across frequencies, we investigated how dFC between hubs of the salience and executive control networks (SN, ECN) varies across different frequency bands.
Methods: We acquired 3T anatomical and 10-minute rs-fMRI scans from 47 healthy subjects (20-31 yo), who provided informed written consent (Kucyi et al., 2013). Following pre-processing (without temporal filtering), separate rs-fMRI datasets were created by temporally filtering into previously defined slow waves (SWs): 6 (0.0052 – 0.01 Hz), 5 (0.01 – 0.027 Hz), 4 (0.027 – 0.073 Hz) and 3 (0.073 – 0.198 Hz) (Zuo et al., 2010). We then extracted the timecourse from a seed in each subject's anterior mid-cingulate cortex (aMCC) of the SN, and dorsolateral prefrontal cortex (DLPFC) of the ECN (Seeley et al., 2007), and plotted the power spectrums using a periodogram. The dynamic conditional correlation (Lindquist et al., 2014) was calculated between the timeseries of the aMCC and DLPFC. dFC was quantified as the standard deviation of the dynamic conditional correlation. Two separate repeated-measures ANOVAs were run across subjects for: 1) aMCC-DLPFC dFC, and 2) aMCC-DLPFC FC across frequency bands as the within-subject factor ($p < 0.05$). Post-hoc paired sample t-tests were then conducted.
Results: Power decreased with increasing frequency in both the aMCC and DLPFC of each subject. Group mean aMCC-DLPFC dFC decreased across frequency bands from the slowest to the fastest (SWs 6-3 respectively: 0.295, 0.247, 0.206, 0.089). Within-subject decreases in

aMCC-DLPFC dFC across frequency bands was statistically significant ($p < 0.05$). Paired samples t-tests revealed a statistically significant reduction in aMCC-DLPFC dFC progressing from SWs 6-3 (6 and 5, 5 and 4, 4 and 3; all $p < 0.05$). In contrast, FC of the aMCC-DLPFC ($p > 0.05$) did not differ across frequency bands.
Conclusion: Dynamic SN-ECN inter-network functional connectivity is strongest in the 0.0052 – 0.01 Hz ultraslow SW6 and diminishes at higher frequencies. However, static FC is not significantly different from 0.0052-0.198Hz. These findings will inform future work of dFC related to pain and cognition.

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Presentation Number: 577.08

Topic: D.02. Somatosensation: Pain

Support: NCCIH R21AT007530-01

NINDS K24NS064050-09

Title: Functional and biochemical placebo analgesia mechanisms in migraine patients and healthy subjects

Authors: C. LINNMAN[1], C. CATANA[2], L. BECERRRA[1], *D. BORSOOK[1];
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Abstract: Background: The underlying biological pathways that lead to placebo analgesia have generally been studied in healthy subjects, and mechanisms of placebo analgesia in chronic pain patients have yet to be compared to healthy subjects. Placebo analgesia constitutes opportunity to improve clinical outcomes. It is also a challenge for clinical drug trials. These issues are especially true in migraine. In the United States, approximately 30 million people have one or more migraine headaches per year (18% of females and 6% of males). Meta-analysis of placebo controlled double blind migraine prophylaxis studies indicate 23% of patients experience a reduction in migraine attacks of > 50% from placebo.
Major advances in our understanding of the placebo response have been made in the last decade: fMRI studies defining alterations in blood oxygenation level dependent (BOLD) signal, correlated to neuronal activation; PET studies have identified endogenous neurotransmitter

release (notably endorphines and dopamine) associated with placebo analgesia. The magnitude of the placebo response and related endogenous opioid release varies substantially between subjects, possibly predictable by baseline endogenous neurotransmitter tone or resting state functional connectivity, but such predictors remain to be established.
Methods: Placebo responses in inter-ictal migraine patients and in healthy subjects were measured using a repeated-measure (no drug or placebo) conditioning paradigm with intravenous placebo administration. Functional responses to placebo were measured with resting state functional connectivity and pain-evoked responses using fMRI, and simultaneous [^{11}C]-diprenorphine PET imaging to determine opioid receptor distribution.
Results: Our behavioral analysis indicates that migraine patients rated painful and non-painful stimuli as more aversive. Further, administration of placebo led to an 18% decrease in VAS ratings in healthy subjects, and a 26% decrease in the migraine population (n.s.). Structural analysis indicates decreased gray matter density in the dorsal anterior cingulate and the bilateral insula in migraine patients. Insula and medial prefrontal volumes further predicted the magnitude of placebo response. Placebo did not, however, induce any significant changes in resting state functional connectivity.
Conclusions: Clear changes were observed at both a behavioral and structural interactions with placebo responses. Further analysis (ongoing) relates differences in opioid receptor distribution to placebo magnitude and functional alterations in patients and controls.

Disclosures: C. Linnman: None. **C. Catana:** None. **L. Becerrra:** None. **D. Borsook:** None.

Nanosymposium

577. Pain Imaging

Location: SDCC 5B

Time: Tuesday, November 15, 2016, 1:00 PM - 3:30 PM

Presentation Number: 577.09

Topic: D.02. Somatosensation: Pain

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CIHR

Title: White matter structure in chronic migraine is related to disease characteristics

Authors: M. MOAYEDI[1], V. A. MATHUR[2], C. S. HUBBARD[2], M. CEKO[3], *D. A. SEMINOWICZ[2];
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Abstract: Chronic migraine is characterized as a pain disorder with at least 15 headache days per month. Previous studies have reported abnormal brain structure and function in episodic migraine (fewer than 15 headache days per month) and other types of headaches. We have previously shown that chronic migraine affects cortical processing of experimental pain: patients had blunted responses in the modulatory regions as stimulus intensity increased, compared to controls. These aberrant pain-related brain responses in the insula of patients with chronic migraine were associated with pain catastrophizing, pain intensity and migraine frequency. These findings suggest that there is nociceptive-related plasticity in chronic migraine. Here, we sought to investigate whether white matter in migraine patients showed abnormalities associated with migraine disease characteristics and compared to healthy subjects. We used diffusion-weighted imaging and tract-based spatial statistics (TBSS) to relate white matter fractional anisotropy (FA; a measure of white matter integrity) to disease characteristics (disease duration, headache frequency, migraine pain intensity, pain catastrophizing) in 14 migraine patients (11 F, 3 M, age (SD) 40.8 (11.9)). We also compared whole-brain voxel-wise FA between patients and 14 matched controls (11 F, 3 M, age (SD) 38.9 (12.5)). We found a negative correlation between FA and disease duration in the right mid-insula and a positive correlation between FA and pain catastrophizing in the left mid-insula. There were no significant group differences in FA. Taken together with our previous work, the findings suggest that abnormal structure and function are related to chronic migraine pathophysiology, particularly in the insular cortex.

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Nanosymposium

577. Pain Imaging

Location: SDCC 5B

Time: Tuesday, November 15, 2016, 1:00 PM - 3:30 PM

Presentation Number: 577.10

Topic: D.02. Somatosensation: Pain

Title: Comparison of the hemodynamic response to thermal and non-thermal pain stimuli measured by functional near infrared spectroscopy.

Authors: *D. O. OMIRE-MAYOR, P. KONATH ALVALAPPIL, A. WONG, K. POURREZAEI;
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Abstract: As pain is one of the most frequently encountered symptoms in daily medical practice, investigating objective quantifiable measures to aid in pain treatment is of great interest; however, current methods of measuring hemodynamic modulations to pain have not yet

identified objective biomarkers of pain that can be practically applied in clinical settings. Functional near infrared spectroscopy (fNIRS) is a non-invasive imaging modality with the potential to bridge this gap. The fNIRS continuous-wave imaging system penetrates tissue up to a few centimeters within the 700 to 900nm optical light window to track cerebral hemodynamic changes. In this window, at ~700nm, deoxy-hemoglobin is maximally absorbed and at ~900nm, oxy-hemoglobin is maximally absorbed. Based on modified Beer-Lambert law calculations, a ratio is obtained that gives the changes in oxy and deoxy-hemoglobin, relating to their baseline in the dorsolateral prefrontal cortex region. Previous research in our lab has investigated the hemodynamic response to painful stimuli through the use of the cold pressor test (CPT); subjects were successfully classified into groups of high pain and low pain. Current research furthers this investigation by exploring the hemodynamic response to non-thermal pain stimuli, in particular, electrical stimuli. As different pain types activate different afferents, this will potentially lead us to identify different hemodynamic responses generated by the sympathetic nervous system as a result of different kinds of stimulation. Electrical stimuli was delivered using a FlexTENS (Electrostim Medical Services, Inc., Tampa, FL) neuromuscular stimulator to the dominant hand of subjects at two levels: an electrical stimulus that gave a pain level of 3, which is considered non-painful/innocuous, and an electrical stimulus that gave a pain level of 7, which is considered painful/noxious. Pain levels were found by starting with 80Hz electrical stimulation at 0mA and incrementally increasing (+2.7mA at a time). The fNIRS system monitored the hemodynamic response to each pain level respectively. This data was then compared to previously collected fNIRS data from the CPT. Results show that there are distinguishing features in the signal response to the different types of pain respectively. This data demonstrates the efficacy of fNIRS as a potential diagnostic tool to objectively quantify pain as it can differentiate between types of pain experienced by a subject. Furthermore, it solidifies the fact that the autonomic response to pain, as measured through the hemodynamic response to thermal and non-thermal stimuli, differs based on the type of pain delivered.

Disclosures: **D.O. Omire-Mayor:** None. **P. Konath Alvalappil:** None. **A. Wong:** None. **K. Pourrezaei:** None.

Nanosymposium

578. Reward and Decision Making: Clinical Implications

Location: SDCC 2

Time: Tuesday, November 15, 2016, 1:00 PM - 2:45 PM

Presentation Number: 578.01

Topic: H.02. Human Cognition and Behavior

Support: CIHR Training Program in Genetics, Child Development and Health and the Alberta Children's Hospital Research Institute for Child and Maternal Health

Alberta Children's Hospital Foundation

SickKids Foundation

URGC Seed grant, University of Calgary

Title: Neural correlates of variable learning in adolescents with ASD

Authors: *M. SCHUETZE, I. CHO, S. RAHMAN, S. VINETTE, K. RIVARD, K. TEN EYCKE, A. MCCRIMMON, D. DEWEY, S. BRAY;
Univ. of Calgary, Calgary, AB, Canada

Abstract: Treatment programs for children with Autism Spectrum Disorder (ASD) show varying success rates, potentially because they rely on reinforcement learning strategies. Previous research has shown atypical neural responses towards social rewards in individuals with ASD; however, less is known about responses to restricted interests. This is surprising as restricted interests are a core symptom of ASD and highly motivating for individuals with ASD. Furthermore, few studies have investigated responses to rewarding stimuli in the context of associative learning. 27 ASD (mean age: 16.3 yrs) and 24 TD (mean age: 16.7 yrs) participants and their parents were asked about their specific interests and dislikes. Based on those responses, individual stimulus sets of high- and low-interest pictures were created. These pictures served as rewards in a learning task that participants performed while being scanned with functional MRI. In each of 120 trials, participants chose one of two differently coloured doors (4 in total) to view a picture outcome. Doors differed in their probability of showing high or low-interest pictures ("high-reward" vs. "low-reward" door) and participants learned associations implicitly. The TD group - but not the ASD group - showed significant activity in the ventromedial prefrontal cortex (vmPFC, $p = 0.017$, FDR corrected) and posterior cingulate cortex (pCC, $p=0.029$, FDR corrected) in response to high-, relative to low-interest, outcomes. However, group differences did not reach significance. Learning behaviour showed that ~50% of the ASD sample failed to learn the association, though 'non-learners' rated the outcomes equally pleasant to the 'learners'. Interestingly, region of interest (ROI) analyses on the vmPFC and pCC showed significantly less activity in the ASD non-learners, relative to ASD learners, for both regions (vmPFC, $p=0.05$; pCC, $p=0.029$). Our results suggest that variability in learning behaviors in the ASD population may be related to hypo-activity in regions known to represent stimulus value. This work is important for understanding the heterogeneity in responses to behavioral treatments in ASD, and may suggest avenues for improving treatment programs.

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Nanosymposium

578. Reward and Decision Making: Clinical Implications

Location: SDCC 2

Time: Tuesday, November 15, 2016, 1:00 PM - 2:45 PM

Presentation Number: 578.02

Topic: H.02. Human Cognition and Behavior

Support: NIMH K01 MH101326-01

NIMH R01MH095790

NIH R01AG033406

Title: Value-based decision making in hoarding and comorbid obsessive-compulsive and hoarding disorders.

Authors: *H. PUSHKARSKAYA[1], D. F. TOLIN[2], D. HENICK[1], L. RUDERMAN[1], C. PITTENGER[1], I. LEVY, 06520[1];
[1]Yale Univ. Sch. of Med., New Haven, CT; [2]Anxiety Disorders Ctr., Inst. of Living, Hartford Hosp., Hartford, CT

Abstract: Individuals with hoarding disorder (HD) and individuals with obsessive compulsive disorder (OCD) share a tendency to doubt and a general impairment with making everyday decisions. HD symptoms and OCD symptoms significantly correlate in both clinical and nonclinical samples, and up to a third of OCD patients report HD symptoms. Some practitioners and researchers have suggested that ‘‘pure’’ HD patients are distinct from those with comorbid OCD. The objective of this study was to compare value-based decision making patterns in “pure” HD with that in comorbid OCD/HD. We used a well-validated choice task, grounded in behavioral economic theory, to investigate differences in valuation and value-based choice during decision making under uncertainty in 19 unmedicated participants with HD and 19 unmedicated participants with comorbid OCD/HD. Two control groups, 19 OCD without HD and 45 healthy participants (HC) were included in the study. Individuals with comorbid OCD/HD and individuals with HD differed neither from each other nor from both control groups (OCD and HC) in how they valued uncertain options when outcome probabilities were known (risk). When these probabilities were imprecisely specified (ambiguity), individuals with comorbid OCD/HD and individuals with OCD were more likely to avoid uncertain options than individuals with HD and HC. Compared to individuals with HD and HC, individuals with comorbid OCD/HD and individuals with OCD were less consistent in their choices and less able to identify options that should be clearly preferable. This might indicate similar basic impairments in value-based decision making (i.e. assigning subjective value to available alternatives, comparing the alternatives) in individuals with OCD and individuals with comorbid OCD/HD, while in individuals with HD value based decision making appears to be intact. Future

research focused on the neural valuation network, which is implicated in value-based computations, may provide new neurocognitive insights into the pathophysiology of comorbid OCD/HD, and how it may be distinct from that of HD. Deficits in decision-making processes may represent a target for therapeutic intervention.

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Nanosymposium

578. Reward and Decision Making: Clinical Implications

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Presentation Number: 578.03

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant F32DA039648-01

NIH Grant R01DA038063-02

Title: Week-to-week fluctuations in risky decision making track heroin use in treatment-seeking opioid users

Authors: *A. B. KONOVA[1], S. LOPEZ-GUZMAN[1], A. URMANCHE[1], J. DENNISON[1], S. ROSS[2], K. LOUIE[1], J. ROTROSEN[2], P. W. GLIMCHER[1];
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Abstract: The degree to which opioid replacement therapy (e.g., methadone), the gold standard in opioid addiction management, is effective at reducing illicit opioid use depends on how well titrated it is for the current needs of an individual. However, good proximal predictors of when an individual is at risk for relapse—and therefore in need of additional clinical/pharmacological intervention—are currently lacking. Here, we use standard computationally driven decision making measurements to decompose the risk taking behavior of opioid users undergoing opioid replacement therapy, as a way to identify behavioral markers that might predict illicit opioid use. We had our subjects perform simple and easy-to-automate monetary decision making tasks weekly over several months of treatment. We established when our subjects returned to illicit opioid (or any drug) use by both self-report and urine testing. A matched sample of drug-free community controls also completed the decision making tasks. These subjects both served as a baseline control group as well as allowed us to assess the test-retest reliability of our measurements. The measurements we used are based on a standard neuroeconomic model which decomposes the behavior of each individual into two parameters: “risk attitude” and “ambiguity

attitude", indexing how sensitive that individual is to known and unknown risks, respectively. We find a high degree of test-retest reliability across the study sessions for both parameters. Attesting to the distinct aspects of risky decision making captured by these parameters, we find that only sudden increases in an opioid-dependent subject's willingness to take risks in our task correlated with, and in some cases preceded, illicit opioid use. But importantly, we find both parameters are not stationary in the opioid-dependent subjects: both parameters fluctuate as individuals approach and recover from opioid use events in a way not seen in controls. Both risk and ambiguity tolerance increases surrounding opioid use, albeit at different rates. These data suggest that risk attitudes, which can be quickly and easily measured by our behavioral tasks, might be suitable behavioral markers—and perhaps even predictors—of relapse in opioid addiction.

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Nanosymposium

578. Reward and Decision Making: Clinical Implications

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Title: The role of dopamine in dynamic connectivity during learning: evidence from Parkinson's disease

Authors: *R. T. GERRATY[1], M. SHARP[1], A. BUCH[1], D. S. BASSETT[2], D. SHOHAMY[1]; [1]Psychology, Columbia Univ., New York, NY; [2]Bioengineering, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Parkinson's patients have difficulty learning from feedback, a deficit that is consistent with a central role for dopaminergic inputs to the striatum for updating predictions during reinforcement learning. Recent evidence suggests that reinforcement learning additionally involves dynamic changes in coupling between the striatum and large-scale cortical networks. It is unclear, however, to what degree dopamine might play a role in modulating network dynamics. Here, we address this gap with two questions: (i) How do large-scale brain circuits centered on the striatum reconfigure during learning from feedback in patients with Parkinson's disease? (ii) How do dopaminergic medications modulate this reconfiguration? We tested participants with Parkinson's disease ON and OFF dopaminergic medications on a probabilistic learning task during the acquisition of multi-band fMRI data with high temporal resolution. In order to more directly probe the association between incremental learning and network dynamics, we perturbed learning partway through the task by including a single reversal of contingencies. We used tools from the emerging field of dynamic network neuroscience to obtain time-resolved descriptions of network coordination during learning as well as at rest. We predicted that Parkinson's patients OFF medications would show altered network coupling and that the degree to which network dynamics would be improved by dopaminergic medications would be associated with medication-related improvement of learning. Preliminary data support these predictions and indicate that dopamine modulates the degree to which the striatum couples dynamically with distributed brain networks. These results suggest that the established role of striatal dopamine in learning from feedback may, in part, be related to its modulatory effect on large-scale brain networks.

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Nanosymposium

578. Reward and Decision Making: Clinical Implications

Location: SDCC 2

Time: Tuesday, November 15, 2016, 1:00 PM - 2:45 PM

Presentation Number: 578.05

Topic: G.03. Emotion

Title: Acquired alexithymia disrupts reward valuation: A human lesion study

Authors: *J. HOGEVEEN[1,2], F. KRUEGER[4,5], J. GRAFMAN, 60611[1,2,3];
[1]Cognitive Neurosci. Lab., Rehabil. Inst. of Chicago, Chicago, IL; [2]Physical Med. & Rehabil., [3]Neurol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [4]Dept. of Psychology, [5]Mol. Neurosci. Dept., George Mason Univ., Fairfax, VA

Abstract: Emotional states help to shape reward processes and guide motivated behavior, but the functional role of subjective emotional experience (i.e. feelings) in reward valuation remains unclear. Alexithymia-a subclinical condition characterized by an inability to interpret and communicate one's feelings-offers a unique opportunity to establish the functions of conscious emotional experience. Here, we measured alexithymia levels and reward valuation abilities in a large sample of patients with focal brain injuries and a group of demographically-matched control participants. We computed patients' degree of damage to two regions-of-interest: anterior insula (AI; previously linked to emotional awareness), and ventromedial prefrontal cortex (vmPFC; previously linked to reward valuation). Our results suggest that pronounced AI damage impairs reward valuation indirectly via elevated levels of acquired alexithymia. In contrast, vmPFC damage was directly associated with impaired reward valuation, but did not impact emotional awareness scores. This pattern of results remained significant after controlling for a variety of potential confounds, including anxiety, depression, posttraumatic stress disorder, nonconscious emotional contagion, intelligence, and total lesion volume. Therefore, the present evidence from patients with AI damage and acquired alexithymia suggest that subjective emotional experience plays a role in shaping the reward processes that drive motivated human behavior.

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Nanosymposium

578. Reward and Decision Making: Clinical Implications

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Presentation Number: 578.06

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

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Title: Age-related factor including smoking history predicts lower response for anticipated intrinsic incentive in the ventral striatum

Authors: *Y. OGURA[1,2], Y. WAKATSUKI[1], N. HASHIMOTO[1], T. MIYAMOTO[1], Y. NAKAI[1,2], A. TOYOMAKI[1], Y. TSUCHIDA[3], S. NAKAGAWA[1], T. INOUE[4], I. KUSUMI[1]; [1]Grad. Sch. of Med., Hokkaido Univ., Sapporo, Hokkaido, Japan; [2]Japan Society for Promotion of Sci., Tokyo, Japan; [3]Ryukyu Univ., Okinawa, Japan; [4]Dept. of Psychiatry, Tokyo Med. Sch., Tokyo, Japan

Abstract: Dopamine dysfunction is an important biological background of depressive disorders. Functional brain imaging study report that depressive patients showed suppressed activation in the ventral striatum during reward anticipation (Stoy et al. 2012), which can be linked to dopamine dysfunction (Juckel et al. 2006).
On the other hand, sociopsychological factors, such as affective temperatments and childhood abuse, are also suggested to be an important background of depressive disorders (Nakai et al. 2014, Toda et al, 2015). We investigated the relationship between sociopsychological factors and functional brain response to reward anticipation.
Healthy adults (N=99; M:F=53:46) completed a behavioral task in a fMRI scanner and a questionnaire survey. As a behavioral task, we used the modified version of "monetary incentive delay task" (Knutson et al. 2000). We focused on the beta value of "\500" (the largest reward) minus "\0" (no reward) during the anticipation phase in the ventral striatum. The questionnaire survey contained 30 items, including basic demographic information, TEMPS-A (Temperament Evaluation of Memphis, Pisa, Paris and San Diego-Autoquestionnaire version; Akiskal et al. 2005) and CATS (Child Abuse and Trauma Scale; Sanders and Becker-Lausen 1995).
We identified 5 factors underlying the questionnaire data; (1) "depression", (2) "anxiety", (3) "hyperthymia", (4) "social status" and (5) "age-related". A multiple linear regression analysis revealed that higher score of age-related factor predicted lower response to reward anticipation in the ventral striatum (estimated coefficient: -0.5406, p= 0.0024). In the "age-related" factor, age (0.57), education years of mother (-0.56) and father (-0.52), and smoking history (0.48) had relatively higher factor loading. Childhood abuse had intermediate (0.25-0.31) factor loadings. Affective temperatures had low (<0.10) factor loadings in the "age-related" factor. Although the temperatures had higher factor loadings in the "depression" or "hyperthymia" factors, but neither factors had significant correlation with the response in the ventral striatum.
The result suggest that affective temperaments might have a distinct biological background from the dopaminergic system. In the clinical population, however, depressive temperature had a correlation with response for anticipated reward magnitude (Wakatsuki, personal communication). Difference in biological background between healthy and clinical population may underlie the different contribution of the depressive temperament to dopamine system.

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Eli Lilly, Eisai, Ono Pharmaceutical. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); GlaxoSmithKline, Eisai, Pfeizer, Daiichi-Sankyo, Meiji Seika Pharma, Ono Pharmaceutical, Eli Lilly. **T. Inoue:** 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; GlaxoSmithKline, Eli Lilly, Mochida Pharmaceutical, Mitsubishi Tanabe Pharma. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); GlaxoSmithKline, Pfeizer, Astellas, Eli Lilly, Mitsubishi Tanabe Pharma, Mochida Pharmaceutical, Otsuka Pharmaceutical, Meiji Seika Pharma, Asahi Kasei Pharma, Shionogi, Janssen Pharmaceutical, Takeda Pharmaceutical, Yoshitomi Pharmaceutical, Otsuka Pharmaceutical. **I. Kusumi:** 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; Takeda Pharmaceutical, Astellas, Dainippon Sumitomo Pharma, Mitsubishi Tanabe Pharma. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Eli Lilly.

Nanosymposium

578. Reward and Decision Making: Clinical Implications

Location: SDCC 2

Time: Tuesday, November 15, 2016, 1:00 PM - 2:45 PM

Presentation Number: 578.07

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant T32 MH018399

Title: Posttraumatic stress disorder is associated with exaggerated error-driven learning: a computational study probing an arousal-linked cognitive process

Authors: *J. R. HOWLETT[1], H. HUANG[2], M. P. PAULUS[2];
[1]UCSD, La Jolla, CA; [2]Laureate Inst. for Brain Res., Tulsa, OK

Abstract: Humans adjust the rate of error-driven learning (*learning rate*) based on the rate of environmental change. High learning rates imply greater reactivity to new stimuli. Learning rate is linked to norepinephrine (NE) signaling in locus coeruleus (LC) and to dorsal anterior cingulate cortex (dACC), a major LC input. Posttraumatic stress disorder (PTSD) is also linked to elevated NE signaling and dACC hyperactivity. A hyperactive dACC-LC circuit may therefore contribute to hyper-reactivity in PTSD via exaggerated error-driven learning, and this circuit dysfunction could be measured using computational methods. This study aimed to characterize error-driven learning in PTSD with the hypothesis that learning rates would be

exaggerated. 20 PTSD subjects and 32 controls performed a task in which subjects attempted to predict the location of a target stimulus. At intervals, the distribution changed such that a new location was most likely to contain the target. We calculated learning rates using the Rescorla Wagner (RW) model of error-driven learning, as well as a behavioral analogue of this learning rate. To determine whether PTSD subjects began the task with a different learning rate compared to controls, we plotted behavioral learning rate across block 1. PTSD subjects exhibited significantly higher behavioral learning rates ($p=0.0026$) and RW learning rates ($p=0.012$) than controls. PTSD subjects began block 1 with a higher behavioral learning rate than controls ($p=0.006$). PTSD is associated with a bias toward exaggerated error-driven learning. This process could serve as a computational marker for the development of treatments targeting the arousal-linked dACC-LC circuit.

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Nanosymposium

579. Executive Functions of the Human Brain

Location: SDCC 1B

Time: Tuesday, November 15, 2016, 1:00 PM - 3:45 PM

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Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust Grant WT098282

Title: Motivation by Contingent and Non-Contingent Rewards

Authors: *S. G. MANOHAR[1], D. FINZI[2], D. DREW[3], M. HUSAIN[4];
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Abstract:
Rewards motivate better performance. This often involves energising faster actions, a phenomenon known as “motivational vigour”. There are two distinct reasons why incentives might drive faster behaviour. First, they increase *expected reward*, so that time carries an opportunity cost. Second, they increase the value difference between successful and unsuccessful performance, thus introducing greater *contingency*. In previous human studies of motivation, these two aspects of vigour - stimulated by non-contingent vs. contingent incentives - have never directly been compared. The present study aimed to establish whether it is the mere presence of reward itself, or rather the need to act in order to obtain it, that controls motivational vigour.
Method:

We asked participants to shift gaze to a target as fast as possible, after hearing a cue indicating how outcomes would be determined. To index vigour, we measured the peak velocity of rapid eye movements (saccades), which we have previously shown to increase with reward (Manohar et al. 2015). In the present studies, participants heard a cue before each movement that indicated how reward would be determined. *Contingent motivation* was measured by comparing conditions in which the fastest half of trials were rewarded, with a condition in which half of trials were randomly rewarded, such that the conditions were matched for value and uncertainty. *Non-contingent motivation* was measured by comparing a condition in which reward was certain irrespective of movement speed, with a condition in which no reward was available.
Results:
When the cue indicated that reward would be contingent on performance, movements were faster than if reward was random ($p<0.05$). But even when the cue indicated that reward was guaranteed regardless of speed, movement was still faster than when no reward was available ($p<0.05$). Motivation by contingent and certain rewards was uncorrelated across individuals, consistent with the view that these two drives constitute *independent* facets of motivation. Arousal, signalled by pupil dilatation, was driven primarily by contingent motivation. In a further study, contingent *penalties* speeded movement similarly to contingent rewards ($p<0.05$), but guaranteed penalties did not. Although penalty and reward effects were tightly correlated ($p<0.05$), contingent and non-contingent effects remained distinct. Thus we isolated two separable components of motivation by incentives: performance improvements due to *reward expectation* itself, and due to reward being *contingent on performance*.

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Nanosymposium

579. Executive Functions of the Human Brain

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Support: NIH Grant DA026452

Title: Testing whether visual search involves inhibitory response control to deal with salient distractors

Authors: ***F. MARINI**, C. M. LEWIS, J. SCOTT, A. R. ARON;
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Abstract: Task-irrelevant yet perceptually salient visual stimuli that are presented during search for a target visual stimulus often capture attention and the eyes, thereby making behavioral task performance slower and more error prone. Oculomotor capture may occur automatically, but it must be kept under control during covert search tasks, in which the eyes must fixate the center while searching for a peripheral target stimulus. Here, we investigated whether this form of control involves motoric suppressive mechanisms. Specifically, we hypothesized that inhibitory motor control is recruited during covert visual search in the presence of salient task-irrelevant peripheral distractors, possibly in order to prevent oculomotor capture. Participants made foot-pedal responses to a unique shape (singleton target) among non-target shapes while ignoring a unique colored non-target (singleton distractor) that was presented on 33% of trials. The color of the distractor was alternated among eight equiluminant colors to maximize capture effects. In Experiment 1 (behavior), we validated this paradigm for inducing a singleton-distractor cost, which consisted of slower reaction times (RT) and more errors on trials with (vs. without) distractors (n=16; RT: $p<.001$, errors: $p=.05$). In Experiment 2, we probed motor excitability during covert search by delivering single-pulse transcranial magnetic stimulation (TMS) over the left motor cortex at five post-stimulus onset asynchronies (range: 110-230 ms) while measuring motor-evoked potentials (MEP) from the right hand. As responses were made with the feet, the hand was task-irrelevant, and any MEP reduction would reflect a global motor suppression process which has already been documented for outright stopping eyes, speech and hands. Behavioral results replicated the finding of a singleton-distractor cost (n=11; RT: $p<.001$, errors: $p<.05$). In the preliminary TMS analysis, contrary to our hypothesis, we did not observe any MEP reduction in presence of distractors. Although we instructed participants to maintain fixation, no eye-tracking procedure was used and thus a key issue was whether participants actually moved their eyes during the task. We are now replicating the TMS study with eye-tracking and with a larger sample of participants. Our prediction is that, on trials with a distractor where the participant does not move the eyes, there will be global motor suppression (MEP reduction from the hand) at about 140-170 ms post-stimulus onset. This would suggest the involvement of inhibitory control processes in the unfolding of events subsequent to the visual presentation of a salient, yet irrelevant, distracting stimulus.

Disclosures: **F. Marini:** None. **C.M. Lewis:** None. **J. Scott:** None. **A.R. Aron:** None.

Nanosymposium

579. Executive Functions of the Human Brain

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Title: Mind-wandering as a memory retrieval process? Behavioral, neural and modeling approaches to understanding mind-wandering

Authors: *M. K. VAN VUGT, N. BROERS, S. HUIJSER, N. TAATGEN;
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Abstract: Mind-wandering refers to thought processes that occur when people do not perform their main task. Most of the time, the content and mode of these thought processes are not specified, and very often they are even just considered as "blank" states. More recently, people have started to think about the cognitive processes that are involved in mind-wandering, considering it as an alternative "task" the participant can be doing. On the basis of this idea, we have developed a computational model of mind-wandering. Participants engage in the mind-wandering task of retrieving memories and mental simulation when this task goal becomes more important than the main task goal. This mind-wandering model can then distinguish between more narrowly-focused mind-wandering akin to rumination (which is often self-referential or focused on specific concerns) and more fluid mind-wandering (such as daydreaming) by manipulating the parameters of the mind-wandering memory retrieval process. Data from both a simple sustained attention task and a complex working memory task support the hypothesis that these two dimensions of mind-wandering exist and have different behavioral and eye tracking correlates. Preliminary ECoG data further provide support for the idea that mind-wandering is associated with memory retrieval, since it is associated with increased gamma oscillations in the hippocampus. Together, these findings help to develop a detailed theory of the cognitive processes underlying mind-wandering.

Disclosures: **M.K. van Vugt:** None. **N. Broers:** None. **S. Huijser:** None. **N. Taatgen:** None.

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NICHD R01 HD060628

Title: Prenatal factors and newborn functional brain systems predict executive functioning skills at 24-months-of-age

Authors: *A. GRAHAM[1], M. D. RUDOLPH, 97215[1], C. BUSS[2,3], J. RASMUSSEN[4], S. ENTRINGER[2,3], P. D. WADHWA[4], R. NARDOS[1], D. FAIR[1];
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Abstract: Due to the importance of executive functioning (EF) for children's mental health, and academic and social functioning, it has been recognized as an important target for early intervention. Advances in behavioral assessment allow for reliable measurement of EF by 24 months-of-age. However, brain systems underlying eventual EF skills begin to develop prenatally, and the earliest influences on EF development are poorly understood. Here, we take a data-driven approach to identify prenatal factors and properties of the newborn brain that predict emerging EF skills at 24-months. Participants were recruited in early pregnancy, and maternal biological, psychological and nutritional/health data were collected in each trimester. Resting state functional connectivity MRI (rs-fcMRI) data was collected in newborn infants during natural sleep (gestational age at birth=39.3+/-1.7 weeks, scan age=26.1+/-12.1 days). At 24-months-age children (N=67; Mean age=23.7+/-.993 months) completed laboratory EF assessments including Spin the Pots (working memory), Snack Delay (impulse control) and the EF Scale for Early Childhood (task switching). We used partial least squares regression (PLSR) combined with cross-validation to generate and test predictive models of EF skills in independent training and test sets. Prediction based on maternal factors during pregnancy was well above chance for impulse control and working memory (working memory *mean r=0.331*, impulse control *mean r=0.289*). Robust maternal psychological and biological predictors included 1st trimester pregnancy specific distress, 3rd trimester general psychological stress, and 3rd trimester inflammation (indexed by maternal interleukin-6 concentrations). Maternal nutritional/health factors, and specifically regular exercise and percentage of dietary saturated fat, were the strongest predictors. Prediction of 24-month EF skills based on newborn rs-fcMRI data was also above chance for several brain systems. For example, dorsal attention and salience system connectivity significantly predicted 24-month impulse control. Taking a data-driven approach and employing predictive modeling represents an important step towards identifying the earliest risk and protective factors for healthy development of EF. It is possible to predict emerging EF skills in young children based on prenatal factors, and the status of the brain at birth. The next phase of this work involves testing these models on an independent dataset, and identifying robust predictors across samples. This will inform focused preventive interventions to improve children's mental health and school readiness.

Disclosures: **A. Graham:** None. **M.D. Rudolph:** None. **C. Buss:** None. **J. Rasmussen:** None. **S. Entringer:** None. **P.D. Wadhwa:** None. **R. Nardos:** None. **D. Fair:** None.

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Topic: H.02. Human Cognition and Behavior

Support: FAPERJ 111.429/2014, 111.529/2013, 111.030/2013

Title: Neuropsychological correlates of self-kindness on late adolescence: increased cognitive flexibility and emotional regulation.

Authors: ***N. MOTA**, E. ROCHA, M. ANTUNES, R. BORGES, V. DAUDT;
Fundaments of Psychology, Univ. Do Estado Do Rio De Janeiro, Rio de Janeiro, Brazil

Abstract: Background: Self-kindness involves feelings of care and non-judgmental understanding towards oneself in response to situations of inadequacy or suffering. Its training has showed positive effects on different psychiatric conditions (depression, anxiety and eating disorders), however its related neuropsychological mechanisms remain unclear. As it implies positive response upon situations that trigger negative emotion, it is hypothesized that kindness is a self-regulatory process, that requires cognitive flexibility and emotional regulation, both dependent on the medial frontal cortical and subcortical activation, still under development during late adolescence.
Methods: Forty six students (18 - 21 years-old) from different courses of the University of the State of Rio de Janeiro attended a clinical interview and a comprehensive neuropsychological assessment, which included the Wisconsin Card Sorting Task (WCST) and the Stroop (measures of cognitive flexibility), as well as the Emotional Regulation Task. Exclusion criteria included: acquired brain injury or neurological disorder, pre- and perinatal complications, psychiatric disorders (DSM-V), regular/intensive drug consumption, and uncorrected sensorimotor deficit. Partial correlations (2-tailed) were performed, controlling for social desirability (Marlowe-Crowne Social Desirability Scale).
Results: Higher self-kindness increased the possibility of higher adaptive choices according to the circumstances (WCST % Conceptual Level Responses, $r = .352$, $p = .021$; WCST % Errors, $r = -.329$, $p = .031$), and was not related to the inhibition of the response to predominant but irrelevant information (STROOP Interference Score, $r = -.203$, $p = .198$). When exposed to emotionally negative images, although participants who reported more self-kindness didn't present differentiated levels of negative emotional activation, $r = -.294$ $p = .115$, they were more prone to effectively diminish it deliberately $r = -.412$, $p = .024$.
Discussion: Self-kindness on late adolescence might increase the proneness to the goals achievement, through a favorable interaction with the environmental unforeseen or unpleasant circumstances. Future perspectives include its consideration as a potential protective factor

against the neurocognitive manifestation of neuropathologic processes, as well as a resource for neurorrehabilitation.

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Dana Foundation

Title: Action potential-theta coherence encodes conflict in the human dorsolateral prefrontal cortex.

Authors: *E. H. SMITH[1], C. B. MIKELL[1], T. G. DYSTER[1], S. L. PULLMAN[2], Q. YU[2], G. M. MCKHANN, Jr.[1], S. A. SHETH[1];
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Abstract: Cognitive control processes have been associated with theta-range EEG activity arising from the dorsal anterior cingulate cortex (dACC) in humans. The neural mechanisms underlying cognitive control are still poorly understood. Specifically, there is still debate about where cognitive control signals originate, and little is known about the neuronal computations underlying cognitive control. We hypothesized theta oscillations entrain dorsolateral prefrontal cortex (dlPFC) neuronal firing during increased cognitive demand.
We recorded single neuron and local field potential (LFP) signals in the dlPFC of patients undergoing deep brain stimulation surgery for movement disorders while they performed the Multi-Source Interference Task, a Stroop-like task characterized by multiple levels of conflict. We examined firing rate coding of conflict using an adaptive-kernel firing rate estimate, and temporal coding of conflict using spike-field coherence for LFP frequencies ranging from 0.3 to 50 Hz using multi-taper methods. We isolated 32 neurons over nine subjects and examined stimulus-evoked activity. Of those neurons, 20 exhibited no significant firing rate change, nine exhibited significant firing rate suppression, and three exhibited significant firing rate enhancement, when compared with a baseline period (Mann-Whitney U test, $p < 0.01$). Only two neurons, both with enhanced firing, exhibited significantly different firing rates across conflict

levels (ANOVA, $p < 0.01$). Upon examining stimulus-evoked spike-field coherence, however, we found that 20 neurons exhibited significantly more theta coherence for high conflict trials (shuffle test, 1000 permutations, $p < 0.01$). These results suggest temporal coding is more widespread and informative than rate coding in human dlPFC. Our previous work showed information transfer from dACC to dlPFC. Entrainment of neuronal firing in dlPFC may thus be an electrophysiological mechanism by which dACC instantiates control processes in dlPFC.

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Topic: H.02. Human Cognition and Behavior

Support: National Natural Science Foundation of China (Grant No. 31471068)

Title: Dissociated functions of prefrontal cortex in metacognition

Authors: ***X. WAN**, L. QIU, Y. NI, Y. BAI, J. SU, X. LI;
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Abstract: Metacognition is referred to as cognition about cognition, a high-level cognitive control process of monitoring and controlling the foregoing cognitive processes. Although the prefrontal cortex has been proposed to play critical roles in metacognition, its neural mechanism of metacognition remains unknown. Here we designed a novel experimental paradigm of "decision-redecison" to separate the foregoing cognitive processes and the metacognitive processes. There were two tasks: one was the rule-based decision (Sudoku) task, and the other was the perceptual decision (random dot motion, RDM) task. The subjects made a decision and then once again on the same problem. We found the inferior frontal junction (IFJ) was actived during the decision phase; the frontoparietal network was actived during the redecision phase, but not during the decision phase in both tasks using fMRI. The frontoparietal network consisted of frontopolar cortex (FPC), DLPFC, ACC, anterior insula cortex (AIC) and intraparietal loble (IPL). The activities of these areas were correlated with foregoing decision uncertainty, and were also associated with performance adjustment. The AIC and ACC activities were correlated with the subjects' uncertainty sensitivity in both tasks, but the FPC activities were correlated with the subjects' accuracy improvement only in the Sudoku task. Therefore, AIC and ACC was mainly associated with uncertainty monitoring across both tasks, but FPC was associated with

performance adjustment only in the Sudoku task. To dissect the dynamic processes of metacognition, we concurrently caried out EEG experiments. We found alpha-band (8-12 Hz) appeared in the IFJ area during the deicison phase and theta-band (4-8 Hz) appeared in the ACC area during confidence phase in both tasks, while beta-band (20-30 Hz) appeared in the FPC area during redecision phase only in the Sudoku task. The EEG results again demonstrated dissociated functions of prefrontal cortex in metacognition. To further test the causality of prefronal cortex in metacogntion, we systematically interferenced these areas using online-TMS. At the IFJ area the resposne time of decision was delayed; At the ACC area, the resposne time of confidence reporting became faster; At the FPC area, the accuracy improvement during the redecicson phase was impaired in the Sudoku task, while at the DLPFC area there were no effects oberserved. Taken together, our results show that the prefrontal cortex in metacognition was dissociated. The IFJ was only involved in cognitive process; the ACC (and AIC) was involved in uncertainty monitoring, and the FPC was involved in performance adjustment.

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Nanosymposium

579. Executive Functions of the Human Brain

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Topic: H.02. Human Cognition and Behavior

Title: Investigating the sub-regional functional organization of prefrontal cortex

Authors: ***S. L. COOKSON**, E. H. SCHUMACHER;
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Abstract: Prefrontal cortex (PFC) is involved in many cognitive processes important for complex, flexible human behavior (Duncan & Owen, 2000). Recent research has posited at least two axes of functional organization in PFC: a rostro-caudal axis, along which the PFC processes tasks of varying abstractness or complexity (Badre 2008); and a dorso-ventral axis, along which the PFC handles spatial versus nonspatial task information (Goldman-Rakic, 1995). However, it remains unclear how these two axes may interact with one another, as well as with other known organizational principles in PFC (viz., lateralization of motor control). The present experiment aimed to address these questions in a novel “hierarchical precuing” task that combined a traditional cuing procedure with a hierarchical mapping structure in an event-related fMRI design. Participants made one of four possible judgments about pairs of stimuli based on simple spatial and nonspatial characteristics shared by the pair. Two judgments related to spatial

features of the stimuli, and two to nonspatial features. One spatial judgment and one nonspatial judgment were mapped to each hand. Cues presented at the start of each trial allowed participant to prepare sets of different levels of complexity based on whether they received information about the upcoming judgment type, response hand, both, or neither. The cues produced a stair-step effect on reaction time as a function of the amount of information presented a priori. The fMRI data demonstrated segregation of activity in PFC at the cue time point for the main effects of each factor: a rostro-caudal distribution as a function of cue information; a dorso-ventral distribution according to judgment domain; and lateralization of activity as a function of response hand. We then investigated how different combinations of the amount of cue information, the domain of task processing, and the response hand interacted to influence the distribution of activity within these regions of interest. These results demonstrate how the functional structure of the PFC integrates these different axes across the cortex and validate the hierarchical precuing task as a procedure for integrating multiple cognitive factors into a single event-related task design.

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Nanosymposium

579. Executive Functions of the Human Brain

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Topic: H.02. Human Cognition and Behavior

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Title: Neurophysiological markers of conscious detection of partial errors and their correction

Authors: *S. C. FICARELLA, N. ROCHET, B. BURLE;
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Abstract: In a constantly changing environment, cognitive control is necessary to flexibly adjust our behavior to perform goal-directed actions. In conflicting situations, environmental stimuli can elicit unwanted responses that can be tracked, with a millisecond resolution with Electromyography (EMG) (Hasbroucq et al., 2001, *Psychophysiology*; Burle et al., 2002, *Psychol. Res.*). Such subthreshold motor activations, called *partial errors* need to be suppressed to perform the correct action. Rochet et al. (*Cogn Affect Behav Neurosci*, 2014) showed that most partial errors (about 70%) remain undetected by subjects. The aim of this study is understand what differentiate detected from undetected partial errors, and investigate the mechanisms

underlying their conscious detection. We used high density EEG, coupled with EMG, while participants (N=18) performed a conflict task (Simon task). At the end of each trial, subjective confidence of having committed a partial error was orally collected. Behavioral results replicate the findings of Rochet et al. (2014). EEG data show two main results: a partial error conscious detection-dependent modulation of the *Error-Negativity* (Ne, or ERN) and of the amplitude of the contralateral primary motor cortex (M1). The Ne, an electrophysiological component associated to error detection, was also found in partial errors (Bonini et al., 2014, *Science*; Burle et al. 2008, J. Cognitive Neurosci.). The amplitude of this component was significantly smaller in undetected, compared to detected, partial errors. Just before partial errors, the M1 contralateral to the incorrect motor activation is transiently activated. Importantly, its activity is reduced for undetected partial error, specifically 40ms following the onset of the partial error. This result suggests that the motor commands responsible for detected and undetected partial error generation are initially the same, but undetected partial errors are interrupted earlier than detected ones. Hence, detected partial errors elicit a stronger M1 activation and they are inhibited later than undetected partial errors. This M1's modulation could be at the origin of the partial error (un)detection. In fact, motor activations elicit an *efference copy* (Angel, 1976, *Neurology*) which, in the case of partial errors, is stronger for detected than undetected ones. The *efference* copy is sent to the Supplementary Motor Area (SMA), the main generator of the *Ne* (Bonini et al., 2014). When incorrect M1 activity is stronger, SMA is more strongly activated through the *efference* copy and hence generates a larger alarm signal (*Ne*), resulting in an increased likelihood of partial errors conscious detection.

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Nanosymposium

579. Executive Functions of the Human Brain

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Presentation Number: 579.10

Topic: H.02. Human Cognition and Behavior

Title: An fronto-basal ganglia inhibitory control mechanism delays response emission during response conflict

Authors: *J. R. WESSEL[1,2], J. D. GREENLEE[3];
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Abstract: The ability to adapt ongoing behavior to situational demands is key to cognitive control. For example, when two incompatible actions are simultaneously triggered (e.g., in the

Simon task), motor responding is delayed to resolve the ensuing response-conflict. The neural mechanism underlying this motor delay is unknown. We hypothesized that during response-conflict, a well-characterized neural mechanism for motor inhibition delays the initiation of motor activity. This inhibitory control mechanism is usually studied in the stop-signal task (SST), where it allows rapidly cancelling motor responses following stop-signals. Crucially, this mechanism involves a purported hyperdirect pathway that connects fronto-central cortical regions with the subcortical basal ganglia, specifically the subthalamic nucleus (STN). In the current study, we present two experiments. In Experiment 1, we measured the human scalp-encephalogram during Simon and stop-signal tasks, which we analyzed using independent component analysis. From the SST, we identified a well-characterized fronto-central independent component (IC) that reflects motor inhibition. Crucially, this same IC was active during delayed response emission on incongruent trials in the Simon task. Furthermore, its single-trial activity, specifically in the theta-band (5-8Hz), predicted delayed sensorimotor activity (namely, the onset of mu-suppression over contralateral M1) on such trials. In Experiment 2, we measured subcortical STN activity using depth electrodes and cortical M1 activity using subgaleal electrodes from Parkinson's patients undergoing deep-brain stimulation surgery. These data show that the STN is also active during incongruent Simon-task trials. Similar to the fronto-central scalp component in Experiment 1, increased STN activity on individual incongruent trials predicted the delayed onset of mu-suppression over contralateral M1. Taken together, this study shows that a universal brain mechanism for response inhibition is recruited to delay the onset of motor activity during response-conflict. We hypothesize that this happens via a fronto-basal ganglia hyperdirect pathway involving the mesial frontal cortex and the STN.

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R01 MH106700-01A1

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Title: Conflict sensitivity in human anterior cingulate cortex revealed by intracranial local field potentials

Authors: *M. YATES, E. SMITH, S. SHETH;
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Abstract: Cognitive control refers to the ability to flexibly adjust behavior and information processing in the service of goal achievement. The prefrontal cortex (PFC) is believed to play a central role in cognitive control, however there is debate regarding the contributions of different regions within the PFC. Conflict Control Loop Theory (Krug & Carter, 2012) proposes that medial and lateral PFC work together to achieve cognitive control. The role of medial PFC is to detect conflict (e.g. the existence of two competing potential responses) which indicates a need for cognitive control. This information is then transmitted to lateral PFC, which implements cognitive control. This theory predicts, firstly, that medial PFC should be sensitive to level of conflict, and secondly, that conflict-related activity should emerge earlier in medial PFC compared to lateral PFC. We tested these predictions using intracranial recordings in four human subjects. Local field potentials were simultaneously recorded from multiple sites within anterior cingulate cortex (ACC, n =14) and dorsolateral PFC (DLPFC, n = 14) while patients performed the Multi Source Interference Task, a number-based Stroop-like task with high and low conflict trials. In high conflict trials, correct responding requires inhibition of pre-potent response tendencies. The first prediction of the Conflict Control Loop Theory was confirmed by our data - event-related potentials at ACC sites showed differential activity for high versus low conflict trials. The second prediction, however, was not supported - conflict-differentiated responses did not emerge earlier at ACC sites compared to DLPFC sites.

Disclosures: **M. Yates:** None. **E. Smith:** None. **S. Sheth:** None.

Nanosymposium

580. Advanced Imaging Methods and Probes

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Title: Two-photon imaging of neuronal voltage dynamics using genetically encoded voltage indicators

Authors: *F. ST-PIERRE[1,2,3], H. Y. YANG[3], S. CHAMBERLAND[4], M. PAN[3], M. CHAVARHA[3], Y. YANG[3], C. SALESSE[4], H. WU[3], J. C. WU[3], K. TOTH[4], T. R. CLANDININ[3], M. Z. LIN[3];
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Abstract: A longstanding goal in neuroscience is to understand how spatiotemporal patterns of neuronal electrical activity underlie brain function, from sensory representations to decision making. An emerging technology for monitoring electrical dynamics — voltage imaging using genetically encoded voltage indicators (GEVIs) — couples the power of genetics with the advantages of light. We recently reported a novel GFP-based GEVI, Accelerated Sensor of Action Potentials 1 (ASAP1), and showed it can report electrical activity in vitro and in brain slices using one-photon illumination. Given that two-photon microscopy has established itself as a key method for deep-tissue imaging of neural activity, we sought to characterize the performance of ASAP1 and ASAP1-derived sensors under two-photon illumination. We report that the ASAP family of sensors enables two-photon imaging of action potentials and subthreshold potentials in brain slices, and of graded electrical activity in *Drosophila* axonal terminals. We further report ASAP2, an ASAP1 variant with improved sensitivity. We anticipate that these results will inspire two-photon voltage imaging studies aimed at understanding the neural processes that underlie behavior.

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Presentation Number: 580.02

Topic: I.04. Physiological Methods

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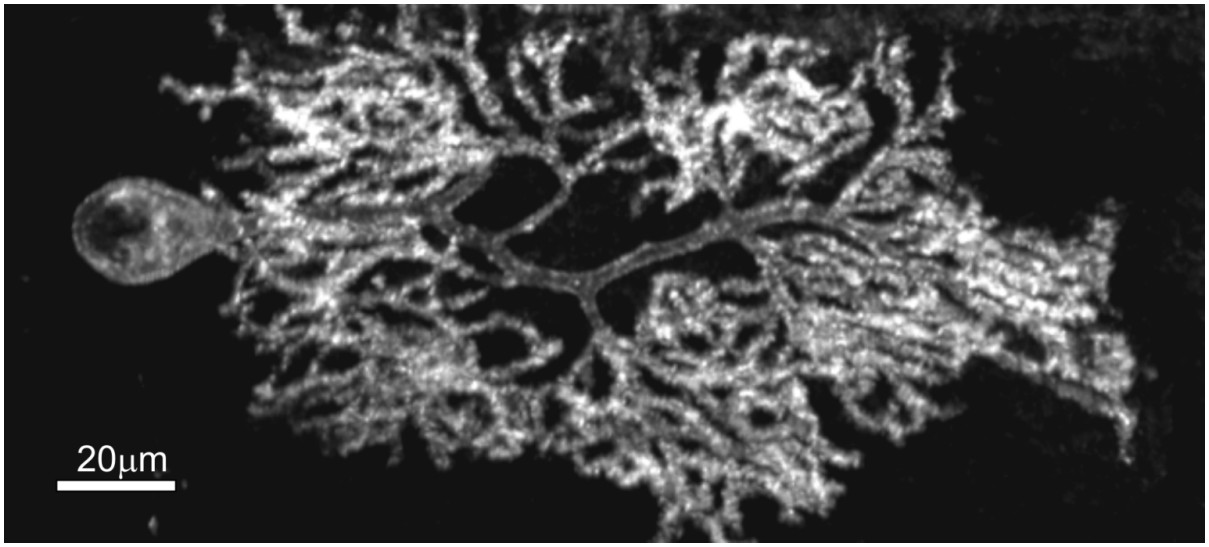
Title: Genetically encoded voltage indicator imaging of GABAergic cell classes in the mouse brain

Authors: *C. SONG[1], E. POWER[2], K. POTAPOV[2], D. M. PISCOPO[3], C. NIELL[3], R. M. EMPSON[2], T. KNOPFEL[1];
[1]Med., Imperial Col. London, London, United Kingdom; [2]Dept. of Physiol., Univ. of Otago, Dunedin, New Zealand; [3]Univ. of Oregon, Eugene, OR

Abstract: GABAergic neurons contribute to diverse neurophysiological and behavioural functions despite constituting only approximately 20-30% of the neuronal populations in the brain. The functional roles of GABAergic neurons in shaping cerebral and cerebellar circuit dynamics had been technically difficult to investigate using traditional imaging approaches. The fast spiking properties and subthreshold activity of cortical GABAergic neuron populations and constant, rhythmic firing of cerebellar Purkinje neurons means that satisfactorily detecting their behaviour using the popular calcium imaging approach is challenging. Therefore, directly monitoring the voltage activity of these cells is necessary. Furthermore, in the cortex and cerebellum, classical voltage-sensitive dye imaging is dominated by activity from the glutamatergic neuronal population. Genetically encoded voltage indicators (GEVIs) targeted to GABAergic cell population offer a solution to this problem.
Here we express two GEVIs (VSFP Butterfly 1.2 and chimeric VSFP Butterfly) in mice to successfully use optical imaging to monitor GABAergic interneuron circuit dynamics in the brain. We first establish transgenic approaches to achieve controlled targeted indicator expression in defined GABAergic neuronal populations. Two strategies created specific expression of VSFP Butterfly 1.2 in cerebellar Purkinje cells (VGat-Cre; Pcp2-tTA; Ai78, Figure), and of chimeric VSFP Butterfly in GABAergic neurons across cortical layers (VGat-Cre; ztTA; chiVSFP).
We performed *ex vivo* and *in vivo* imaging respectively from these mice. *Ex vivo* imaging from sagittal cerebellar slices demonstrated robust parallel fibre and climbing fibre synaptic responses specifically from cerebellar Purkinje neuron dendrites. *In vivo* voltage imaging of cortical GABAergic neuronal population demonstrated distinct circuit dynamics during pentobarbiturate-induced slow wave sleep.



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Nanosymposium

580. Advanced Imaging Methods and Probes

Location: SDCC 7B

Time: Tuesday, November 15, 2016, 1:00 PM - 4:15 PM

Presentation Number: 580.03

Topic: I.04. Physiological Methods

Support: Brain Initiative grant

Title: Selective targeting of a genetically encoded voltage indicator to label neuronal soma and proximal processes

Authors: *M. CHAVARHA[1], M. Z. LIN[2];
[1]Bioengineering, Stanford Univ., palo alto, CA; [2]Neurobiology, Bioengineering, Stanford Univ., Palo Alto, CA

Abstract: To unravel complexities of the mammalian brain we need tools to follow electrical activity of large defined populations of neurons in behaving animals over long periods of time. Genetically encoded voltage indicators optically report direct changes in membrane potential. However, the membrane localized nature of GEVIs poses a challenge for AP detection and timing. Recently-reported novel class of GEVIs, ASAP1, is efficiently targeted to the membrane and densely labels the neuropil. To study the individual firing patterns of neurons with high

temporal precision, it would be desirable to concentrate ASAP1 signal in the easily identifiable cell body. We have created fusions of ASAP to a soma targeting domain from an ion channel and show the enrichment of fusions in the soma and proximal processes in neurons. The new soma-targeted GEVIs will allow higher signal/background ratios with denser GEVI expression in vivo, particularly with one-photon imaging.

Disclosures: M. Chavarha: None. **M.Z. Lin:** None.

Nanosymposium

580. Advanced Imaging Methods and Probes

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Time: Tuesday, November 15, 2016, 1:00 PM - 4:15 PM

Presentation Number: 580.04

Topic: I.04. Physiological Methods

Support: NIH Grant DC005259

NIH Grant DC012981

WCI 2009-003

Title: The olfactory bulb contributes to the concentration invariance of odor perception

Authors: *D. A. STORACE[1], L. B. COHEN[1,2];
[1]Cell. and Mol. Physiol., Yale Univ., New Haven, CT; [2]Ctr. for Functional Connectomics, Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: The goal of the present study was to identify the function(s) of the olfactory bulb by comparing its output with its input. The input olfactory receptor neurons were anatomically targeted via nasal infusion with an organic calcium sensitive dye. In the same preparation, genetically encoded voltage or calcium indicators were targeted to the output mitral and tufted cells using Cre-dependent AAV transduction in a transgenic mouse (Pcdh21) that expresses Cre recombinase in mitral and tufted cells. Wide-field epifluorescence imaging was used to measure odor-evoked activity in glomeruli across ~2 log units of odorant concentration in freely breathing anesthetized mice. Remarkably, the output maintained a relatively stable representation of odor quality over the tested concentrations, while the number of activated input glomeruli and the input amplitude markedly declined. This provides the first direct evidence that the mammalian olfactory bulb participates in generating the perception of concentration invariance of odor quality. These imaging methods should also be useful for determining the transform from input to output in other regions of the mammalian brain.

Disclosures: **D.A. Storace:** None. **L.B. Cohen:** None.

Nanosymposium

580. Advanced Imaging Methods and Probes

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NIH grant R21 NS081507

Stanford Interdisciplinary Graduate Fellowship for H.H.Y.

Allen Foundation (M.Z.L.)

Title: Subcellular imaging of voltage signals reveals neural processing *In vivo*

Authors: ***H. H. YANG**[1], F. ST-PIERRE[2], M. Z. LIN[1], T. R. CLANDININ[1];
[1]Dept. of Neurobio., Stanford Univ., Stanford, CA; [2]Dept. of Neurosci. and Dept. of Electrical and Computer Engin., Baylor Col. of Med. and Rice Univ., Houston, TX

Abstract: A mechanistic understanding of neural computation requires determining how information is processed as it passes through neurons and across synapses. However, it has been challenging to measure membrane potential changes in axons and dendrites *in vivo*. We use *in vivo*, two-photon imaging of ASAP-family genetically encoded voltage indicators to measure sensory stimulus-evoked signals in the *Drosophila* visual system with subcellular resolution. We observe major transformations in the kinetics, amplitude, and sign of voltage responses to light. With molecular and circuit manipulations, we are dissecting the mechanisms that implement this processing of visual information. By combining voltage imaging with genetic manipulations, we illuminate where and how critical computations arise.

Disclosures: **H.H. Yang:** None. **F. St-Pierre:** None. **M.Z. Lin:** None. **T.R. Clandinin:** None.

Nanosymposium

580. Advanced Imaging Methods and Probes

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Presentation Number: 580.06

Topic: I.04. Physiological Methods

Support: ERC 322699

NIH GM085808

Title: The precision and accuracy of time super resolution imaging by event correlation microscopy

Authors: *Q. FANG[1], Y. ZHAO[1], M. LINDAU[1,2];
[1]Max-Planck Inst. For Biophysical Chem., Goettingen, Germany; [2]Applied Engin. Physics, Cornell Univ., Ithaca, NY

Abstract: The time resolution of fluorescence imaging is limited by the exposure time and readout time of the camera. Although many biological events are very fast on the time scale of ~1 ms, due to the weak fluorescence signal, the exposure times are typically 100-200 ms, much longer than many time scales of interest. The problem is somewhat similar to that in localization microscopy where a fluorescent spot needs to be determined with a precision that is much higher than that of camera pixels size. To beat the time resolution of the image frames, we developed an ECOM (Event COrrelation Microscopy), taking advantage of the fact that for an intensity step change the intensity in a certain region of interest reported by an imaging frame depends on the time at which the intensity change occurred. To do the ECOM assay, intensity traces with 1 ms per point were constructed for each image recording assigning the intensity of a given frame to all points spanning this frame. Averaging a certain number of recordings leads to an average intensity change that spans the time of two frames reaching 50% of the intensity change at the time of the intensity step.
To determine the accuracy of the ECOM method, its results were compared to direct high time resolution measurements using high intensity test signals generated by a laser shutter and a fura-2 calcium signal change after a short stimulation pulse. In the laser shutter experiment, the delay between the shutter trigger pulse and opening of the shutter determined by the ECOM method from 100 ms imaging frames was ~14ms, very close to that of a fast photodiode measurement (~13 ms). Using ECOM the 50% time point a fura-2 fluorescence signal change was found to occur 15 ms after onset of a 10-ms stimulation pulse, which was confirmed by rapid imaging using a very small region of interest. At high signal-to-noise ratio the accuracy of the ECOM method is thus ≤1 ms. The dependence of the precision of ECOM analysis on the signal to noise ratio was determined using computer simulations. For a 200-ms exposure time imaging experiment, the precision (SD) of ECOM timing reaches 10 to 2 ms for a signal to noise ratio

range of 20-100, respectively.
The optimal choice of exposure time depends on the signal-to-noise ratio of the fluorescence image, where the signal is the time dependent intensity change. At low signal-to-noise ratio the precision of time resolution obtained with ECOM was found to deteriorate considerably for exposure times <100 ms. ECOM thus allows to optimize time superresolution information based on the choice of exposure times and number of averages used in the analysis.

Disclosures: **Q. Fang:** None. **Y. Zhao:** None. **M. Lindau:** None.

Nanosymposium

580. Advanced Imaging Methods and Probes

Location: SDCC 7B

Time: Tuesday, November 15, 2016, 1:00 PM - 4:15 PM

Presentation Number: 580.07

Topic: I.04. Physiological Methods

Support: HHMI

Title: Megapixel two-photon imaging at kHz framerates

Authors: ***K. PODGORSKI**;
Janelia Res. Campus, HHMI, Ashburn, VA

Abstract: To image neuronal and synaptic activity over large volumes in awake mice, we have developed a high speed two-photon microscope that takes advantage of compressive sensing. Unlike random access microscopy, this approach is insensitive to sample motion and requires no preselection of areas of interest. The microscope obtains diffraction-limited images of over 1 million pixels at framerates over 1 kHz, while maintaining the optical sectioning and insensitivity to scattering of conventional two-photon imaging. The degree of compression and input power requirements are easily scaled over a wide range while maintaining diffraction-limited resolution. We present the optical design and image reconstruction methods as well as several applications of the microscope to particle tracking and neuronal activity imaging in single planes at >1kHz and over large 3D volumes at >20Hz.

Disclosures: **K. Podgorski:** None.

Nanosymposium

580. Advanced Imaging Methods and Probes

Location: SDCC 7B

Time: Tuesday, November 15, 2016, 1:00 PM - 4:15 PM

Presentation Number: 580.08

Topic: I.04. Physiological Methods

Support: LFF 'ReAdMe'

Title: Hybrid circuits for optical probing of axon activity via semiconductor micro tubes

Authors: *R. H. BLICK[1,2], A. KOITMAE[1,2], J. HARBERTS[1,2], M. MUELLER[1,2], C. BAUSCH, PhD[1,2], R. ZIEROLD[1,2], W. HANSEN[1], C. HEYN[1], G. LOERS[3];
[1]Univ. of Hamburg, Hamburg, Germany; [2]Ctr. for Hybrid Nanostructures, Hamburg, Germany; [3]Universitaets Klinikum Eppendorf (UKE), Hamburg, Germany

Abstract: The challenge in retrieving data from axons via semiconductor probes it to adhere to the 3-dimensional environment of the brain. Typically, most of the devices suggested employ electrical feedback methods to detect action potentials (APs), such as microelectrode arrays (MEAs) for 2-dimensional probing of APs. However, for over a decade the emerging research field of optogenetics has created new promising wireless devices for neural stimulation and activation detection. We present a combined approach for local guided adhesion, assisted growth across a large area and probing of synthetic neural circuits via optically active semiconductor microtubes. The electric fields caused by the alteration of the membrane potential during AP generation propagate in the wall of the microtube thus changing the band structure of the semiconductor and the emission wavelength (WL) of the microtube. Our simulations and first data provide support that a shift of about 2 nm in the emission WL of during AP propagation suggesting a promising device for wireless AP detection.

Disclosures: **R.H. Blick:** None. **A. Koitmae:** None. **J. Harberts:** None. **M. Mueller:** None. **C. Bausch:** None. **R. Zierold:** None. **W. Hansen:** None. **C. Heyn:** None. **G. Loers:** None.

Nanosymposium

580. Advanced Imaging Methods and Probes

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Topic: I.04. Physiological Methods

Support: European Research Council (ERC) grant #641171

Israel Science Foundation (ISF) grant #1725/13

Title: Advanced multiphoton methods for in-vitro and in-vivo functional imaging of mouse retinal neurons

Authors: *S. SHOHAM[1,2], A. SCHEJTER BAR-NOAM[1], N. COHEN[1], N. FARAH[3]; [1]Technion, Haifa, Israel; [2]Neurosci. Inst., NYU, New York City, NY; [3]Bar Ilan Univ., Ramat Gan, Israel

Abstract: Studying the responses of retinal ganglion cell (RGC) populations is of major importance to the fields of neuroscience and vision research. Multiphoton excitation of optogenetic probes has specific advantages for imaging retinal activity during visual stimulation, because it leads to reduced direct excitation of the photoreceptors. However, this method is not straightforward: point-by-point scanning leads to repeated neural excitation, while optical access through the rodent eye for *in vivo* imaging has proven highly challenging. Here, we present two enabling optical solutions that facilitate multiphoton imaging of responses to visual stimuli in genetically transduced mouse retinas expressing GCaMP6 calcium indicators. First, we present the first demonstration of two-photon imaging of RGC activity in the live mouse retina. Following a design based on optical modeling, we were able to obtain images of retinal structures with sub-cellular resolution and without the need for incorporating adaptive optics. Our system can remotely depth-scan the retina without translating any of the microscope parts by utilizing an electronically tunable lens (ETL) controlled by the computer. The properties of two-photon excitation, together with our optical design, enable to obtain high-resolution images at different depths. Next, we present an imaging solution based on Scanning Line Temporal Focusing (SLITE) for very rapidly imaging retinal neuronal activity. In this technique, the retina is scanned with a temporally focused line rather than a point, increasing the scan speed and reducing the impact of repeated excitation, while maintaining high optical sectioning. SLITE is used to capture the responses of RGCs populations *in vitro* to different visual patterns.
In sum, the new optical designs presented here overcome a number of outstanding obstacles, allowing the study of rapid calcium signals both *in vitro* and *in vivo*, thereby bringing us a step closer toward distributed monitoring of retinal neural activity during vision.

Disclosures: **S. Shoham:** None. **A. Schejter Bar-Noam:** None. **N. Cohen:** None. **N. Farah:** None.

Nanosymposium

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Support: KIBM Innovative Research Grant 2014-010

Qualcomm Institute's Calit2 Strategic Research Opportunities Program CITS145

Title: Flyception: imaging brain activity in freely walking fruit flies

Authors: *D. GROVER, T. KATSUKI, R. J. GREENSPAN;
Kavli Inst. For Brain and Mind, LA Jolla, CA

Abstract: The development of optically detectable calcium and voltage indicators has made it possible to record neural activity from large numbers of neurons at high temporal resolutions. However, linking neural activity to behavior remains a challenge, because most existing imaging paradigms and model organisms suffer from at least one of the following issues: 1) behaviorally restrained subjects, 2) poor coverage of the brain, or 3) limited behavioral and cognitive complexity. Consequently, it remains largely unknown how complex brain functions such as memory, sensations, emotions, and other fundamental behaviors arise from the coordinated actions of large numbers of neurons.

Here, we describe flyception, a system that enables imaging of brain activity from unrestrained freely walking *Drosophila*, approximately resolving all of the above-mentioned issues. To gain optical access to the brain, we created a chronic imaging window on the dorsal side of the fly head by removing the cuticle and sealing the opening with biologically inert clear silicon. This window provides a view of the protocerebrum, the dorsal half of the fly brain that includes antennal lobes, central complex, and the mushroom bodies. A fly with an imaging window is placed in a concave elliptical arena surrounded by a water-filled moat and allowed to walk freely and voluntarily interact with other flies. We developed a galvanometer-mirror based tracking system that accurately locates and stabilizes the brain of the walking fly on multiple imaging sensors at 1000 Hz, allowing for imaging of calcium dynamics at 1x optical magnification. We will demonstrate that our system robustly measures neuronal activity during sensory (odor responses) and socially-evoked behaviors (courtship), from as few as ~20 neurons per hemi-brain. We believe that this technology shifts our methodological paradigm of brain imaging and behavioral neuroscience by allowing for the study of neural activity in naturally behaving flies.

Disclosures: D. Grover: None. **T. Katsuki:** None. **R.J. Greenspan:** None.

Nanosymposium

580. Advanced Imaging Methods and Probes

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Support: National Natural Science Foundation of China 81227901

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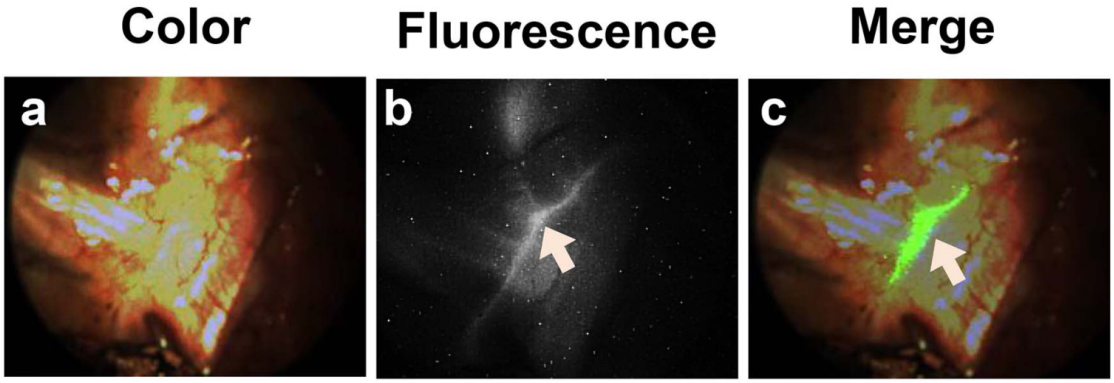
Title: High-sensitivity anatomical imaging of thoracic sympathetic nerve with a novel fluorescence thoracoscopic system

Authors: *C. CHI[1,2], Y. MAO[2], K. HE[2], F. YANG[3], J. ZHOU[3], H. HUI[2], X. YANG[1], J. TIAN[2]; [1]Inst. of Automation, Chinese Acad. of Scienc, Beijing City, China; [2]Key Lab. of Mol. Imaging, Inst. of Automation, Chinese Acad. of Sci., Beijing, China; [3]Dept. of thoracic surgery, Peking university people's hospital, Beijing, China

Abstract: Introduction: In thoracic surgery, accidental thoracic sympathetic nerve injury leads to facial paralysis, which seriously affects the patients' quality of life. Gross appearance and anatomical location is the traditional methods. However, it is hard to avoid iatrogenic damage of nerves due to the similar appearance of nerves and surrounding tissues and anatomy structure differences for various patients. In recent years, fluorescence molecular imaging is introduced into nerves visualization for real-time avoidance of nerve damage. Imaging method and clinical translations are the major challenges for intraoperative usage. This study demonstrates a clear identification of thoracic sympathetic nerve with our self-developed high-sensitivity thoracoscopic fluorescence imaging system (SUPEREYE) for the superior outcome of thoracic surgery.
Methods: This study uses a novel self-developed florescence-light-optimized imaging system (SUPEREYE). 6 patients were recruited for this study. 5 mg/kg indocyanine green (ICG) was intravenously injected according to patient's weight with 24 hours before surgery. Video-rate color, fluorescence and merge images were displayed for anatomical imaging nerves and recorded for analysis.
Results: The representative color and fluorescence images collected by our system were shown in Fig. 1. Higher signal to background (SBR) was calculated in the group imaged with our system (2.4±0.1) when compared with common white-light thoracoscopy.
Conclusion: The improvement of SBR provides surgeons with a clearer identification of thoracic sympathetic nerve, and demonstrates our system can improve the precision of thoracic sympathetic nerve identification.

Fig. 1. The color, fluorescence and merged images of thoracic sympathetic nerve were collected by our system (SUPEREYE). The SBR of Fig. 1b were measured and calculated. Our system showed a high SBR (2.4±0.1).



Disclosures: **C. Chi:** None. **Y. Mao:** None. **K. He:** None. **F. Yang:** None. **J. Zhou:** None. **H. Hui:** None. **X. Yang:** None. **J. Tian:** None.

Nanosymposium

580. Advanced Imaging Methods and Probes

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DARPA SIMPLEX N66001-15-C-4032

U.S. Army Research Office W911NF-12-1-0594 (MURI)

Title: Three dimensional holographic imaging and optogenetics with spatial light modulators

Authors: *W. YANG[1], L. CARRILLO-REID[1], J.-E. K. MILLER[1], E. PNEVMATIKAKIS[2,3], L. PANINSKI[1,3], D. S. PETERKA[1], R. YUSTE[1];
[1]Columbia Univ., New York, NY; [2]Simons Ctr. for Data Analysis, Simons Fndn., New York, NY; [3]Dept. of Statistics, Ctr. for Theoretical Neuroscience, and Grossman Ctr. for the Statistics of Mind, Columbia Univ., New York, NY

Abstract: Two-photon microscopy provides a powerful platform to image and manipulate neural circuits *in vivo* with cellular resolution, which could be important for understanding brain function. In conventional two-photon microscopes, a single laser beam is serially scanned across the sample in a raster pattern or with a specified trajectory that intersects targets of interest along the path in a two dimension plane. To image or photoactivate targets at different focal planes, one needs to change the focus. This serial scanning and focusing poses a limit in the temporal resolution of imaging and photostimulation, which becomes slower with increases in the number of cells or focal planes. Here, we discuss the usage of spatial light modulators (SLMs) in two-photon microscopes for beam multiplexing, which enables simultaneously imaging a large population of cells across multiple focal planes, and photostimulating a groups of neurons in three-dimension (3D), all with cellular resolution. The SLM generates a 3D holographic excitation pattern, which is projected on the mouse cortex for multi-target imaging or photoactivation. Furthermore, the spatial light modulator can switch patterns in high speed, facilitating time-multiplexing. We demonstrate simultaneous imaging of the neural activity in layer 2/3 and 5 of mice visual cortex *in vivo*. Novel statistical algorithms are used to extract the signal from each individual plane. Furthermore, we perform 3D patterned photoactivation of groups of target neurons on mice cortex. SLM-based two-photon microscopy establishes an all-optical platform to study the neural circuits in 3D.

Disclosures: W. Yang: None. **L. Carrillo-Reid:** None. **J.K. Miller:** None. **E. Pnevmatikakis:** None. **L. Paninski:** None. **D.S. Peterka:** None. **R. Yuste:** None.

Nanosymposium

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Presentation Number: 580.13

Topic: I.04. Physiological Methods

Support: NIH R15GM110690

Title: Optical control of glutamate transport with a photoswitchable inhibitor

Authors: D. SHCHEPAKIN[1], B. CHENG[2], L. KALACHEV[1], D. TRAUNER[2], *M. P. KAVANAUGH[1];
[1]Univ. of Montana, Missoula, MT; [2]Dept. of Chem. and Ctr. for Integrated Protein Science , LMU Munich, Munich, Germany

Abstract: Excitatory amino acid transporters (EAATs) clear glutamate from the synaptic cleft and play a critical role in glutamatergic neurotransmission. Their differential roles in astrocytes, microglia, and neurons are poorly understood due in part to a lack of pharmacological tools that can be targeted to specific cells and tissues. We now describe ATT (Azo-TFB-TBOA), a photoswitchable glutamate transporter inhibitor designed via 'azologization' of the *N*-phenyl benzamide moiety of trifluoromethylbenzoylamino-benzyloxyaspartate (TFB-TBOA; Shimamoto et al. Mol Pharm. 2004 65:1008). ATT interacts with the major mammalian forebrain transporters EAAT1-3 in a manner that can be reversibly switched between *trans* (high-affinity) and *cis* (low-affinity) configurations using light of different colors. In the dark, ATT competitively blocked transport currents induced by 30 μM L-Glu in a subtype-selective manner (IC50 values: EAAT2=0.9±0.1nM; EAAT1=8.3±1.2 nM; EAAT3=212.9±15.9 nM; n>5). In experiments with the major glial glutamate transporter isoform EAAT2, brief pre-exposure of ATT to 350 nm light resulted in an ~13-fold reduction in the apparent affinity of the blocker (IC50=12.7±1.4nM). Photoisomerization of the high-affinity *trans*-configuration by 350 nm light could also be induced in the docked transporter complexes within a discrete illuminated spatial domain, resulting in an increase in the blocker off-rate monitored in the presence of glutamate. The results demonstrate that ATT can be used to reversibly manipulate glutamate transporter activity with light. This tool may be useful to gain insights into the physiological roles of glutamate transporters in the brain, as well as to study the dynamic molecular interactions of transporters with ligands.

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Nanosymposium

663. Mechanisms of Fragile X Syndrome

Location: SDCC 25A

Time: Wednesday, November 16, 2016, 8:00 AM - 11:30 AM

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Topic: A.07. Developmental Disorders

Support: NIH NIMH Grant MH104227

NIH NIMH Grant MH094449

NIH NINDS Grant NS078791

Title: Astrocytic contributions to synaptic and learning abnormalities in a mouse model of Fragile X Syndrome

Authors: *J. HODGES[1], X. YU[3], A. GILMORE[2], H. BENNETT[2], M. TJIA[2], J. PERNA[2], C.-C. CHEN[2], X. LI[4], J. LU[2], Y. ZUO[2];
[1]Mol. Cell & Developmental Biol., [2]Univ. of California Santa Cruz, Santa Cruz, CA; [3]Univ. of California Los Angeles, Los Angeles, CA; [4]Columbia Univ., New York, NY

Abstract: Fragile X Syndrome (FXS) is the most common type of mental retardation attributable to a single-gene mutation. It is caused by *FMR1* gene silencing and the consequent loss of its protein product, Fragile X Mental Retardation Protein (FMRP). Despite the prevailing neuron-centric view of brain function, many lines of evidence suggest that astrocytes are important contributors to developmental and degenerative neurological diseases. To investigate astrocytic contributions to the progression of synaptic abnormalities and learning impairments associated with FXS, we generated and characterized mice in which FMRP is selectively deleted or exclusively expressed in astrocytes. We performed *in vivo* two-photon imaging to track spine dynamics/morphology along dendrites of neurons in the motor cortex and examined associated behavioral defects. We found that adult astrocyte-specific *Fmr1* KO mice displayed an increased spine density in the motor cortex and impaired motor-skill learning. The learning defect coincided with a lack of enhanced spine dynamics in the motor cortex that normally occurs in response to motor skill acquisition. While spine density was normal at one month of age in astrocyte-specific *Fmr1* KO mice, new spines formed at an elevated rate, which preceded the overabundance of spines and behavioral impairments found in adulthood. Furthermore, the behavioral and synaptic phenotypes in astrocyte-specific *Fmr1* KO mice mimicked those observed in the global *Fmr1* KO mice, thereby revealing a significant role for astrocytes in FXS etiology. However, expression of FMRP only in astrocytes was insufficient to rescue spine or behavioral defects. Thus, FMRP expression is indispensable in both neurons and astrocytes and suggests a joint astrocytic-neuronal contribution to FXS pathogenesis.

Disclosures: J. Hodges: None. **X. Yu:** None. **A. Gilmore:** None. **H. Bennett:** None. **M. Tjia:** None. **J. Perna:** None. **C. Chen:** None. **X. Li:** None. **J. Lu:** None. **Y. Zuo:** None.

Nanosymposium

663. Mechanisms of Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: NIH RO1 NS070526

MOD research grant #6-FY12-378

FRAXA

Title: FMRP-miRNA controls activity-dependent translation of BDNF

Authors: *Y. FENG, W. FENG, W. LI, A. LAU;
Emory Univ., Atlanta, GA

Abstract: The fragile X mental retardation protein (FMRP) is known to regulate translation of numerous mRNA targets in brain neurons that play key roles in controlling neuronal network development and synaptic plasticity. The loss of FMRP results in deregulation of protein synthesis, which is responsible for the intellectual disability in fragile X syndrome patients. However, precise molecular mechanisms by which FMRP regulates protein synthesis in response to neuronal activity changes still remain elusive. Here we report novel mechanisms by which FMRP co-operates with microRNA that underlie neuronal activity-dependent translation of the brain derived neurotrophic factor (BDNF). FMRP specifically targets a BDNF mRNA isoform that carries a long 3'untranslated region (3'UTR) derived from alternative polyadenylation, which is translationally silenced and transported to dendrites in resting neurons but undergoes translational de-repression upon neuronal activation. The loss of FMRP results in aberrantly increased basal levels of BDNF translation in the hippocampus of Fmr1 ko mice. In addition, expression of tissue plasminogen activator (tPA) and Zn-deposition to the mossy fiber axons are dysregulated in the Fmr1 ko hippocampus, both of them govern the proteolytic cleavage of pro-BDNF and formation of mature BDNF for TrkB signaling. Moreover, the loss of FMRP leads to failures of translational activation of BDNF upon neuronal activation. Together, our studies revealed a functional link between the FMRP-miRNA pathways with BDNF-TrkB signaling, both play pivotal roles in controlling synaptic plasticity, thus suggesting that deregulation of BDNF translation is an important mechanism for Fragile X pathogenesis.

Disclosures: Y. Feng: None. **W. Feng:** None. **W. Li:** None. **A. Lau:** None.

Nanosymposium

663. Mechanisms of Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: NIH Grant AG044714

NIH Grant AG033514

NIH Grant TR000427

FRAXA Research Foundation

DOD Grant PR152034

Title: The APP theory of fragile X.

Authors: *C. J. WESTMARK;
Univ. Wisconsin, Madison, WI

Abstract: Fragile X syndrome (FXS) is a debilitating genetic disorder with no cure and few therapeutic options. Excessive signaling through metabotropic glutamate receptor 5 (mGluR5) in FXS leads to increased translation of numerous synaptic proteins and exaggerated long-term depression (LTD). Two of the overexpressed proteins are amyloid-beta protein precursor (APP) and its metabolite amyloid-beta, which have been well-studied in Alzheimer's disease (AD). Accumulating evidence suggests that dysregulated levels of APP and its catabolites contribute to the impaired synaptic plasticity and seizure incidence observed in several neurological disorders including FXS. We hypothesize that pharmaceuticals under study for the modulation of APP and amyloid-beta in AD might be viable therapeutic strategies for FXS. Specifically, we are studying the efficacy of BACE1 inhibitors in reducing amyloid-beta levels and the corresponding effects on seizure, learning & memory, and sleep phenotypes in Fmr1KO mice. In addition, APP and its proteolytic fragments are emerging as biomarkers for neurological health. Multiple recent FXS clinical trials have failed on their primary endpoints indicating that there is a compelling need for validated biomarkers and outcome measures in the field. Thus, we further hypothesize that APP and amyloid-beta may be viable blood-based biomarkers that are responsive to drug treatment in FXS. Our studies, in conjunction with work from the Lahiri, Tan, Wegiel, Bagni and Erickson laboratories, to understand the role of APP metabolites in developmental conditions such as FXS and autism are a quantum leap for the neuroscience field, which has traditionally restricted any role of APP to AD and aging.

Disclosures: C.J. Westmark: None.

Nanosymposium

663. Mechanisms of Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: NIH grant 1U54HD082008

FRAXA Research Foundation

Title: Role of mmp-9 in the development of PV interneurons and auditory processing deficits in fragile x syndrome mouse model

Authors: *I. M. ETHELL[1], S. AFROZ[2], T. WEN[3], S. REINHARD[3], J. LOVELACE[3], D. BINDER[2], K. RAZAK[2];
[1]Univ. California Riverside Sch. of Med., Riverside, CA; [2]Biomed. Sci. and Neurosci., [3]Univ. of California Riverside, Riverside, CA

Abstract: Fragile X Syndrome (FXS) is the most prevalent cause of inherited intellectual disability and autism. Symptoms include cognitive and communication deficits, delayed language, hyperactivity, abnormal social interactions, hypersensitivity to sensory stimuli and seizures. The *Fmr1* knockout (KO) mouse is an established model of FXS as it displays similar behavioral impairments, including hypersensitivity to tones that is seen in humans with FXS. Our study revealed abnormal responses to sound in auditory cortex of *Fmr1* KO mice, which showed impaired repetition rate-dependent habituation to sound. However, the mechanisms underlying the auditory deficits are not known. Recent clinical trials show that minocycline improves FXS-associated behaviors and normalizes cortical responses to repeating sound in humans with FXS. Beside its antibiotic and anti-inflammatory activity, minocycline can reduce the levels and activity of matrix metalloproteinase-9 (MMP-9) in the brain. MMP-9 is an extracellular protease that is known to cleave extracellular matrix and its translation is negatively regulated by FMRP. Our data show that Mmp-9 levels are abnormally high in the auditory cortex of *Fmr1* KO mice, suggesting that elevated Mmp-9 levels may be responsible for the abnormal auditory deficits in *Fmr1* KO mice. Indeed, impaired repetition rate-dependent habituation to sound was reversed in *Fmr1* KO mice by a genetic deletion of *Mmp-9*. MMP-9 can impact the inhibition by disrupting perineuronal nets (PNN) that are critical in supporting Parvalbumin (PV) interneurons. Our preliminary results support this hypothesis and show a decrease in the number of PNN positive PV cells, PV cell density and impaired PNN distribution around PV cells in the layer 4 of P21 auditory cortex of *Fmr1* KO mice as compared to WT mice. We also found a restoration of PV and PNN cell density in the auditory cortex of Fmr1 KO mice following a full and partial deletion of Mmp-9. Density of PV/PNN positive neurons and the percentage of PV cells enwrapped with PNN were also restored to WT levels in Fmr1/MMP9 double KO mice. These findings indicate that elevated Mmp-9 levels may be responsible for the abnormal development and maturation of PV/PNN in the auditory cortex of Fmr1 KO mice leading to impaired excitatory-inhibitory balance and the development of auditory processing deficits.

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Nanosymposium

663. Mechanisms of Fragile X Syndrome

Location: SDCC 25A

Time: Wednesday, November 16, 2016, 8:00 AM - 11:30 AM

Presentation Number: 663.05

Topic: A.07. Developmental Disorders

Title: Dissecting the role of a lipid kinase in peripheral nerve myelination

Authors: *A. ALVAREZ-PRATS[1], I. BJELOBABA[4], Y. KIM[1], Z. ALDWORTH[2], D. ABEBE[1], S. STOJILKOVIC[3], M. STOPFER[2], T. BALLA[1];
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Abstract: Myelination of axons is essential for proper neuronal signal propagation. Our recent studies identified phosphatidylinositol 4-kinase III-alpha (PI4KA) as a key regulator of phosphatidylserine (PS) metabolism in mammalian cells. Since PS and phosphatidylethanolamine (PE) are key components of the myelin sheath, we decided to evaluate the role of this kinase in myelination using a mouse model.
We created Schwann cell-specific PI4KA knockout mice by crossing mice that have floxed alleles of pi4ka with a line that expresses Cre recombinase controlled by the myelin protein zero (P_0) (MPZ) promoter. MPZ is a glycoprotein located only in Schwann cells of the peripheral nervous system, but not in sensory neurons. Mice of both sexes were randomly divided into three groups: Pi4ka(fl/fl)Cre+; Pi4ka(fl/wt)Cre+; and Pi4ka(wt/wt)Cre+, the latter two groups serving as controls.
Pi4ka(fl/fl)Cre+ mice show subtle gait abnormalities 30 days after birth, but by day 60 they exhibit dramatic impairment in using their hind legs. These abnormalities are not observed in the two control groups. Immunohistochemical analysis of sciatic nerves shows greatly reduced S100β staining as well as decrease of myelin proteins, MPZ and MBP, in the affected animals. EM analysis, also performed on sciatic nerves, shows dramatic decrease of myelin thickness and onion bulbs formation, hallmarks of demyelination diseases. Likewise, lipidomic analysis of the sciatic nerves showed greatly reduced phospholipid content that disproportionally affected PS and PE, and sphingomyelin, another important component of myelin, in the Pi4ka(fl/fl)Cre+ mice compared to controls.
To our knowledge, this is the first study on the role of PI4KA in the peripheral nervous system, shedding important light on the involvement of this enzyme in myelination. Further efforts are focused on elucidating the underlying cause of the phenotype using cellular and molecular approaches. Understanding the control of phospholipid synthesis and transport in myelin

formation could help to identify new targets for the treatment of myelination disorders in the future.

Disclosures: **A. Alvarez-Prats:** None. **I. Bjelobaba:** None. **Y. Kim:** None. **Z. Aldworth:** None. **D. Abebe:** None. **S. Stojilkovic:** None. **M. Stopfer:** None. **T. Balla:** None.

Nanosymposium

663. Mechanisms of Fragile X Syndrome

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Presentation Number: 663.06

Topic: A.07. Developmental Disorders

Support: 5R01HD052731-08

Title: FMRP regulates activity-dependent priming of hippocampal synaptic plasticity: Mechanisms and implications for cognition

Authors: K. KIM, J.-Y. JOO, T.-K. KIM, *K. M. HUBER;
Neurosci., Univ. of Texas Southwestern Med. Ctr. at Dallas, Dallas, TX

Abstract: Fragile X Mental Retardation Protein (FMRP) is an RNA binding protein that regulates translation of brain mRNAs, including those in dendrites. Importantly, FMRP regulates a form of synaptic plasticity that relies on rapid translation of new proteins in dendrites; specifically, long-term synaptic depression induced by activation of Group 1 metabotropic glutamate receptors (mGluR-LTD) in hippocampal CA1 neurons. In the mouse model of Fragile X Syndrome, *Fmr1* KO, the magnitude of mGluR-LTD is enhanced, and is independent of new protein synthesis, in contrast to wildtype (WT) mice. The contribution of hippocampal mGluR-LTD to cognitive function or dysfunction in *Fmr1* KO mice is unknown. Brief exposure to a novel environment enhances induction of LTD in CA1 as measured with chronic *in vivo* recordings in awake rodents or in acute hippocampal slices suggesting a role of mGluR-LTD in learning of novel environments. To study the mechanisms and role of FMRP in novelty-induced priming of mGluR-LTD, we developed an *in vitro* model. We used optogenetics to fire CA1 neurons in organotypic hippocampal slice cultures in a pattern mimicking that observed during exploration of a novel environment. CA1 neurons in slice culture, were sparsely (<1%) transfected with ChETA, and stimulated with blue light to fire action potentials in brief bursts of high-frequency action potentials, or Burst Photo-Stimulation (BPS). In WT cultures, BPS alone did not affect excitatory synaptic transmission, as measured by evoked and miniature (m) EPSCs, but enhanced subsequent induction of mGluR-LTD, similar to what is observed with novel experience. In slice cultures of *Fmr1* KO mice, LTD magnitude is robust regardless of

prior BPS, indicating that *Fmr1* KO synapses are insensitive to the activity history of the neuron. Brief novel experience and neuronal activity induce the immediate early gene, *Arc*, a dendritic mRNA and FMRP target. Data indicate that FMRP suppresses translation of dendritic *Arc* mRNA upon induction and forms a necessary translational switch for activity-dependent priming of mGluR-LTD. These results predict a deficit in novelty-related priming of mGluR-LTD in Fragile X Syndrome which may contribute to alterations in learning associated with novel stimuli or environments.

Disclosures: **K. Kim:** None. **J. Joo:** None. **T. Kim:** None. **K.M. Huber:** None.

Nanosymposium

663. Mechanisms of Fragile X Syndrome

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Presentation Number: 663.07

Topic: A.07. Developmental Disorders

Support: NIH MH093661

Spastic Paralysis Research Foundation of Illinois-Eastern Iowa District of Kiwanis International

Title: Molecular requirements for FMRP and the RNA helicase MOV10 in translation regulation of cobound RNAs

Authors: *S. CEMAN, P. J. KENNY, M. C. LANNOM, G. SKARIAH;
Univ. Illinois, Urbana, IL

Abstract: Fragile X syndrome is the most common form of inherited cognitive impairment and is caused by the absence of the Fragile X Mental Retardation Protein, FMRP. FMRP is an RNA binding protein that associates with approximately 4% of brain mRNAs and regulates their translation through a variety of mechanisms. There is evidence that FMRP stalls translation and directly binds ribosomes. In addition, FMRP is associated with the microRNA pathway. We showed that FMRP functionally associates with the RNA helicase MOV10. MOV10 binds RNA intramolecular G-quadruplexes and functionally associates with RISC factor Argonaute 2. Like FMRP, MOV10 is expressed throughout brain until adolescence. FMRP is required for recruitment of MOV10 to commonly bound RNAs although when both proteins bind the same sequence in the 3'UTR, association with Argonaute is blocked. Thus, FMRP acts as a positive regulator of expression. We will elucidate the mechanism of this positive translational regulation by mapping the interacting domains of FMRP and MOV10. We have already determined that it

is the N-terminal domain of MOV10 that directly interacts with FMRP. We are also examining how association of FMRP and MOV10 regulates target RNA binding and association with Argonaute 2, particularly the role of the RGG box of FMRP. We will also determine how MOV10 binding to FMRP affects RGG box association with RNAs bearing G- quadruplexes: a structure recognized by both FMRP and MOV10. In brain, FMRP and MOV10 bind a common subset of RNAs and MOV10 preferentially binds cytoskeletal RNAs. We are characterizing the role of FMRP and MOV10 on dendritic process formation through these commonly bound RNAs in cultured neurons.

Disclosures: **S. Ceman:** None. **P.J. Kenny:** None. **M.C. Lannom:** None. **G. Skariah:** None.

Nanosymposium

663. Mechanisms of Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: Spastic Paralysis Research Foundation of the Illinois-Eastern Iowa District of Kiwanis International

The Jerome LeJeune Foundation

Title: Elevated VEGF-A expression mediates Fragile X Syndrome neuronal and behavioral abnormalities

Authors: ***R. GALVEZ**[1], A. BELAGODU[2];
[1]Psychology, Univ. of Illinois, Urbana-Champaign, Urbana, IL; [2]Neurosci., Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: The Fragile X Mental Retardation Syndrome (FXS) is the leading form of inherited mental retardation. Although the general cause of the syndrome is understood [absence of the fragile X mental retardation protein (FMRP)], the mechanism(s) by which the absence of FMRP causes cognitive abnormalities remains elusive. In our efforts to characterize abnormalities in FXS, our recent studies have demonstrated that adult FXS mice exhibit elevated brain vascular endothelial growth factor A (VEGF-A) expression (Belagodu et al., Submitted). VEGF-A has been classically associated with vascular growth; however, recent studies have demonstrated that VEGF receptors are located on the post-synaptic density of neurons and play a key role in modulating learning (Rossi et al., 2016). Furthermore, studies have shown that altering VEGF-A expression can cause LTD like conditions (McCloskey et al., 2005) as well as directly modulate

many neuronal properties, such as neurite outgrowth, axonal sprouting and cell proliferation, independent of its effects on vasculature (Sondell, M. et al., 1999; Sondell, M. et al., 2000; Matsuzaki, H. et al., 2001; Jin et al., 2002). Interestingly, these VEGF-A induced neuronal properties are consistent with abnormalities observed in FXS [increased LTD, axonal material, cell proliferation, and number of immature spines (Slegtenhorst-Eegdeman et al., 1998; Huber et al. 2002; Galvez & Greenough 2005; Antar et al 2006)] suggesting that excessive VEGF-A expression facilitates FXS abnormalities. The current presentation outlines a series of experiments examining the mechanism for and effects of elevated VEGF-A expression on various FXS abnormalities. Our studies have demonstrated that elevated VEGF-A expression in FXS is mediated through the mTORC1 pathway that has been shown to be overactive in FXS (Sharma et al., 2010). Using pharmacological manipulations we have also demonstrated that blocking VEGF-A binding to its receptor can decrease elevated synapse density in FXS. Our subsequent behavioral studies have revealed that blocking VEGF-A binding to its receptor also alleviates FXS learning deficits and abnormalities in vocalization patterns. These studies demonstrate that VEGF-A plays a critical role in mediating FXS abnormalities and more importantly provide insight into a novel mechanism that can easily be manipulated for future interventions.

Disclosures: **R. Galvez:** None. **A. Belagodu:** None.

Nanosymposium

663. Mechanisms of Fragile X Syndrome

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Time: Wednesday, November 16, 2016, 8:00 AM - 11:30 AM

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Topic: A.07. Developmental Disorders

Support: Simons Grant 336605

NARSAD Young Investigator Award

Title: The study of homeostatic network excitability in Fragile X mouse model

Authors: ***N.-P. TSAI**[1], K. A. JEWETT[2];
[1]Univ. of Illinois At Urbana-Champaign, Urbana, IL; [2]Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Fragile X syndrome (FXS) is the most common inherited form of mental retardation and autism, with a population prevalence of about 1/4,000 males and 1/8,000 females. FXS is caused by transcriptional silencing or loss-of-function mutations in the *Fmr1* gene, which

encodes for the Fragile X Mental Retardation Protein (FMRP). FMRP is an RNA-binding protein which regulates mRNA transport and translation in dendrites. In FXS patients and the mouse model of FXS, *Fmr1* KO mice, multiple signs of neuronal hyperexcitability are observed. Many molecular and cellular mechanisms have been proposed to describe the hyperexcitability. However, it is unclear whether and why homeostatic mechanism fails to reduce excitability in FXS. In the current study, we employed multiunit electrophysiology recording system to evaluate neural network activity in cultured cortical neurons from *Fmr1* KO mice. We first revealed that homeostatic network activity downscaling, which is induced by chronic stimulation with neuronal activity, is impaired in *Fmr1* KO cultures. We further showed that such homeostatic mechanism requires Murine Double Minute 2 (Mdm2)-mediated degradation of tumor suppressor p53, which is also impaired in *Fmr1* KO cultures. Using a p53 inhibitor to mimic activity-induced inhibition of p53, we partially but significantly correct homeostatic network activity downscaling in *Fmr1* KO cultures. These data provide valuable information on the deficits of neuronal and circuit excitability in FXS. They also introduce novel therapeutic targets for treating, or controlling, the symptoms in FXS.

Disclosures: **N. Tsai:** None. **K.A. Jewett:** None.

Nanosymposium

663. Mechanisms of Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: FRAXA program grant

Title: Rectification of synaptic and network alterations associated with delayed gaba polarity switch in fragile x mice

Authors: ***Q. HE**[1], E. D. ARROYO[2], C. PIOCHON[1], C. PORTERA-CAILLIAU[2], A. CONTRACTOR[1];
[1]physiology, Northwestern Univ. Dept. of Physiol., Chicago, IL; [2]Neurol. and neurobiology, David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: Disruptions in GABA signaling are prevalent in Fragile X syndrome (FXS) and may contribute to the alterations in synaptic development and cortical hyperexcitability in *Fmr1*$^{-/y}$ mice. We have recently reported a delay in the normal polarity switch in $GABA_AR$ reversal potential (E_{GABA}) in cortical neurons during the first two postnatal weeks of development in *Fmr1*$^{-/y}$ mice (He et al., 2014). This delay in E_{GABA} switch is caused by accumulation of

intracellular [Cl^-] due to elevated expression of the juvenile chloride cotransporter NKCC1. Here we used chronic postnatal administration of bumetanide, a known NKCC1 inhibitor, to determine whether normalizing intracellular [Cl^-] could reverse the developmental alterations in cortical circuits. *Fmr1*$^{-/y}$ and littermate control (*Fmr1*$^{+/y}$) pups were injected daily with bumetanide (0.2mg/kg) or vehicle solution beginning at postnatal day (P)0. E_{GABA} were recorded using perforated patch in layer 4 spiny stellate neurons. In vehicle injected *Fmr1*$^{-/y}$ mice, E_{GABA} was depolarized at -49 ± 7 mV. In contrast, bumetanide injected *Fmr1*$^{-/y}$ mice exhibited hyperpolarized E_{GABA} of -84 ± 9 mV ($p < 0.05$) similar to E_{GABA} in vehicle treated *Fmr1*$^{+/y}$ (-73 ± 8 mV). We next determined whether chronic bumetanide administration could also rectify the persistence in LTP at the end of the critical period for plasticity in layer IV neurons (Harlow et al., 2010). Thalamocortical synapses in *Fmr1*$^{-/y}$ mice demonstrate delayed developmental features including extended LTP beyond the end of the first postnatal week. In saline treated *Fmr1*$^{-/y}$ at P7 there was a persistent LTP of 129 ± 7 % at these synapses, but not age matched *Fmr1*$^{+/y}$ (92 ±11 % $p < 0.05$). In contrast, bumetanide treated *Fmr1*$^{-/y}$ animals exhibited no significant potentiation at this age (88 ± 5 %, $P < 0.05$), similar to vehicle treated *Fmr1*$^{+/y}$. This data demonstrates that the persistent LTP at the end of the critical period is corrected by chronic bumetanide administration. Finally, we tested whether the exaggerated whisker-evoked network responses in the barrel cortex of Fmr1$^{-/-}$ mice could be rescued by bumetanide (Arnett 2014). We performed optical imaging of intrinsic signals at 2 weeks and 2 months of age to assess cortical response to single whisker stimulation. We found that the size of the whiskers maps was significantly larger in vehicle-treated *Fmr1*$^{-/y}$ mice than *Fmr1*$^{+/y}$ at both ages, and that this could be rectified by two-week long daily treatment of bumetanide from birth. Taken together our results demonstrate that chronic treatment with an NKCC1 inhibitor during the postnatal critical period can rectify the plasticity time window in layer IV and can rescue cortical network defects in *Fmr1*$^{-/y}$ mice.

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Nanosymposium

663. Mechanisms of Fragile X Syndrome

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Presentation Number: 663.11

Topic: A.07. Developmental Disorders

Support: NIH Grant R01NS095311

Title: Inhibitory control of cortical hyper-excitability in fragile x syndrome

Authors: *M. M. HUNTSMAN[1], C. A. CEA-DEL RIO[1], S. FREEDMAN[1], A. NUNEZ-PARRA[2], D. RESTREPO[2];
[1]Dept. of Pharmaceut. Sci., [2]Dept. of Cell and Developmental Biol., Univ. of Colorado at Denver - Anschutz Med. Campus, Aurora, CO

Abstract: Fragile X Syndrome (FXS) patients exhibit behavioral phenotypes reflective of hyperexcitable circuitry. A prominent phenotype is a hypersensitivity to sensory stimuli. Our previous work illustrated faulty activation of Somatostatin-positive (Sst) interneurons by glutamate via metabotropic glutamate receptors (mGluRs). Here we combined both *in vitro* and *in vivo* electrophysiological approaches. First, we performed whole cell patch clamp recordings of Sst interneurons and pyramidal cells (PCs) from layer 2/3 of the somatosensory cortex from *Fmr1* KO and WT mice. In *Fmr1* KOs we found: 1) sIPSC frequency in PCs is higher, 2) application of DHPG (an mGluR agonist) fails to induce high frequency action potential firing in Sst interneurons and sIPSC frequency in PCs, 3) no induction of slow self-inhibition (SSI), an auto-inhibitory mechanism that operates in response to mGluR activation, and 4) no inhibitory long-term depression (LTDi) in the presence of DHPG. Thus, mGluR-mediated inhibitory plasticity is altered in *Fmr1* KO mice suggesting an increased inhibitory drive onto PCs. Next, we tested neuronal activation and sensory responsiveness to stimulation of whiskers in layer 2/3 of the somatosensory cortex in anesthetized and awake *Fmr1* KO and WT mice. Single unit analysis revealed the baseline rate of recorded units is higher in *Fmr1* KOs consistent with overall hyperexcitability. Furthermore, when contralateral whiskers were stimulated with an air puff, subsets of cells responded differentially with either increases or decreases in firing rate. Importantly, a higher percentage of units recorded from *Fmr1* KOs decrease their spike rate in response to whisker stimulation than those in WTs, suggesting the possibility of an enhanced inhibitory drive. Next we trained animals in a go-no go task by changing the stimuli to different air-flow rates (S+, rewarded; S-, unrewarded). We then performed tetrode recordings from layer 2/3 in these animals. Single units responded with a more transient increase in firing in the correct rejection trials (correct rejection: refrain from licking during S-) in *Fmr1* KOs, suggesting a faster recruitment of inhibitory drive. This evidence indicates that the cortex in *Fmr1* KOs is hyperexcited at a basal state. In addition, when the network is activated via whiskers the period of increased excitability rapidly comes back to basal levels suggesting an increased inhibitory drive during periods of active behavioral task discrimination. Taken together these data suggest that the failure of mGluR-mediated mechanisms compromise the normal inhibitory control of the network and further contribute cortical hyperexcitability.

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Nanosymposium

663. Mechanisms of Fragile X Syndrome

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Presentation Number: 663.12

Topic: A.07. Developmental Disorders

Support: NIH grant R01 MH096832

Title: Critical period synaptic pruning mediated by shrub in a fragile x syndrome model

Authors: *D. J. VITA, K. S. BROADIE;
BioSci, Vanderbilt Univ., Nashville, TN

Abstract: Fragile X Mental Retardation Protein (FMRP) is an RNA-binding translational regulator whose loss causes Fragile X syndrome (FXS), the most common form of heritable intellectual disability (ID) and autism spectrum disorder (ASD). A hallmark phenotype in FXS patients and animal models is overelaborated synaptic processes, both pre- and postsynaptically. This apparent increase in connectivity may be attributed to an inability to prune synapses during early-use, critical period developmental windows. Using the well-characterized *Drosophila* FXS model, we employed developmental proteomics to identify strong transient misregulation of the endosomal sorting complex required for transport III (ESCRT-III) protein Shrub (human CHMP4B). Shrub functions in membrane scission, acting at both the cell surface and in the formation of internal multivesicular bodies (MVBs). Shrub has been implicated in neural development and synaptic pruning, with mutants strongly resembling the *Drosophila* FXS model. We therefore predicted Shrub misregulation in the FXS disease state is causative in synaptic pruning defects occurring during critical periods of circuit refinement. Our RNA immunoprecipitation (RIP) analyses reveal that FMRP binds Shrub mRNA, supporting a direct interaction. Our brain Western blot analyses show a striking transient increase in Shrub expression in the *Drosophila* FXS model, with peak Shrub levels occurring during the early-use period immediately following eclosion. In the central brain, we are targeting our study to olfactory Projection Neurons (PNs) providing input into the well-defined Mushroom Body (MB) learning and memory circuit. Our brain imaging results show that Shrub over-expression mimics the FXS state of maintained, over-elaborated PN synaptic inputs into the MB calyx, and that Shrub knockdown during the early-use critical period prevents connectivity defects in the FXS model. We are currently using transmission electron microscopy to investigate the synaptic pruning mechanism within the MB calyx, testing the hypothesis that Shrub is acting locally in membrane scission. Our data support the working hypothesis that FMRP directly interacts with Shrub mRNA to suppress translation during the early-use critical period to regulate synaptic pruning and optimize circuit connectivity. This work is entirely supported by NIH grant R01 MH096832 to Kendal Broadie.

Disclosures: **D.J. Vita:** None. **K.S. Broadie:** None.

Nanosymposium

663. Mechanisms of Fragile X Syndrome

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Presentation Number: 663.13

Topic: A.07. Developmental Disorders

Support: Astellas project grant

Title: Effects of GABA-B agonist baclofen on neural oscillations and behaviour in the FMRP1 knockout mouse model of Fragile X syndrome

Authors: ***D. SINCLAIR**[1,2], R. FEATHERSTONE[2], S. AKUZAWA[3], M. MATSUMOTO[3], S. J. SIEGEL[2];
[1]Schizophrenia Res. Laboratory, Neurosci. Re, Randwick, Australia; [2]Translational Res. Program, Univ. of Pennsylvania, Philadelphia, PA; [3]Neurosci. Res. Unit, DDR, Astellas Pharma Inc., Tsukuba-shi, Japan

Abstract: Fragile X syndrome (FXS) results from mutation of the FMR1 gene and concomitant loss of FMRP protein, and is the most common known genetic cause of both autism and inherited intellectual disability. Current treatment options have limited success in alleviating key symptoms of the disorder, such as altered sensory sensitivity and impaired cognition. In this study, we investigated auditory sensory processing and cognitive function in FMR1 knockout mice, a rodent model of FXS with high construct validity. We also targeted the γ-aminobutyric acid (GABA) neurotransmitter signalling pathway, using the GABA-B agonist baclofen, in order to normalize abnormalities in these domains. Male and female FMR1 knockout mice (n=18 per group) had increased relative power of baseline high frequency gamma (30-80Hz) oscillations as measured by electroencephalography (EEG) relative to wild-type C57BL/6 controls, suggestive of increased background 'noise' in the brain. Phase-locked gamma oscillations evoked by auditory stimuli, which are generated during early stimulus processing, were also increased in FMR1 knockout mice. The increased power of baseline and evoked gamma oscillations in FMR1 knockout mice were reversed by treatment with 2.5mg/kg baclofen. These treatment-responsive abnormalities of neural oscillations were accompanied by impaired working memory (decreased spontaneous T maze alternation) and decreased anxiety-like behaviour (increased open field centre time) in FMR1 knockout mice, both of which were also were improved by baclofen treatment. Furthermore, animals showing the greatest therapeutic evoked gamma response to baclofen displayed the greatest improvement in working memory. These findings shed further light on the neural underpinnings of sensory disturbances in FMR1 knockout mice and their

relationship to altered cognitive function. They suggest that auditory-evoked changes in high-frequency oscillations, which (to our knowledge) are yet to be investigated in FXS, may be a useful objective measure for assessing sensory processing/sensitivity in the disorder. Furthermore, these findings highlight the potential for treatment-induced changes in neural oscillations to be used as indices of potential behavioural treatment responsiveness in a clinical setting.

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Nanosymposium

663. Mechanisms of Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: NIH Center Grant (U54) to G.B

R21MH103748 to C.G

Title: Use of a p110β subunit selective inhibitor to rescue behavioral impairments in a mouse model of fragile x syndrome

Authors: *A. BANERJEE[1], C. GROSS[2], R. A. RIVERO[3], S. L. GOURLEY[4], G. BASSELL[5]; [1]Dept. of Cell Biol., Emory Univ., Tucker, GA; [2]Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [3]Flexible Discovery Unit, GlaxoSmithKline, Philadelphia, PA; [4]Dept. of Pediatrics, Emory Univ. Sch. of Medicine, Yerkes Natl. Primate Res. Ctr., Atlanta, GA; [5]Dept. of Cell Biol., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability and monogenic cause of autism. Several studies have demonstrated increased signaling through gp1 mGlu1/5 receptors in animal models of fragile x syndrome, and mGlu5 antagonists rescue FXS-associated phenotypes, although clinical trials have not been successful. Further work is needed to better understand the molecular mechanism of exaggerated mGlu5 signaling in FXS and to develop alternative therapeutic strategies. Our recent work has revealed that the activity of the PI3K signaling complex is elevated in animal models of FXS and human patient

cells. Our work further suggests that elevated levels of p110β, the catalytic subunit of class 1A PI3K, may be responsible for increased activity of the PI3K signaling complex that is directly associated with mGlu5 receptors. The design and use of subunit selective inhibitors for p110β have advanced to clinical trials for certain cancers and might be repurposed for fragile x syndrome. An advantage of subunit-selective inhibitors is to preserve overall PI3K activity. An additional advantage of targeting the catalytic subunit p110β in FXS is that it is a direct mRNA target of FMRP and is overexpressed in *FMR1* KO mice and human FXS patient cells, which may directly contribute to dysregulation of the PI3K/mTOR pathway. In this study, GSK2702926A, a novel GlaxoSmithKline subunit-selective p110β inhibitor that has optimized selectivity, potency, pharmacokinetics and CNS penetration, was evaluated for its ability to rescue FXS associated behavioral phenotypes in a mouse model of FXS. Wildtype and *FMR1* KO mice were divided into four groups- WT/vehicle, WT/GSK2702926A, *FMR1*KO/vehicle, *FMR1*KO/GSK2702926A. A single dose of 5 mg/kg GSK2702926A or vehicle was administered to the animals intraperitoneally, one hour before each behavioral assay. At this time point we observed a decrease in phosphorylated AKT relative to total levels in the hippocampus and cortex, suggesting reduction in PI3K signaling. One hour following drug injection, mice were tested in a battery of behavior tests including open field, three chambered social behavior, self-grooming, auditory and contextual fear conditioning. Application of GSK2702926A was able to rescue the increased time spent in the center time and number of entries in an open field assay in *Fmr1* KO mice. Application of GSK2702926A was able to rescue reduced social behavior in a three-chambered social apparatus. Lastly, the aberrant contextual and auditory fear learning in *FMR1* KO mice was rescued by GSK2702926A. These findings motivate further development of p110β-targeted therapy for FXS.

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Nanosymposium

664. Microglia in Nervous System Diseases

Location: SDCC 30B

Time: Wednesday, November 16, 2016, 8:00 AM - 11:00 AM

Presentation Number: 664.01

Topic: B.12. Glial Mechanisms

Support: NIH Grant R01 NS048216

Title: Targeted knockout of neuronal or microglial Na^+/H^+ exchanger isoform 1 in mice has differential effects on reducing brain injury and functional recovery after stroke

Authors: *S. SONG[1], S. WANG[1,2], V. PIGOTT[1], W. ZHU[1], Y. SHI[1], K. CARNEY[1], Y. CHEN[3], W. GAN[4], G. E. SHULL[3], D. SUN[1,5];
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Abstract: Na^+/H^+ exchanger isoform-1 (NHE1) is ubiquitously expressed in all cell types in the central nervous system (CNS) and plays a role in ischemic brain damage. We previously reported that transgenic global NHE1 knockout mice exhibited reduced infarct volume and less neuronal and astrocytic damage. Microglial activation and pro-inflammatory responses were also decreased in global *Nhe1*$^{-/-}$ knockout mice. To differentiate the specific function of NHE1 in each cell type, *CamKIICre*$^{+/-}$*;Nhe1*$^{flox/flox}$ and *Cx3cr1CreER*$^{+/-}$*; Nhe1*$^{flox/flox}$ mice were established to evaluate the roles of NHE1 in neurons and microglia, respectively. To induce conditional knockout of *Nhe1* in microglia, *Cx3cr1CreER*$^{+/-}$*;Nhe1*$^{flox/flox}$ mice at P30-34 were treated with either corn oil (3.75ml/kg body weight) or tamoxifen (Tam, 75mg/kg body weight/day, i.p., for 5 days). Ischemic stroke was induced at P60-70 with 60 min of transient middle cerebral artery occlusion (tMCAO). *CamKIICre*$^{+/-}$*; Nhe1*$^{flox/flox}$ mice showed a 58.5% reduction in infarct volume compared to *CamKIICre*$^{+/-}$ control mice (33.0 ± 9.2 mm^3 vs. 79.6 ± 9.8 mm^3; $p < 0.01$) and significant improvements in neurological function during the first week recovery after ischemic stroke ($p < 0.05$). On the other hand, no significant difference of ischemic infarct volume was detected in the oil-treated and Tam-treated *Cx3cr1CreER*$^{+/-}$*;Nhe1*$^{flox/flox}$ mice (82.8 ± 14.2 vs. 74.0 ± 6.4 mm^3, $p > 0.05$) with TTC staining at 48 h after ischemic stroke. However, selective deletion of microglial *Nhe1* in the Tam-treated *Cx3cr1CreER*$^{+/-}$*;Nhe1*$^{flox/flox}$ mice did result in 42.0% less $CD11b^+/CD45^{low\text{-}medium}$ microglial cells than the oil-treated control mice (79.8 ± 4.8 vs. 137.3 ± 11.5 cells/brain, $p < 0.01$) in the non-ischemic brain at 30 days after injection. Interestingly, Tam-treated *Cx3cr1CreER*$^{+/-}$*;Nhe1*$^{flox/flox}$ mice showed significantly faster neurological function recovery reflected in neurological function scoring and corner tests at 1-5 days post-stroke ($p < 0.01$). These findings suggest that neuronal NHE1 and microglial NHE1 play differential roles in acute ischemic brain injury formation and post-stroke recovery. Our new transgenic mouse models allow us to further investigate the underlying cellular and molecular mechanisms.

Disclosures: **S. Song:** None. **S. Wang:** None. **V. Pigott:** None. **W. Zhu:** None. **Y. Shi:** None. **K. Carney:** None. **Y. Chen:** None. **W. Gan:** None. **G.E. Shull:** None. **D. Sun:** None.

Nanosymposium

664. Microglia in Nervous System Diseases

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Presentation Number: 664.02

Topic: B.12. Glial Mechanisms

Support: NSFC81272576

NSFC 81471249

Title: TREM2 protects against cerebral ischemia/reperfusion injury

Authors: *Y. TANG;
Sun Yat-Sen Mem. Hospital, Sun Yat-Sen Univ., Guangdong, China

Abstract: Although post-ischemic inflammation induced by the innate immune response is considered an essential step in the progression of cerebral ischemia injury, the role of triggering receptor expressed on myeloid cells 2 (TREM2) in the pathogenesis of ischemic stroke remains to be elucidated. Here, we found that the transcriptional and post-transcriptional levels of TREM2 were increased in cultured primary microglia after oxygen-glucose deprivation and reoxygenation and in the ischemic penumbra of the cerebral cortex after middle cerebral artery occlusion (MCAO) and reperfusion in mice. TREM2 was mainly expressed in microglia, but not in astrocytes, neurons,oroligodendrocytes in mice subjected to MCAO. Manipulating TREM2 levels *in vitro* and *in vivo* significantly regulated the production of pro- and anti-inflammatory mediators after ischemic stroke. TREM2 overexpression markedly suppressed the inflammatory response and neuronal apoptosis. By contrast, TREM2 gene silencing intensified the inflammatory response, increased neuronal apoptosis and infarct volume, and further exacerbated neurological dysfunction. This study shows for the first time that TREM2 protects against cerebral ischemia/reperfusion injury in ischemic stroke. Pharmacological targeting of TREM2 to suppress the inflammatory response may provide a new approach fordeveloping therapeutic strategies inthe treatment of ischemic stroke and other cerebrovascular diseases.

Disclosures: Y. Tang: None.

Nanosymposium

664. Microglia in Nervous System Diseases

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Presentation Number: 664.03

Topic: B.12. Glial Mechanisms

Support: NINDS grant NS076620

Title: IFNbeta-mediated neuroprotection against ischemia is dependent on microglia

Authors: *A. MCDONOUGH, J. R. WEINSTEIN;
Dept. of Neurol., Univ. of Washington, Seattle, WA

Abstract: Ischemic preconditioning (IPC) is a brief period of ischemia that confers robust neuroprotection against subsequent ischemic events. Microglia, the resident immune cells in the CNS, play a significant role in the neuroinflammatory response to ischemia. Although microglial activation has typically been considered a pro-inflammatory process, recent publications suggest that microglia could play a protective role in IPC. Recent studies, including from our group, have implicated innate immune pathways, including Toll-like receptors (TLRs) and type 1 interferon (IFN) signaling in IPC-mediated protection. We have shown that intact interferon signaling in microglia is critical for protection in white matter models of ischemia. In this study, we aim to further characterize the importance of microglia and interferon signaling in IPC-mediated neuroprotection in mouse models of focal ischemia. We show that after IPC, microglia initiate a robust interferon-stimulated gene (ISG) response that is dependent on IFNAR1. We also show that IFNβ administration induces a similar ISG response and confers IPC-like neuroprotection against stroke. In this study we will characterize the potent IPC-like neuroprotective effects of IFNβ administration prior to stroke using a variety of methods, including transgenic mice with either systemic IFNAR1 knockout or microglial-specific IFNAR1 knockdown. These studies will help us better understand the role of type 1 IFN signaling in IPC with a focus on microglia specifically, which we hypothesize are the primary mediators of IPC-mediated neuroprotection. Our results suggest that type 1 IFN signaling is a critical component of IPC, and IPC-like neuroprotective effects can be achieved using non-invasive pharmacological agents.

Disclosures: A. McDonough: None. **J.R. Weinstein:** None.

Nanosymposium

664. Microglia in Nervous System Diseases

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Presentation Number: 664.04

Topic: B.12. Glial Mechanisms

Support: NINDS grant NS076620

Title: Ischemia modulates type 1 interferon signaling in microglia

Authors: ***J. R. WEINSTEIN**[1], A. MCDONOUGH[2], S. NOOR[2], R. LEE[2], T. MOELLER[2]; [1]Neurol., Univ. Washington, Seattle, WA; [2]Univ. of Washington, Seattle, WA

Abstract: ***Background***: Ischemic preconditioning (IPC) is a robust neuroprotective phenomenon in which a brief period of cerebral ischemia confers transient tolerance to subsequent ischemic challenge. Type 1 interferons (IFN), include the IFNα's and IFNβ, and are key cytokines in the innate immune response. We recently demonstrated that IPC-mediated neuroprotection in white matter was dependent on type 1 IFN signaling specifically in microglia (Hamner et al., *J. Neurosci* 2015). Here we explore the effects of ischemia on interferon signaling in microglia both *in vivo* and *in vitro*. ***Methods***: We performed 15 min middle cerebral artery occlusion (MCAO) or sham surgery on 12 - 14 week old *wild-type* or *IFNAR1*$^{-/-}$ male mice following established in vivo paradigms for IPC. We used *ex vivo* flow cytometry to isolate populations of microglia in cortex and quantified interferon stimulated gene (ISG) expression using qRT-PCR. *In vitro*, we exposed cultured mouse microglia to ischemia-like conditions and characterized ISG expression. We also examined ischemia-induced and IFNβ-induced changes in IFNAR1 surface expression and phosphorylation of STAT1 by flow cytometry. ***Results***: Both *in vivo* and *in vitro* assays demonstrated robust expression of ISGs in ischemia-exposed microglia. Prototypical ISGs including *ifit2*, *irf7*, *oas3* and *usp18* were up-regulated between four- and 32-fold *in vivo* and two- and eight-fold *in vitro*. Ischemia-induced ISG expression in microglia was completely dependent on expression of IFNAR1. Both ischemia-like conditions and IFNβ treatment *in vitro* induced complete elimination of IFNAR1 surface expression and phosphorylation of STAT1 in microglia. Stimulation with IFNβ also induced a broad and robust ISG response in microglia as well as dose-dependent release of ISG chemokines CXCL10 and CCL5. ***Conclusions***: Both our *in vivo* and *in vitro* microglia-specific, ischemia-targeted, datasets converged to indicate a robust ISG expression in microglia in response to ischemia. This finding is of particular significance given that IPC-mediated neuroprotection both in white matter and gray matter is dependent on type 1 IFN signaling.

Disclosures: **J.R. Weinstein:** None. **A. McDonough:** None. **S. Noor:** None. **R. Lee:** None. **T. Moeller:** None.

Nanosymposium

664. Microglia in Nervous System Diseases

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Support: JSPS KAKENHI 15H02522

Toray Science Foundation

Title: Spinal cord microglia activated by purinergic receptors and neuropathic pain

Authors: *M. TSUDA;
Kyushu Univ., Fukuoka, Japan

Abstract: In contrast to physiological pain, pathological pain is not dependent on the presence of tissue-damaging stimuli. One type of pathological pain - neuropathic pain - is often a consequence of nerve injury or of diseases. Optimal treatment of neuropathic pain is a major clinical challenge because the underlying mechanisms remain unclear and currently available treatments are frequently ineffective. A growing body of evidence has shown that this aberrant excitability may not merely be a consequence of changes in neurons, but rather of multiple alterations in microglia, which are resident macrophages in the central nervous system. In my talk, I highlight recent advances in our understanding of the mechanisms that underlie neuropathic pain caused by peripheral nerve injury with a specific focus on purinergic signaling in spinal cord microglia. These provide convincing evidence for a crucial role for microglial purinergic signaling in the pathogenesis of neuropathic pain, and P2 receptors may be potential therapeutic targets for managing neuropathic pain.

Disclosures: M. Tsuda: None.

Nanosymposium

664. Microglia in Nervous System Diseases

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Title: Spinal inhibition of microglial caspase-6/p38 signaling reduces neuropathic pain in male but not female mice: Sex-dependent microglial signaling in the spinal cord.

Authors: *T. BERTA[1], G. CHEN[2], S. TAVES[2], R. TONELLO[1], R.-R. JI[2];
[1]Anesthesiol., Univ. of Cincinnati, Cincinnati, OH; [2]Anesthesiol. and Neurobio., Duke Univ. Med. Ctr., Durham, NC

Abstract: Microglia are the resident immune cells in the spinal cord and brain. Mounting evidence suggests that activation of microglia plays an important role in the pathogenesis of chronic pain, including neuropathic pain after peripheral nerve injury. Our previous study has shown that caspase-6, a secreted protease that is expressed in dorsal root ganglia axonal terminals surrounding microglia, is a robust activator of microglia and induces profound release of TNF-α from microglia via activation of p38 MAP kinase. The activation of this microglial caspase-6/p38 signaling cascade consistently contributes to the generation of chronic pain in male mice.

Microglial activation was thought to be similar in both sexes, however, recent findings suggest distinct microglial contributions to chronic pain in male and female mice. Here we demonstrate that the caspase-6/p38 signaling in spinal microglia is sex-dependent in the chronic constriction injury (CCI) animal model of neuropathic pain. Our data show that (1) CCI induces similar increases in the expression levels of caspase-6 in dorsal root ganglia and microglial marker IBA1 in spinal cord of both sexes, but (2) microglial p38 activation is primarily observed in male mice and (3) intrathecal injection of selective p38 or caspase-6 inhibitors reduced neuropathic pain in male but not female mice. Sex-dependent role of caspase-6 in neuropathic pain was also confirmed in caspase-6 knockout mice. Furthermore, spinal cord expression of TNF-α was substantially reduced in caspase-6 male knockout mice.

These data suggest that targeting the CASP6/p38 MAPK/TNF-α signaling pathway may offer a new approach for the management of chronic pain by modulating microglial signaling. However, they also demonstrate a clear sexual dimorphism of this microglial signaling in mice emphasizing the importance of studying both sexes in pain research and the requirement of distinct approaches for the treatment of chronic pain in men versus women.

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Nanosymposium

664. Microglia in Nervous System Diseases

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Topic: B.12. Glial Mechanisms

Support: NIH GrantR01 NS088627

China NSFC U1201223

Title: Microglial regulation of region-specific synaptic alterations contributes to chronic pain and memory deficits

Authors: *Y. LIU[1,2,3], L.-J. ZHOU[2,3], J. WANG[2], M. MATTSON[4], L.-J. WU[3], X.-G. LIU[2]; [1]Lab. of Neurosci., Natl. Inst. On Aging, Baltimore, MD; [2]Zhongshan Sch. of Med. of Sun Yat-sen Univ., Guangzhou, China; [3]Rutgers, the State Univ. of New Jersey, Piscataway, NJ; [4]Lab. of Neurosci., Natl. institute on aging, Baltimore, MD

Abstract: Chronic pain patients have memory deficits and reduced hippocampal volume but the underlying mechanisms are poorly understood. Here we show that structural and functional synaptic connectivity and brain derived neurotrophic factor (BDNF) expression are significantly reduced in the hippocampus but enhanced in spinal dorsal horn in a mouse model of neuropathic pain after spared nerve injury (SNI). The SNI-induced region-dependent alterations were prevented by genetic deletion of tumor necrosis factor receptor 1 (TNFR1) in *vivo* and mimicked by TNF-α *in vitro*. Inhibition or ablation of microglia also prohibited the region-dependent changes and neuropathic pain and memory deficits induced by SNI. Our data suggests that microglial activation and TNF-α upregulation oppositely regulate the synaptic connectivity in the hippocampus and in dorsal horn. Therefore, inhibition of TNFR1 and microglia may provide a therapeutic benefit for both chronic pain and cognitive impairment caused by peripheral nerve injury.

Disclosures: **Y. Liu:** None. **L. Zhou:** None. **J. Wang:** None. **M. Mattson:** None. **L. Wu:** None. **X. Liu:** None.

Nanosymposium

664. Microglia in Nervous System Diseases

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Topic: B.12. Glial Mechanisms

Support: NIH Grant R01NS079166

NIH Grant R01DA036165

Title: Microglia activation contributes to HIV-1-gp120 induced synapse degeneration

Authors: *W. RU[1], S.-J. TANG[2];
[1]The Univ. of Texas Med. Br., Galveston, TX; [2]The Univ. of Texas Med. Br. At Galveston, Galveston, TX

Abstract: HIV-1 infection of the central nervous system (CNS) can lead to synaptic degeneration that likely underlies the development of cognitive impairments in HIV-1/AIDS patients. However, the mechanism(s) by which HIV-1 causes the synaptic degeneration is unclear. We hypothesize that microglia, the major immune responsive cells in the CNS, contribute to removing the damaged synapse on live neurons after HIV-1 infection. To test this hypothesis, we have determined the effects of HIV-1 coat protein gp120 on neuronal synapses in primary cortical cultures and mouse spinal cord. Using western blotting analysis, we detected the decrease of pre- and post-synaptic makers synapsin I and PSD95 following gp120 exposure. Interestingly, we found that gp120 caused microglial activation that was concomitant with the decrease of synaptic proteins. Blockage of microglia activation abolished the gp120-induced synapse loss. In addition, we observed that fractalkine, a chemokine that is specifically expressed in neurons and can regulate microglia activation, was up-regulated in gp120-treated primary cortical cultures. Disruption of FKN signaling attenuated gp120- induced synapse degeneration. These findings indicate that HIV-gp120 induces synapse degeneration via fractalkine-mediated microglial activation.

Disclosures: W. Ru: None. **S. Tang:** None.

Nanosymposium

664. Microglia in Nervous System Diseases

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Support: Deutsche Forschungsgemeinschaft (SFB TRR43)

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Title: Experimental cortical spreading depression induces NMDA receptor dependent potassium currents in microglia

Authors: *S. WENDT[1], E. WOGRAM[2], L. KORVERS[1], H. KETTENMANN[1];
[1]Cell. Neurosci., Max-delbrück-Center Berlin Buch, Berlin, Germany; [2]Inst. of Physiol. and Pathophysiology, Univ. of Heidelberg, Heidelberg, Germany

Abstract: Cortical spreading depression (CSD) is a propagating event of neuronal depolarization which is considered as the cellular correlate of the migraine aura. It is characterized by a change in the intrinsic optical signal and by a negative DC potential shift. Microglia are the resident macrophages of the central nervous system and act as sensors for pathological changes. In the present study we analyzed whether microglial cells might sense CSD by recording membrane currents from microglia in acutely isolated cortical mouse brain slices during an experimentally induced CSD. Coincident with the change in the intrinsic optical signal and the negative DC potential shift we recorded an increase in potassium conductance predominantly mediated by $K_{ir}2.1$ which was blocked by the NMDA receptor antagonist D-AP5. Application of NMDA and an increase in extracellular K^+ mimics the CSD induced K_{ir} activation. Application of D-AP5, but not the purinergic receptor antagonist RB2, blocks the NMDA induced K_{ir} activation. The K^+ channel blocker Ba^{2+} blocks both, the CSD and the NMDA triggered increase in K_{ir} channel activity. In addition we could confirm previous findings that microglia in the adult brain do not express functional NMDA receptors by recording from microglia cultured from adult brain. From these observations we conclude that CSD activates neuronal NMDA receptors which lead to an increase in extracellular $[K^+]$ resulting in the activation of K_{ir} channel activity in microglia.

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Nanosymposium

664. Microglia in Nervous System Diseases

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Topic: B.12. Glial Mechanisms

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CSCR15ERG015

Title: P2Y12 purinergic receptor mediates microglia-neuron communication in epilepsy

Authors: *L.-J. WU;
Cell Biol. & Neurosci., Rutgers The State Univ. of New Jersey, Piscataway, NJ

Abstract: Epilepsy represents a neurological disorder that can manifest in uncontrolled seizures in patients. Microglia are exquisitely sensitive to disruptions in the central nervous system. Since epilepsy is characterized by neuronal hyperactivity rooted in excessive glutamate release and ionic imbalance, it is conceivable that microglia respond to and regulate neuronal activities during the pathology. Here, we found an increased number of microglial primary processes in the hippocampus during kainic acid-induced seizure activity. Consistently, global glutamate induced robust microglial process extension (MPE) towards neurons making increased contact with neurons in both brain slices and in the intact brain in vivo. The mechanism of the glutamate-induced MPE involves the activation of neuronal NMDA receptors, calcium influx, subsequent ATP release, and microglial response through P2Y12 receptors. In addition, we serendipitously found that extracellular Ca^{2+} reduction induced microglial processes to converge at distinct sites, a phenomena we termed microglial process convergence (MPC). This novel MPC also happens in mouse cortex following kainic acid-induced seizure. Our studies further revealed that MPC occurs independent of astrocytic functions and are not directed towards astrocytes but target neuronal dendrites. Similar to glutamate-induced MPE, extracellular Ca^{2+}-dependent MPC is also mediated by ATP and microglial P2Y12 receptor. Finally, we found that P2Y12 deficiency abolished seizure-induced MPE and MPC but worsened kainic acid-induced seizure behaviors. These studies are the first to investigate the microglial dynamics and discovered MPE and MPC during acute epilepsy. Our results elucidate the molecular mechanisms underlying microglia-neuron communication that may be potentially neuroprotective in the epileptic brain. Studying microglia-neuron communication in epilepsy informs the development of novel therapies targeting microglia in the treatment of epileptic disorders.

Disclosures: L. Wu: None.

Nanosymposium

664. Microglia in Nervous System Diseases

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Presentation Number: 664.11

Topic: B.12. Glial Mechanisms

Support: New Jersey Commission on Brian Injury Research Fellowship CBIR14FEL001

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Title: The polarity protein Par1 in regulating microglia activation following TBI

Authors: *V. L. DIBONA[1], K. KRAUSE[1], M. SHAH[1], A. RAFALIA[1], W. ZHU[1], D. SMITH[2], D. CROCKETT[1], H. ZHANG[1];
[1]Rutgers The State Univ. of New Jersey, Piscataway, NJ; [2]Neurosci. Summer Undergraduate Res. Program (SURP), Rutgers The State Univ. of New Jersey, New Brunswick, NJ

Abstract: Microglia are the brain's resident immune cells that are rapidly activated following injury or illness. However, the mechanisms underlying microglial activation are still unclear. Microglia lose most of their spatial asymmetry and become rounded when they transform from a highly ramified state to an activated state. This raises the exciting possibility that proteins regulating cellular polarity/asymmetry are involved in the microglia activation process.
The partitioning-defective (Par) proteins are central regulators of polarity establishment in many different cellular contexts including embryogenesis, directional motility, axon specification and dendritic spine formation. Here, we show that one of the polarity regulators, the Ser/Thr kinase Par1, plays an important role in microglia activation in a controlled cortical impact (CCI) model of Traumatic Brain Injury (TBI). We show that following CCI injuries, Par1b KO mice show excessive microglia activation that spreads more distally from the injury site than wild-type (WT) controls. Interestingly, even sham-operated and naïve KO mice show changes in microglia activation, suggesting that the microglia in the Par1b KO mice are hypersensitized to insults. Further, knockdown of Par1 in primary microglia cultures resulted in morphological changes from a ramified to an amoeboid shape, reminiscent of activated microglia. Moreover, microglia depleted of Par1 phagocytized significantly more damaged neuronal particles than controls. Lastly, pharmacological stimulation of the Par1 pathway immediately following injury significantly recovers the negative behavioral and cellular outcomes of TBI. Treated mice had significant improvements in learning and memory, nesting behavior, and a reduction in microglia density and activated morphology after CCI. Taken together, these data suggest that decreased Par1 activity and expression leads to increased neuroinflammation and worsens injury outcomes.

Moreover, therapeutic stimulation of the Par1 pathway following injury can recover these negative outcomes.

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Nanosymposium

664. Microglia in Nervous System Diseases

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Presentation Number: 664.12

Topic: B.12. Glial Mechanisms

Title: TDP-43 depletion in microglia promotes phagocytosis of amyloid and induces synaptic loss in mice

Authors: ***R. PAOLICELLI**, A. VALERI, L. RAJENDRAN;
IREM, Inst. of Regenerative Med., Univ. of Zurich, Schlieren, Switzerland

Abstract: TDP-43 aggregates are common features of several neurodegenerative disorders including frontotemporal lobar degeneration (FTLD) and Alzheimer's disease (AD), both characterized by deposits of protein aggregates and synaptic loss. TDP-43 cytoplasmic inclusions are found in neurons and glia, however the specific role of TDP-43 in microglia cells *in vivo* has never been investigated. Microglia, major phagocytes of the brain, remove synapses during development and also mediate clearance of protein aggregates. Here we show that selective TDP-43 depletion in microglia enhanced lysosomal activity, which not only promoted phagocytosis and degradation of amyloid in a mouse model of AD, but also enhanced synaptic loss, suggesting a mechanistic role for microglial TDP-43 in the pathogenesis of neurodegenerative diseases.

Disclosures: **R. Paolicelli:** None. **A. Valeri:** None. **L. Rajendran:** None.

Nanosymposium

665. Therapeutic and Protective Strategies for Alzheimer's Disease

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Presentation Number: 665.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: European Research Council (E.R.C.) advanced grant (232835)

EU Seventh Framework Program (FP7) through the TargetBrain consortium (279017)

Title: Therapeutic potential of immune checkpoint blockade in mouse models of Alzheimer's disease

Authors: *K. BARUCH, N. ROSENZWEIG, M. SCHWARTZ;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder in which chronic neuroinflammation contributes to disease escalation. Nevertheless, although anti-inflammatory and immunosuppressive therapies have demonstrated some efficacy in neurodegenerative disease models, these treatments have largely failed in the clinic. Accordingly, we suggested that under neurodegenerative conditions systemic immune suppression curtails the ability to mount cell-mediated immune responses that are needed for brain repair. We show that boosting adaptive immunity in the 5XFAD and APP/PS1 AD mouse models, either by transient genetic depletion of $Foxp3^+$ regulatory T cells (Tregs), or by utilizing an immune checkpoint blockade approach directed against the programmed death-1 (PD-1) pathway, has a therapeutic effect which includes amyloid-β plaque clearance, mitigation of the neuroinflammatory response and reversal of cognitive decline. We further characterize this process to be associated with mounting an interferon (IFN)-γ-dependent systemic immune response, which affects the brain's choroid plexus, a selective gateway for immune cell trafficking to the CNS, and supports the subsequent recruitment of monocyte-derived macrophages to the brain. Our findings suggest that much like the situation in cancer immunotherapy, in order to mobilize immune cells to fight pathology, peripheral immunity should be boosted, rather than suppressed. Taken together, these findings identify immune checkpoint blockade as a potential therapeutic strategy for AD and, possibly, for other neurodegenerative diseases.

Disclosures: **K. Baruch:** None. **N. Rosenzweig:** None. **M. Schwartz:** None.

Nanosymposium

665. Therapeutic and Protective Strategies for Alzheimer's Disease

Location: SDCC 33C

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Presentation Number: 665.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NINDS NS079637

Title: TREM-2 induced microglial activation promotes the clearance of amyloid-beta in an AD mouse model

Authors: *J. GOOCH[1], T. L. SUDDUTH[1], E. M. WEEKMAN[1], I. TASSI[2], T. SCHWABE[2], S.-J. LEE[2], F. AVOGADRI-CONNORS[2], A. ROSENTHAL[2], D. M. WILCOCK[1];
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Abstract: Neuroinflammation is now recognized as a critical mediator of the neurodegenerative process of Alzheimer's disease and other chronic neurodegenerative conditions. Uncontrolled, chronic inflammatory processes of the central nervous system is likely to contribute to neurodegeneration, however, data also indicates that harnessing the ability of the immune system for clearance of pathological proteins such as amyloid deposits could be a target for therapeutic development. Indeed, anti-Aβ immunotherapy has leveraged such processes for amyloid clearance. Despite preclinical efficacy clinical trials of anti-Aβ immunotherapy, in clinical trials this approach seems to be most appropriate in early stage, preclinical prevention. We hypothesize that targeting neuroinflammation therapeutically may target multiple pathological processes that would provide clinical benefit later in the process.
Triggering receptor expressed on myeloid cells -2 (TREM2) is an innate immune receptor expressed on microglia which signals through DAP12 to trigger phagocytosis. TREM2 SNPs have been identified as significantly increasing risk of AD in GWAS studies. The hypothesis for this increased risk is that there is a loss of function, impairing the innate immune system to clear amyloid deposition efficiently. We hypothesized that activating TREM2 may engage the innate immune system. In the current study, we found that upregulation of TREM2 in vivo leads to the induction of of pro-inflammatory mediators in microglia and to a significant reduction in amyloid deposition. Other outcome measures ongoing include further assessment of neuroinflammatory responses, cerebral amyloid angiopathy (CAA) and cerebrovascular events. These preliminary data suggest that TREM2 may be a promising target for treatment of Alzheimer's disease.

Disclosures: J. Gooch: None. **T.L. Sudduth:** None. **E.M. Weekman:** None. **I. Tassi:** None. **T. Schwabe:** None. **S. Lee:** None. **F. Avogadri-Connors:** None. **A. Rosenthal:** None. **D.M. Wilcock:** None.

Nanosymposium

665. Therapeutic and Protective Strategies for Alzheimer's Disease

Location: SDCC 33C

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Presentation Number: 665.03

Topic: C.02. Alzheimer's Disease and Other Dementias

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Cure Alzheimer's Fund

Rose Hills Foundation

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Title: Enabling Alzheimer's Aβ phagocytosis by targeting IRAK-M innate immunity.

Authors: *A. W. VESLING[1], D. GATE[2], K. R. DOTY[1], B. P. LEUNG[1], J. RODRIGUEZ, Jr.[1], C. VAN'T VEER[3], E. MASLIAH[4], T. TOWN[1];
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Abstract: Alzheimer disease (AD) pathology includes deposition of amyloid-β (Aβ) peptides in β-amyloid plaques, neuronal injury, and chronic activation of brain innate immunity. Aβ engages toll-like receptors (TLRs) to stimulate host defense mechanisms, resulting in activation of microglia. TLRs transduce signals through MyD88 and the serine/threonine IL-1 receptor-associated kinases (IRAKs). Generally, the IRAK family activate TLR signaling-- with the notable exception of one inhibitory kinase, IRAK-M. Brain transcription of IRAK-M has been shown to be dysregulated in aging and in AD. Further, IRAK-M expression is specific to cells of monocytic lineage (including microglia) and plays a critical role in maintenance of innate immune homeostasis by dampening inflammation. Structurally, IRAK-M consists of an N-terminal death domain (DD), a kinase-like domain, and an unstructured C-terminal domain. IRAK-M cleavage removes the DD and has been shown to activate monocytes.
Methods: We evaluated TLR-IRAK-NF-κB signaling in post-mortem hippocampal lysates from AD and age-matched, non-demented controls. To assess the effect of IRAK-M cleavage on microglial signaling in the AD context, we stably (via lentivirus) introduced wild-type (WT) and mutant IRAK-M (lacking the N-terminal DD region; IRAK-MΔDD) into human microglia prior to stimulation with $A\beta_{1\text{-}42}$ micro aggregates.
Results: Western blot revealed significantly decreased full-length IRAK-M, while cleaved IRAK-M was increased in AD vs. control brains. Downstream TRAF6 and MEKK3 protein levels were also significantly higher in AD brains vs. controls. Similar to observations in AD

patients, expression of IRAK-MΔDD increased TRAF6 expression upon stimulation with Aβ and increased amyloid uptake. Furthermore, immunoprecipitation of TRAF6 revealed preferential binding to IRAK-MΔDD compared to WT IRAK-M. Finally, IRAK-MΔDD-expressing cells had robust expression of pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα), and decreased expression of the anti-inflammatory cytokine transforming growth factor-β1 (TGF-β1) after Aβ treatment.
Conclusions: Collectively, these data suggest that the IRAK-M DD critically referees microglial Aβ uptake and inflammatory gene expression. We hypothesize that increased cleavage of IRAK-M in the AD brain is a compensatory, though insufficient, response to cerebral amyloidosis. Activation of TRAF6 by the IRAK-M DD may represent a pharmacological target to enable cerebral innate immune remodeling of amyloid deposits in AD.

Disclosures: **A.W. Vesling:** None. **D. Gate:** None. **K.R. Doty:** None. **B.P. Leung:** None. **J. Rodriguez:** None. **C. van't Veer:** None. **E. Masliah:** None. **T. Town:** None.

Nanosymposium

665. Therapeutic and Protective Strategies for Alzheimer's Disease

Location: SDCC 33C

Time: Wednesday, November 16, 2016, 8:00 AM - 10:00 AM

Presentation Number: 665.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The anti inflammatory morphinan derivative sinomenine reduces tau pathology in P301S mutant tau transgenic mice

Authors: *L. ZHAO[1], M. WONG[1], H. ZHOU[2], R. WU[1], G. A. PETSKO[1], S. M. PAUL[1]; [1]Appel Alzheimer's Dis. Res. Institute, Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY; [2]State Key Lab. of Quality Res. in Chinese Medicine, Fac. of Chinese Med., Macau Univ. of Sci. and Technol., Macau, China

Abstract: Alzheimer's disease (AD) is characterized by progressive deposition of amyloid-β peptides (Aβ), intracellular accumulation of hyperphosphorylated tau-containing neurofibrillary tangles (NFTs), as well as chronic inflammation in affected brain regions. There is compelling evidence that neuroinflammation plays an important role in the pathogenesis of AD as it occurs early in vulnerable regions of AD brain. Epidemiologic studies also suggest that certain nonsteroidal anti-inflammatory drugs (NSAIDs) may lower the risk of AD, although the results have been inconsistent across studies and it is still unclear if (and which) and how NSAIDs reduce AD risk. Sinomenine (SIN) is a natural morphinan derivative extracted from the Chinese medicinal plant, *Sinomenium acutum*, which has potent anti-inflammatory and immunosuppressive activity and has been used to treat rheumatoid arthritis and other

inflammatory diseases for centuries in China & Japan. Pharmacokinetic studies have shown that SIN crosses the blood-brain barrier (BBB) and is readily detected in brain immediately after peripheral administration. Recently SIN has been shown to potently inhibit microglial activation *in vitro* as well as the production of reactive oxygen species, TNF-α and PGE2 via inhibition of NAPDH oxidase. Given the important role of microglia in AD pathogenesis and the potent anti-inflammatory effects of SIN, we examined the effects of SIN on tau pathology in the P301S mouse model of tau pathology. P301S mice develop abundant hyperphosphorylated tau, NFTs, neurodegeneration, and both microgliosis and astrogliosis. Here we report that intraperitoneal administration of SIN (50mg/kg) daily for 4 weeks markedly reduces RAB, RIPA, and 70% formic acid (FA) extractable pSer202/Thr204 tau as well as FA extractable pThr181 tau levels as measured by ELISA. The reduction of these pathological phospho-tau species following treatment with SIN was confirmed by immunostaining and immunoblotting. Total tau levels were not altered by SIN treatment. Furthermore, a significant decrease in expression of both the microglia marker Iba-1 and the astrocyte marker GFAP were observed in brain tissue of P301S mice treated with SIN, suggesting that the reduction in tau pathology is associated with reduced neuroinflammation. Taken together, our study demonstrates that the natural anti-inflammatory morphinan derivative SIN reduces both tau pathology and neuroinflammation in P301S mice. Further investigation of the anti-inflammatory and neuroprotective effects of SIN could shed light on the role glial inflammation plays in AD pathogenesis and may lead to a novel treatment for AD and other tauopathies.

Disclosures: **L. Zhao:** None. **M. Wong:** None. **H. Zhou:** None. **R. Wu:** None. **G.A. Petsko:** None. **S.M. Paul:** A. Employment/Salary (full or part-time): Voyager Therapeutic.

Nanosymposium

665. Therapeutic and Protective Strategies for Alzheimer's Disease

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Alzheimer’s Association Zenith Award ZEN-10-174633

ARCS Foundation and John Douglas French Alzheimer’s Foundation - Maggie McKnight Russell Memorial Postdoctoral Fellowship

Title: Nanoparticle blockade of TGF-β signaling in peripheral macrophages mitigates Alzheimer-like pathology in TgF344-AD rats.

Authors: *T. M. WEITZ[1], D. KIM[2], J. RODRIGUEZ, Jr.[1], T. FAHMY[3], T. TOWN[1]; [1]Zilkha Neurogenetic Institute, Keck Sch. of Med. of USC, Los Angeles, CA; [2]Rangel Col. of Pharm., Texas A&M Univ., College Station, TX; [3]Biomed. Engin., Yale Univ., New Haven, CT

Abstract: Transforming growth factor-beta (TGF-beta), a critical immunoregulatory cytokine, has increased abundance in AD patient brains. Our group has shown that genetic ablation of TGF-beta-Smad 2/3 signaling in peripheral macrophages causes their brain recruitment and resolution of cerebral amyloidosis, which does not come at the cost of damaging neuroinflammation. Currently, we are exploring whether pharmacological blockade of TGF-beta signaling in peripheral macrophages using next-generation nanoparticle technology can re-balance inflammation and mitigate AD-like pathology in the TgF344-AD rat model, which manifests the full spectrum of age-dependent AD pathologies and cognitive disturbance. To specifically target peripheral macrophages, we have developed PEG-PLGA nanoparticles encapsulating SB505124 (a small molecule TGF-beta-Smad 2/3 inhibitor) and the non-toxic fluorescent tracker, Coumarin-6 (designated nano-C6/SB). Results from in vitro experiments show that nano-C6/SB directly targets peripheral macrophages, effectively inhibiting TGF-beta signaling and increasing macrophage Abeta uptake. Two long-term peripheral treatment studies were performed with nano-C6/SB – one beginning treatment prior to plaque accumulation and the other beginning after/during plaque accumulation. Following treatment, aged TgF344-AD rats and controls were behaviorally tested and their brains were analyzed for AD-like pathology. We show that peripheral nano-C6/SB treatment: 1) promotes brain infiltration of C6-positive mononuclear phagocytes that localize to amyloid plaques; 2) attenuates cerebral amyloidosis and tauopathy; and 3) partially remediates cognitive deficits, with treatment regimen dominantly affecting outcome. Our results suggest that PEG-PLGA nanoparticles encapsulating small molecule TGF-beta-Smad 2/3 inhibitors hold pre-clinical promise to directly target peripheral macrophages for cerebral amyloid clearance.

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Nanosymposium

665. Therapeutic and Protective Strategies for Alzheimer's Disease

Location: SDCC 33C

Time: Wednesday, November 16, 2016, 8:00 AM - 10:00 AM

Presentation Number: 665.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CNPQ

Capes

Faperj

ISN

Title: Dopaminergic signaling counteracts cognitive deficits and depressive-like behavior in Alzheimer's disease models

Authors: *D. BECKMAN[1], L. E. SANTOS[1], M. V. LOURENCO[1], J. T. S. FORTUNA[2], A. F. BATISTA[1], S. BOSCHEN[4], J. F. CODOCEDO[5], P. CISTERNAS[5], C. DA CUNHA[4], N. INESTROSA[5], P. F. GARDINO[3], F. G. DE FELICE[1], S. T. FERREIRA[1,3];
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Abstract: Alzheimer's disease (AD) is a common form of dementia, affecting more than 35 million people worldwide and clinically characterized by cognitive deficits and memory loss. Soluble oligomers of the Aβ peptide (AβOs) accumulate in AD brains and in animal models of AD. AβOs are known for mediating synapse damage and memory loss in animal models, and are increasingly regarded as proximal synaptotoxins in AD. Physiologically, dopamine modulates memory and synaptic plasticity, but in the context of AD and memory impairment, its potential roles remain largely unexplored.. Here, we report that dopaminergic signaling is impaired in *in vitro* and *in vivo* models of AD, and selective activation of dopaminergic D1 receptors (D1R) restores behavioral, cognitive and synaptic deficits in such models. In cultured slices of both rodent and human brain cortex, exposure to AβOs resulted in loss of D1R-mediated cAMP production, and subsequent reductions in PKA activation, CREB phosphorylation, and BDNF production. Activation of D1Rs in cultured neurons with a selective agonist, SFK38393, prevented binding of AβOs and ensuing synaptic loss. In APP/PS1 mice, chronic administration of SFK38393 restored memory function and LTP. We further show that bupropione, a dopamine reuptake inhibitor, restores non-cognitive behavioral deficits, including depressive-like behavior and social isolation; important features of the disease, reproduced in different mouse models of AD. AD and major depressive disorder are highly prevalent neuropsychiatric conditions with intriguing epidemiological overlaps. Understanding the role of dopamine in AD may help connect cognitive and neuropsychiatric aspects of the disease, and provide novel targets for therapy.

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Nanosymposium

665. Therapeutic and Protective Strategies for Alzheimer's Disease

Location: SDCC 33C

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Presentation Number: 665.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: EUROSTARS-2 CIIP-20152001 EMTHERAPY

ADDF 20150202

Title: Development of ORY-2001, a dual LSD1/MAOB inhibitor, for the treatment of neurodegenerative disease

Authors: *T. MAES, F. CAVALCANTI, C. MASCARÓ, D. ROTLLANT, C. BUESA; R&D, ORYZON GENOMICS S.A., Cornella de Llobregat, Spain

Abstract: The aging of the Western population is increasing the incidence of neurodegenerative disease; as well as the burden of patient care and medication on families and on governmental budgets. Current drug treatments are essentially symptomatic, and none is able to prevent, halt or much less reverse the neurodegenerative process. ORY-2001 is an orally bioavailable, brain penetrable dual LSD1-MAOB inhibitor in development for the treatment of CNS diseases including Alzheimer's disease (AD). The lysine specific demethylase LSD1 is a component of the CoREST complex that, together with other epigenetic factors like HDAC1/2, is recruited by the transcription factor REST to repress the expression of neuronal genes. By reducing LSD1 activity, we aim to redress or circumvent transcriptional changes or imbalances in AD and other neurodegenerative diseases. MAO-B mediates dopamine metabolism and is a well known target for Parkinson's disease (PD), but increased MAO-B activity is also known to detrimental and to increase GABA production in reactive astrocytes in Alzheimer's disease (AD). We previously reported that ORY-2001 prevents the development of memory defects in the SAMP-8 mouse model for accelerated aging and AD. Treatment with ORY-2001 induced the expression of memory associated genes and reverted part of the transcriptional differences observed in the hippocampus of SAMP-8 mice relative to the SAMR1 reference strain. In particular, ORY-2001 down-regulated the expression of inflammation genes including *S100a9*, a pro-inflammatory protein emerging as an important contributor to inflammation-related neurodegeneration. *S100a9* was over-expressed in the SAMP-8 model, but the protein has also been described to be increased in the brain of patients with AD, postoperative cognitive dysfunction (POCD), traumatic brain injury (TBI), and in multiple sclerosis (MS). Interestingly, knockout or knockdown of *S100a9* has been shown to be beneficial to memory in APP/PS1 and Tg2576 models of AD, and inhibition of the interaction of S100A9 with TLR4 has been shown to be beneficial in a model of experimental autoimmune encephalomyelitis. Here, we evaluate the pharmacological relevance of the anti-inflammatory component of ORY-2001 by hypothesis-

based testing of the compound in additional animal models described to present alterations in the expression of the S100A9 biomarker, and demonstrate the validity of an epigenetics based strategy to modulate *S100a9* activity in CNS disease. ORY-2001 is currently undergoing a Phase I clinical trial in young and elderly healthy volunteers, and long-term GLP toxicology studies to enable future Phase II studies are in progress.

Disclosures: **T. Maes:** A. Employment/Salary (full or part-time): ORYZON GENOMICS EMPLOYEE. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ORYZON GENOMICS SHAREHOLDER. F. Consulting Fees (e.g., advisory boards); ADDF REVIEW BOARD. **F. Cavalcanti:** A. Employment/Salary (full or part-time): ORYZON GENOMICS employee. **C. Mascaró:** A. Employment/Salary (full or part-time): ORYZON GENOMICS S.A. **D. Rotllant:** A. Employment/Salary (full or part-time): ORYZON GENOMICS employee. **C. Buesa:** A. Employment/Salary (full or part-time): ORYZON GENOMICS employee. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ORYZON GENOMICS shareholder.

Nanosymposium

665. Therapeutic and Protective Strategies for Alzheimer's Disease

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Time: Wednesday, November 16, 2016, 8:00 AM - 10:00 AM

Presentation Number: 665.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Collaborative Research to Japan Bio Products, Co., Ltd.

Title: The human placenta-derived drug, Laennec, improves cognitive dysfunction in a mouse model of Alzheimer’s disease

Authors: *C. TOHDA, C. KOGURE;
Inst. of Natural Medicine, Univ. of Toyama, Toyama, Japan

Abstract: Previous studies from our lab identified several compounds derived from herbal medicines that improve memory performance and reduce axonal and presynaptic neuronal degeneration in a mouse model of Alzheimer’s disease (AD). However, all of the herb-derived active compounds identified in those studies were of a low molecular weight and involved different signaling pathways to induce memory enhancement. In this study, we wanted to investigate the range of anti-AD mechanisms by identifying high molecular weight anti-AD compounds. Therefore, we focused on the human placenta drug Laennec because it contains both high (peptides) and low (amino acids) molecular weight compounds. The objective of this study

was to investigate the anti-AD effects of Laennec using the 5XFAD mouse model of AD and Abeta-treated cortical neurons. The Laennec-treated (15 days, i.p. or p.o.) 5XFAD (5 - 7 months old, female) mice showed significant improvement in object recognition memory. There was significant atrophy in the axons and dendrites of the cortical neurons (ddY mice, E14) after 3 days of Abeta (25-35) treatment. Laennec significantly improved the density and dendritic length of the Abeta-treated cortical neurons but did not affect the atrophy of the axons. Furthermore, we separated the components of Laennec according to their molecular weight using ultrafiltration. An approximately 10 kDa fraction of Laennec contained the active compounds for dendrite extension. Using SDS-PAGE, silver staining, and nano LC-MS/MS analysis, we observed that the active fraction contained fragmented human collagen alpha-1(I) chains, which is a protein with several repeated amino acid sequences. We identified one 7 amino acid* active peptide, which improved memory dysfunction in the 5XFAD mice. In conclusion, we found that Laennec improved object recognition memory and dendritic growth in a model of AD. In addition, we found that a short peptide, derived from a human collagen alpha-1(I) chain, may be one of the active substances in Laennec that contributes to improvements in AD. *The sequence of the peptide is not disclosed due to a pending patent.

Disclosures: **C. Tohda:** 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; Japan Bio Products Co., Ltd.. **C. Kogure:** None.

Nanosymposium

666. Therapeutic Potential in Motor Neuron Disease

Location: SDCC 32B

Time: Wednesday, November 16, 2016, 8:00 AM - 9:30 AM

Presentation Number: 666.01

Topic: C.05. Neuromuscular Diseases

Support: Wellcome Trust (089701)

Guy's & St. Thosmas' Clinical Neuroscience PhD studentship

Title: Exploring the use of antisense oligonucleotides in iPSC-derived neurons as a therapy for C9orf72-linked Amyotrophic Lateral Sclerosis/Frontotemporal Dementia

Authors: ***J. GOMEZ**[1,2], Y.-B. LEE[2], A. NISHIMURA[2], J. GREIG[2], J.-M. GALLO[2], C. SHAW[2];
[1]King's Col. London, London, United Kingdom; [2]Basic and Clin. Neuroscience, Kings Col. London, Maurice Wohl, Basic and Clin. neuroscience Inst., London, United Kingdom

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterised by the loss of motor neurons in the brain and spinal cord causing progressive paralysis and death within an average of 3 years from symptom onset. Expansion of an intronic hexanucleotide (G_4C_2) repeat in the chromosome 9 open reading frame 72 (*C9orf72*) gene is the most common cause of ALS as well as of frontotemporal dementia (FTD). The number of repeats varies from 300-3000 in ALS/FTD cases compared to up to 30 repeats in healthy individuals. Hexanucleotide repeat-expanded RNA accumulates into nuclear foci and is also exported to the cytoplasm for translation into dipeptide repeat proteins by repeat-associated non-ATG translation (RAN).We have tested the use of antisense oligonucleotides (ASOs) targeting the C9orf72 transcript as a possible therapeutic strategy for C9orf72-linked ALS/FTD.ASOs were tested in induced pluripotent stem cell (iPSC)-derived cortical neurons from three ALS-C9orf72 expansion carriers and healthy controls. A significant reduction (52%-88%) in the number of nuclear RNA foci was observed after seven day treatment with the ASOs. Neurite length after seven days is culture is reduced by up to 42% in neuroprogenitor cells derived from expansion carriers compared to controls. This mutation-specific phenotype is reversed following seven day incubation with C9orf72-targeting ASOs compared to control ASOs. Our results suggest that C9orf72 targeting ASOs may provide an effective therapeutic strategy for C9orf72-linked ALS/FTD.

Disclosures: J. Gomez: None. **Y. Lee:** None. **A. Nishimura:** None. **J. Greig:** None. **J. Gallo:** None. **C. Shaw:** None.

Nanosymposium

666. Therapeutic Potential in Motor Neuron Disease

Location: SDCC 32B

Time: Wednesday, November 16, 2016, 8:00 AM - 9:30 AM

Presentation Number: 666.02

Topic: C.05. Neuromuscular Diseases

Support: German BMBF

Title: The TGFb System as an important mediator in the disease Progression of ALS

Authors: *S. PETERS, S. KUESPERT, E. ZITZELSPERGER, R. HEYDN, T.-H. BRUUN, U. BOGDAHN;
Dept. for Neurol., Univ. Hosp., Regensburg, Germany

Abstract: Neurodegenerative disorders including amyotrophic lateral sclerosis (ALS) exhibit as a specific characteristic an enhanced pro-inflammatory milieu with increased liquor concentrations of Transforming Growth Factor beta (TGF-ß). In combination with promoting the

degeneration of neurons, these immunological alterations inhibit neurogenesis and drive stem cell quiescence. Despite an increasing knowledge and continuously growing research interest, the heterogenic ethiology of this disorder in combination with a lack of validated biomarkers aggravates the effective treatment of this “orphan disease”.
Here, we first investigated the activation state of the endogenous TGF-ß system in post mortem spinal cord (SC), motor cortex (MC), and occipital lobe (OL) homogenates (kindly provided by Prof. Dr. Thal and Prof. Dr. Petri, both MND network Germany) from ALS patients and controls. Therefore, the expression patterns of TGF-$\beta R_{I,II,III}$, the ligands TGF-β_1, TGF-β_2 and the most important downstream molecules p-Smad 2/3, Smad 4, Smad 1/5, Smad 8 were determined via qRT-PCR and Western Blot analysis.
CTGF as an important downstream-molecule within the TGF-ß system mediates the fibrotic effects of TGF-ß by inducing the deposition of ECM and modulating the reorganization of actin-cytosceleton. Since these pathogenic modifications of the extracellular matrix (ECM) and the actin-cytosceleton often correlate with diseases progression and disease severity, the expression levels of connective tissue growth factor (CTGF) were analysed within the tissue mentioned earlier. In addition, to investigate whether fibrosis is involved in the progression of ALS, we examined the expression profile of the two main components of ECM, Fibronectin (FN) and Collagen IV (CollV). The differences of the ECM and the actin-cytosceleton of ALS patients and controls within the three different tissues were obtained by qRT-PCR, Western Blot analysis, and immunofluoreszense.
Taken together, the results of the current study might shed some light on possible pathways mediating disease progression and provide possible purchases for treatment options in ALS.

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Nanosymposium

666. Therapeutic Potential in Motor Neuron Disease

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Presentation Number: 666.03

Topic: C.05. Neuromuscular Diseases

Title: Evaluating Epha4 as a therapeutic target for amyotrophic lateral sclerosis by antisense-mediated inhibition in the adult mouse CNS

Authors: *K. LING[1], M. NORRBOM[1], A. MCCAMPELL[2], F. RIGO[1];
[1]Ionis Pharmaceuticals, Inc., Carlsbad, CA; [2]Biogen, Cambridge, MA

Abstract: The Epha4 receptor tyrosine kinase is a negative regulator of axonal growth. Genetic ablation of Epha4 in mice has previously been shown to promote regeneration in the central and peripheral nervous system, and attenuate disease phenotypes in a mouse model of Amyotrophic Lateral Sclerosis (ALS), a fatal neurodegenerative disease. To evaluate the therapeutic potential of Epha4 for ALS we developed antisense oligonucleotides (ASOs) to specifically reduce the expression of Epha4 in the CNS of adult mice. Antisense-mediated reduction of Epha4 in the brain and spinal cord of wild-type mice, by intracerebroventricular administration of ASO, promoted re-innervation and functional recovery after sciatic nerve crush. In contrast, lowering of Epha4 in the CNS of a mouse model of ALS ($SOD1^{G93A}$) did not improve their motor function or modify their disease onset, progression and survival. Despite its role in promoting axonal regeneration, our data demonstrates that lowering Epha4 in the adult CNS may not be a viable strategy for treating ALS.

Disclosures: **K. Ling:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals, Inc. **M. Norrbom:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals, Inc. **A. McCampell:** A. Employment/Salary (full or part-time): Biogen. **F. Rigo:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals, Inc..

Nanosymposium

666. Therapeutic Potential in Motor Neuron Disease

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Presentation Number: 666.04

Topic: C.05. Neuromuscular Diseases

Support: NIH/NINDs Grant 1R01NS090962

Title: Human bone marrow stem cell transplantation for repair of the blood-spinal cord barrier in symptomatic ALS mice: optimization of cell dose

Authors: *S. N. GARBUZOVA-DAVIS[1], C. KURIEN[2], N. TAJIRI[2], A. THOMSON[2], D. FALCO[2], S. AHMAD[2], J. STAFFETTI[2], G. STEINER[2], S. ABRAHAM[2], G. JAMES[2], A. MAHENDRASAH[2], S. H. APPEL[4], C. V. BORLONGAN[3];
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a disease characterized by degeneration of motor neurons in the brain and spinal cord. Vascular pathology, including damage of the blood-brain barrier (BBB) and blood-spinal cord barrier (BSCB) via endothelial cell degeneration, is one recently recognized hallmark of ALS pathogenesis. Repairing blood-CNS barrier damage by replacement of endothelial cells via systemic cell administration may be a new therapeutic approach for ALS. The aim of this study was to determine the effect of intravenous transplantation of human bone marrow CD34+ (hBM34+) cells, a source of endothelial progenitor cells, into symptomatic G93A SOD1 mice. Three different doses of hBM34+ cells ($5x10^4$, $5x10^5$, or $1x10^6$) were transplanted via jugular vein into G93A mice at 13 weeks of age. Mice underwent weekly pre-transplant and post-transplant behavioral testing of motor function and monitoring of body weight. Cell-treated, Media-treated, and control mice were perfused at 17 weeks of age, corresponding to 4 weeks post-transplant. The cervical and lumbar spinal cords were removed, post-fixed, and then capillary ultrastructural (electron microscopy, EM), immunohistochemical (anti-human von Willebrand factor), and histological (cresyl violet) analyses were performed. The results demonstrated that systemic administration of hBM34+ cells into symptomatic ALS mice at different doses delayed disease progression as determined by behavioral outcomes and motor neuron survival at 4 weeks post-transplant. The beneficial effects coincided with maintained body weight, delayed deterioration of hindlimb extension, delayed loss of muscle strength, and longer latency on rotarod in all cell-treated ALS mice vs. Media mice for the entire post-transplant period. More significant functional improvements were determined mainly in mice receiving $1x10^6$ cells. Delayed disease progression in G93A mice via hBM34+ cell transplantation at symptomatic stage was confirmed by superior motor neuron survival in the ventral horns of spinal cords in all cell-treated animals. Immunohistochemical analysis revealed that transplanted cells differentiated into endothelial cells and engrafted within the vascular lumen in mice mainly treated with $1x10^6$ cells, possibly leading to repair of BSCB in ALS mice. This repair was confirmed by EM analysis showing restoration of capillary integrity in the spinal cord. Together, our results demonstrated transplantation of hBM34+ cells with optimal cell dose can restore BSCB integrity in symptomatic ALS mice and might have potential as a future therapeutic strategy for ALS patients.

Disclosures: **S.N. Garbuzova-Davis:** None. **C. Kurien:** None. **N. Tajiri:** None. **A. Thomson:** None. **D. Falco:** None. **S. Ahmad:** None. **J. Staffetti:** None. **G. Steiner:** None. **S. Abraham:** None. **G. James:** None. **A. Mahendrasah:** None. **S.H. Appel:** None. **C.V. Borlongan:** None.

Nanosymposium

666. Therapeutic Potential in Motor Neuron Disease

Location: SDCC 32B

Time: Wednesday, November 16, 2016, 8:00 AM - 9:30 AM

Presentation Number: 666.05

Topic: C.05. Neuromuscular Diseases

Support: Academy of Finland

Jane and Aatos Erkko foundation

The Ella and Georg Ehrnrooth foundation

Finnish Cultural foundation

Title: Effect of CDNF administration in mouse model of Amyotrophic Lateral Sclerosis

Authors: *F. DE LORENZO, M. H. VOUTILAINEN, E. MONTONEN, A. SAUKKONEN, M. AIRAVAARA, R. K. TUOMINEN, D. LINDHOLM, M. SAARMA;
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive degeneration of motor neurons (MN) in the ventral horn of the spinal cord, brainstem and motor cortex, which leads eventually to paralysis and muscular atrophy in the affected individuals. Patients usually die within 3-5 years of symptom onset and death is caused, in most cases, by the paralysis of respiratory muscles. Neither cure nor effective therapy is currently available.

The aim of this study was to evaluate the effect of novel cerebral dopamine neurotrophic factor (CDNF) administration in SOD1-G93A mouse model of ALS. Neurotrophic factors (NTFs) are known to promote survival of MNs *in vitro* and *in vivo*; in particular CDNF is highly expressed in muscle, spreads better than other NTFs in brain tissue and it´s crucially involved in the regulation of ER stress, which plays an important role in in the pathophysiology of ALS. Single intracerebroventricular injection of human recombinant CDNF can significantly postpone the appearance of clinical symptoms and increase lifespan in mice, as well ameliorate motor function as assessed by different motor tests. Immunohistochemistry analyses of post-mortem tissues show that CDNF administration can prevent death of MNs in lumbar spinal cord compared to vehicle treated controls. Furthermore, CDNF can preserve neuromuscular junctions (NMJs) innervation and integrity as determined with staining of pre- and post-synaptic terminal in the gastrocnemius muscle.

We conclude that CDNF seems to have a strong protective effect in SOD1-G93A mouse model of ALS, promoting survival of MNs and preservation of NMJs, thus resulting in improved motor

coordination and mice survival. On the base of our encouraging data, CDNF holds promise as a therapeutic candidate for the treatment of ALS.

Disclosures: F. De Lorenzo: None. **M.H. Voutilainen:** None. **E. Montonen:** None. **A. Saukkonen:** None. **M. Airavaara:** None. **R.K. Tuominen:** None. **D. Lindholm:** None. **M. Saarma:** None.

Nanosymposium

666. Therapeutic Potential in Motor Neuron Disease

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Presentation Number: 666.06

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Stiftung Volkswagenwerk

LUMINOUS European Union's Horizon 2020 research and innovation programme under the grant agreement No 686764

Title: Brain machine interface performance in the completely locked-in-state (CLIS)

Authors: *N. BIRBAUMER[1,2], M. PRATS SEDANO[1], A. RAKHIMKULOVA[1], U. CHAUDHARY[1];
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Abstract: Four ALS (amyotrophic lateral sclerosis) patients in CLIS learned to respond with a brain oxygenation and deoxygenation change of frontal brain areas using portable NIRS (near infrared spectroscopy) to short questions requiring a yes or no response presented auditorily within 15 seconds.CLIS duration in the four patients has lasted from 4 months to eight years and was validated with EOG measurement during all sessions.Each session contained 20 to 60 questions (half with yes and half with no answers). All experiments take place at the home of patients. Questions with known answers were used to train a support vector machine classifier (SVM). After achieving 70% correct answers open questions were asked and feedback of the

classified answer was provided to the patients. EEG from 6 electrodes served to control sleep and vigilance decrement: questions were interrupted if sleep-like patterns appeared. 16 to 60 sessions over several months assured stability of communication with an average correct resonse rate of more than 70% to known and 90% correct answers to open questions.Among open questions qualtity of life questions were asked on a weekly basis to three of the patients with longer CLIS duration, all patients report good quality of life as previously reported by our group. Open questions answers are validated by stability over time, information of family and care takers, sentences with semantic errors and face validity (i.e. pain questions during periods of intense pain due to decubitus and other illness related problems). These results suggest that brain machine interfaces using metabolic brain signals may end the unbearble silence of CLIS.

Disclosures: **N. Birbaumer:** None. **M. Prats Sedano:** None. **A. Rakhimkulova:** None. **U. Chaudhary:** None.

Nanosymposium

667. Representation of Features and Objects Along the Ventral Stream

Location: SDCC 1B

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Presentation Number: 667.01

Topic: D.06. Vision

Support: KAKENHI 22135007

KAKENHI 25871171

KAKENHI 16H01683

Title: Representation of glossy material surfaces in common marmoset temporal visual cortex

Authors: ***N. MIYAKAWA**[1], T. BANNO[2], H. ABE[3], T. TANI[3], W. SUZUKI[1], N. ICHINOHE[1,3];
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Abstract: To address the cortical processing of visual information related to material surface, we presented a set of stimuli that have identical three-dimensional shapes (bone, torus or amorphous) but different material appearances (ceramic, glass, fur, leather, metal, stone, wood, or matte) to anesthetized marmoset monkeys (*Callithrix jacchus*), and recorded multi-unit activities from a ventral part of the superior temporal area (FSTv) with multi-shanked, and depth resolved silicone probes. In all 4 animals recorded, we found a small subregion where cells

showed a significant main effect in the material appearance (2-way ANOVA, "object shape" x "material appearance", $p<0.05$), and the best stimulus was glossy materials (glass or metal). In 3 animals, we injected a retrograde fluorescent tracer to the gloss-selective subregion. One week later, we identified multiple retrogradely labeled spots posterior to the injected site by *in vivo* fluorescent imaging. Later histological staining confirmed these spots lay in areas MST, MT crescent (MTc), but not in MT. In 2 animals, we recorded visually evoked activities simultaneously with one probe in the FSTv gloss-selective subregion and another in the retrogradely labeled spot in MTc. Neurons from the retrogradely labeled MTc spot also showed selectivity to glossy images. We regressed the neural responses to multiple low-level image statistics of the stimulus set that relates to shape, color, and parameters of the pixel luminance distribution. The neural responses of gloss-selective subregion in FSTv and of interconnected one in MTc strongly correlated with the skewness of the luminance distribution. Mean, negative kurtosis and variance of the luminance distribution showed a smaller, but significant contribution as well. Responses to pixel-shuffled controls, which have skewed luminance distribution identical to the original images but evoke no percept of glossiness, were reduced largely in the injected subregion in FSTv, but only moderately in the retrogradely labeled MTc spot. Our results suggest that 1) there is neural connection from MTc to FSTv which carries visual information that strongly influence gloss perception, and 2) FSTv neural responses linked to gloss perception seemed to be modulated by unknown feature(s), other than the parameters of pixel luminance distribution.

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Nanosymposium

667. Representation of Features and Objects Along the Ventral Stream

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Presentation Number: 667.02

Topic: D.06. Vision

Support: Burroughs-Wellcome Fund (CRP)

NIH F32EY025523 (TH)

NIH EY016187 (MSL)

Title: End-stopping as a principle for functional organization in areas V1, V4 and posterior IT

Authors: *C. R. PONCE[1], T. HARTMANN[2], M. S. LIVINGSTONE[2];
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Abstract: Primate inferotemporal cortex (IT) is subdivided into domains responsive to naturally occurring categories, such as faces and scenes. With training, IT cortex will also develop domains for artificial categories, such as text. Interestingly, these training-induced domains develop in the same stereotyped IT positions in both humans and monkeys. Previously, we investigated why these regions arise in stereotyped locations within IT regardless of training history, and found that domain positions are partially determined by the shape of their preferred stimuli. Images with curved contours elicit responses in the superior temporal cortex (STS) and along the lower lip of the STS, while images with straight edges elicit strong responses in ventral IT. This raises the question of why curvature preferences should be distributed along IT at all. Using fMRI, we found that this curvature gradient correlates with eccentricity along the visual hierarchy, from V1 through IT. fMRI regions that prefer images located near the center of gaze also prefer images with curvature whereas regions representing more peripheral visual field responded better to rectilinear stimuli. This relationship could be explained if this curvature gradient is driven by receptive field (RF) size distribution. Neurons with central RFs have smaller receptive fields compared to neurons with more peripheral RFs. Because many neurons are end-stopped (or 'hypercomplex' in Hubel & Wiesel's terminology), this size gradient includes not only RF size and spatial frequency, but also selectivity for stimulus length. End-stopping confers selectivity for short, curved, or bent contours compared to long straight contours, proportional to RF size. Consequently, smaller receptive fields may be better at encoding sharp changes in curvature, and larger RFs may be better at encoding long, straight edges. To find out whether curvature selectivity could be explained by end-stopping, we recorded from over 1,100 cortical sites across areas V1, V4 and posterior inferotemporal cortex (five monkeys). We presented banana gabors with different curvatures, orientations and diameters, and analyzed the responses from single- and multiunit sites. We found that units that preferred high curvature also showed a higher degree of end-stopping; this correlation was found in every recorded area. This result suggests that a retinotopic map and the accompanying gradation in receptive field size is a primary determinant for the functional architecture of the ventral stream. Face, body and scene patches acquire their location based on object curvature, which is dictated by receptive field size and eccentricity.

Disclosures: C.R. Ponce: None. **T. Hartmann:** None. **M.S. Livingstone:** None.

Nanosymposium

667. Representation of Features and Objects Along the Ventral Stream

Location: SDCC 1B

Time: Wednesday, November 16, 2016, 8:00 AM - 11:15 AM

Presentation Number: 667.03

Topic: D.06. Vision

Title: Representation of medial axis configurations in lateral occipital complex

Authors: *C. E. CONNOR[1], H. N. TOKOZOGLU[2], A. W. SALI[3], B. A. ANDERSON[3], S. YANTIS[3];
[1]Krieger Mind/Brain Inst., Johns Hopkins Univ., Baltimore, MD; [2]Johns Hopkins Univ., Baltmore, MD; [3]Johns Hopkins Univ., Baltimore, MD

Abstract: Our previous neural recording studies in monkeys have shown that the ventral visual pathway, which culminates in inferotemporal cortex (IT), represents objects as configurations of medial axis and surface components (Yamane et al., Nature Neuroscience, 2008; Hung et al., Neuron, 2012). The putative homologue for IT in the human brain is the lateral occipital complex (LOC), which is functionally defined by differential responses to intact and scrambled object photographs. Here, we used fMRI to test whether human LOC, like IT, represents medial axis structure. Letter-like stimuli were constructed from medial axis components (straight and curved line segments). Stimuli were presented in random order to subjects in the scanner as they performed a one-back shape-matching task. We used a generalized linear model (GLM) to estimate the response pattern of each LOC voxel across stimuli. For each voxel, we used backwards stepwise regression to model the response pattern as a linear combination of partial medial axis configurations that appeared as fragments in multiple stimuli. We constrained model complexity using cross-validation, so that most contained 3-6 fragment terms. For approximately 300 voxels across 3 subjects, these models captured 25-85% of the response variance, and each model captured significantly ($p < 0.05$) more variance than expected by chance based on a randomization test. In contrast, models for voxels in functionally defined Visual Word Form Area (VWFA) were almost uniformly below this significance level. In addition, we showed that population response patterns across voxels could predict the probabilities of fragment presence at levels above chance ($p < 0.05$) for a majority of stimuli. These results indicate that human LOC, like its monkey homologue IT, carries explicit information about the medial axis structure of objects.

Disclosures: C.E. Connor: None. **H.N. Tokozoglu:** None. **A.W. Sali:** None. **B.A. Anderson:** None. **S. Yantis:** None.

Nanosymposium

667. Representation of Features and Objects Along the Ventral Stream

Location: SDCC 1B

Time: Wednesday, November 16, 2016, 8:00 AM - 11:15 AM

Presentation Number: 667.04

Topic: D.06. Vision

Title: Mid-level features are sufficient to drive the animacy and object size organization of the ventral stream

Authors: *B. LONG, T. KONKLE;
Dept. of Psychology, Harvard Univ., Cambridge, MA

Abstract: The ventral stream exhibits a large-scale organization by the conceptual dimensions of animacy and real-world size (Konkle & Caramazza, 2013). However, animates vs. inanimates have different mid-level perceptual features, as do small vs. big objects – animals tend to be curvier than artifacts, and big objects tend to be boxier than small objects (Long et al., 2016). Here, we examined whether these mid-level feature differences are sufficient to drive this animacy and object size organization. We created unrecognizable versions of big animals, big objects, small animals, and small objects using a texture synthesis model. These images preserve mid-level information about texture and form ("texforms"), yet are unrecognizable at the basic-level.

Eight observers underwent functional neuroimaging while viewing recognizable images and texforms. To examine ventral stream organization, we used independent data to isolate object-responsive voxels in occipito-temporal regions outside of early visual cortex. Next, we calculated voxel-wise animacy preferences (animals – objects) and real-world size preferences (small objects – big objects) for both recognizable images and texforms. Finally, we used a map correlation analysis to assess how similar animacy and real-world size topographies were for recognizable images and texforms.

Overall, we found that recognizable images and texforms generated highly similar large-scale organizations across all of occipito-temporal cortex. Voxels had similar animate-preferences for recognizable and texform images within single subjects, resulting in highly reliable map correlations at the group level (M=.74, SD=.063, $t(7)$=20.37, p<0.001). The same same pattern of results was observed for object size (M=.43, SD=.17, $t(7)$=6.88, p<0.001).

These results show that the organization by animacy and object size in ventral cortex does not rely on intact basic-level recognition. Broadly, these results imply that mid-level perceptual information is represented along the ventral stream well beyond early visual areas.

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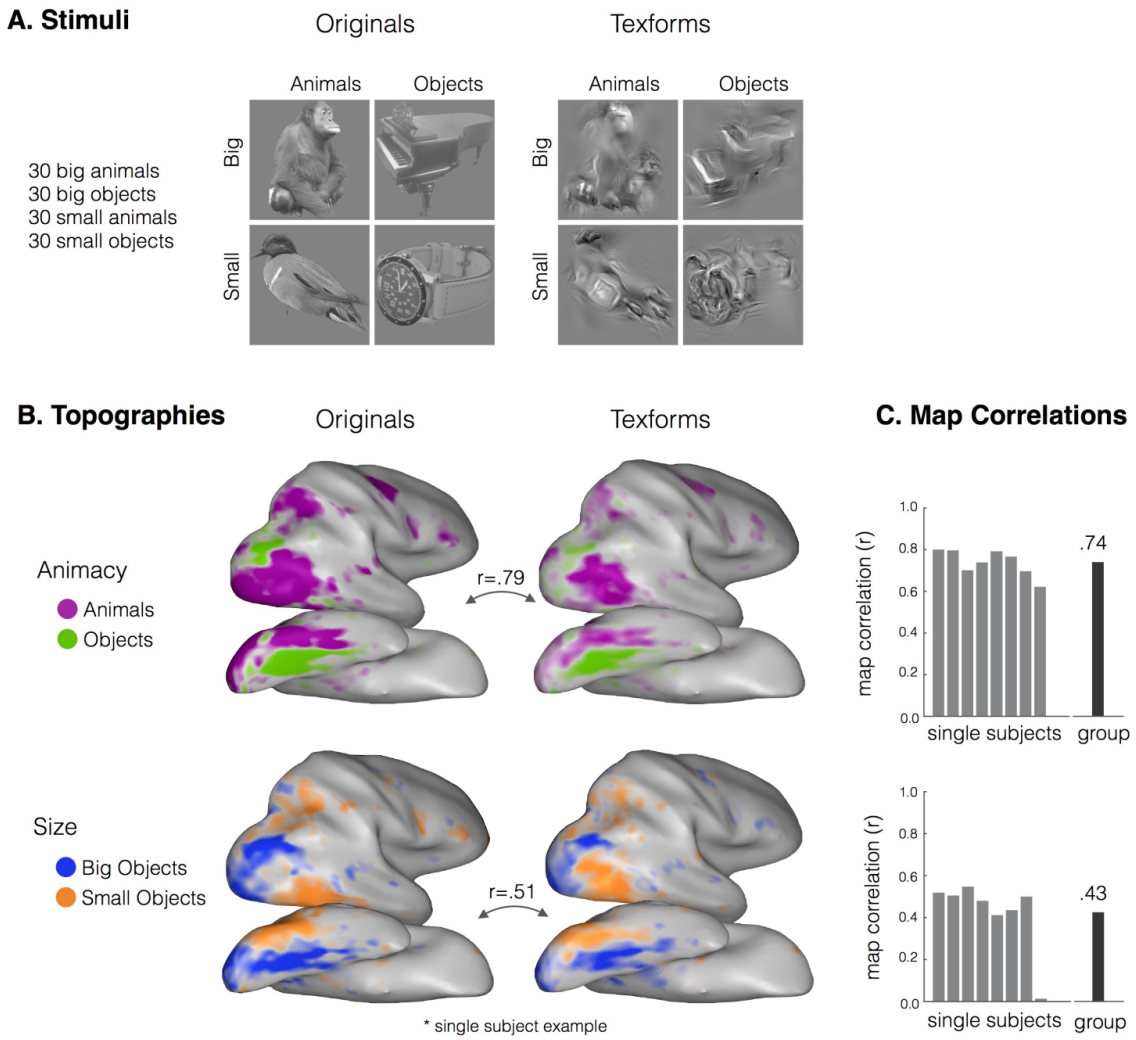


Disclosures: **B. Long:** None. **T. Konkle:** None.

Nanosymposium

667. Representation of Features and Objects Along the Ventral Stream

Location: SDCC 1B

Time: Wednesday, November 16, 2016, 8:00 AM - 11:15 AM

Presentation Number: 667.05

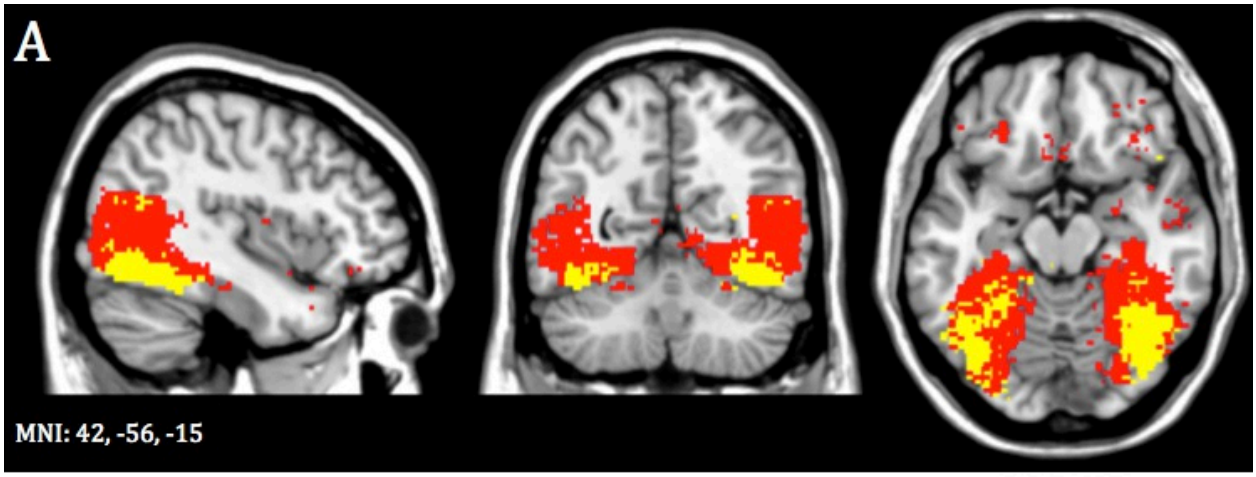
Topic: D.06. Vision

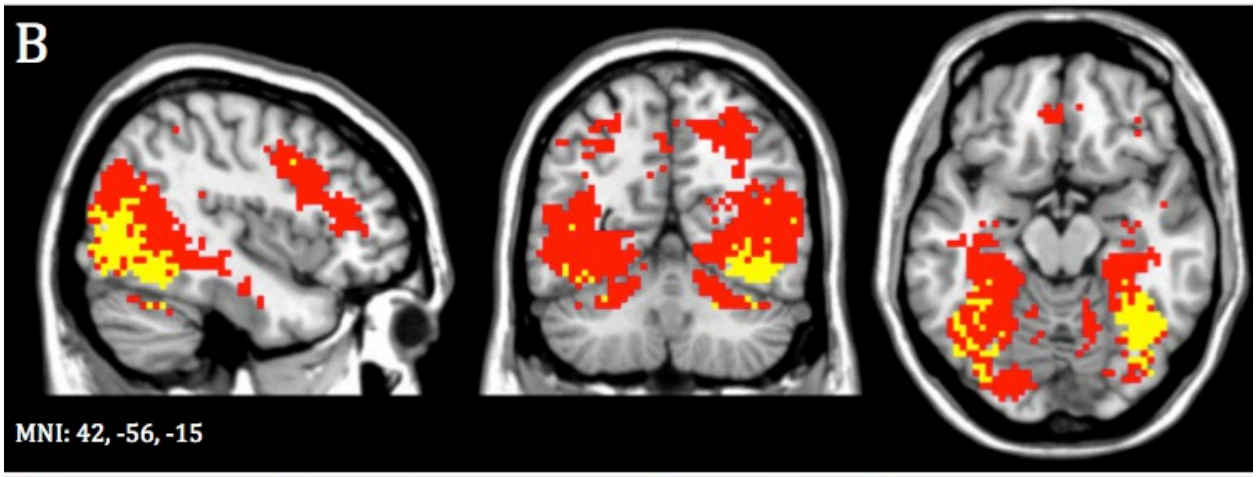
Support: Australian Research Council Future Fellowship FT120100816

Title: Predicting behavior from decoded searchlight representations shows where decodable information relates to behavior

Authors: *T. GROOTSWAGERS[1], R. M. CICHY[2], T. A. CARLSON[1];
[1]Cognitive Sci., Macquarie Univ., Sydney, Australia; [2]Dept. of Educ. and Psychology, Free Univ. Berlin, Berlin, Germany

Abstract: An implicit assumption often made in the interpretation of brain decoding studies is that if information is decodable from a brain region, then the brain is using this information for behavior (but see Williams et al., 2007). In the present study, we sought to study the dissociation between "decodability" and neural correlates of behavior.
We used a support vector machine classifier and searchlight analysis to first identify regions of the brain that could decode whether visually presented objects were animate or inanimate from two fMRI datasets (n=16 and n=15) that used (92/118) different stimuli. A second searchlight analysis was then performed on the same data, where the distance of individual exemplars to the decision hyperplane in each voxel sphere was correlated to human reaction times (RT) on an animacy yes/no categorization task (n=50, collected on Amazon's Mechanical Turk). The decoding and RT-searchlight maps were tested for significance at the group level (FDR controlled).
In both datasets, we found decodable information along the entire ventral-temporal pathway. Regions that also correlated with RT behavior were however restricted to inferior temporal cortex (ITC). These results support ITC's important role in object categorization behavior, consistent with previous region-of-interest based findings (Carlson et al., 2014). Our results further show that our behavioral RT-searchlight method complements standard searchlight decoding analyses by differentiating between information that is merely decodable, and information that is more directly related to behavior.





Subject-averaged searchlight results on the two fMRI datasets (A & B). Voxel centers with significant decoding are marked red, and voxel centers with both decoding and a significant correlation with categorization RT are marked yellow.

Disclosures: **T. Grootswagers:** None. **R.M. Cichy:** None. **T.A. Carlson:** None.

Nanosymposium

667. Representation of Features and Objects Along the Ventral Stream

Location: SDCC 1B

Time: Wednesday, November 16, 2016, 8:00 AM - 11:15 AM

Presentation Number: 667.06

Topic: D.06. Vision

Title: Neural representations in a rapid serial visual presentation task at 17ms per picture

Authors: *D. PANTAZIS[1], S. QIN, 02139[2], Y. MOHSENZADEH[1], Q. LI[3], R. M. CICHY[4]; [1]McGovern Inst. for Brain Research, MIT, [2]MIT, Cambridge, MA; [3]Massachusetts Gen. Hosp., Boston, MA; [4]Free Univ., Berlin, Germany

Abstract: Recent behavioral evidence exemplifies the remarkable robustness of human object recognition: our visual system can capture conceptual information in stimuli presented as fast as 13ms per picture (Potter et al., 2014). Here we explore the neural underpinnings of this exquisite behavior: a) can we decode object information from neural signals in rapidly presented sequences of images? b) what neural representations are compromised at increasingly fast image sequences?

We collected MEG data in a rapid serial visual presentation experiment. Participants (N = 15) viewed sequences of 11 images presented at 17ms (fast condition) or 34ms per image (slow condition) in separate trials. The middle image (target) was randomly sampled from a set of 12 face images or 12 vegetable/fruit images, while the remaining images (masks) comprised different categories of objects. Participants performed a two-alternative forced choice task reporting whether a face image was present in each sequence.

We applied time-resolved multivariate pattern analysis to decode all pairwise combinations of target images separately for the fast and slow conditions. Further, we used the pairwise decoding accuracies to populate time-resolved 24x24 representational dissimilarity matrices (RDMs), enabling comparisons of representational structure across participants.

In agreement with a high behavioral performance of 95% (SE 0.7%) correct face detection, we could reliably decode target images in the slow condition (50ms significant onset time; 0.05 cluster-size permutation test). Surprisingly, despite the substantially lower behavioral performance of 63% (SE 2.9%) correct face detection, decoding performance was robust even in the fast condition with similar onset time. However, decoding accuracies diminished earlier for the fast than the slow condition. Cross-classification (train a classifier in the fast condition and test in the slow condition, and vice versa) indicated neural representations were similar between the two conditions. Comparison (Pearson's correlation) of RDMs across subjects revealed shared representations across individuals with distinct contributions from both across-category stimuli (faces vs. non-faces) and with-category stimuli.

Taken together, our results indicate neural representations are astonishingly robust at rapid presentations of visual information, even at speeds previously shown to disrupt feedback processing. This corroborates evidence from electrophysiological and behavioral studies that a purely feedforward mode of processing is sufficient for recognition of complex objects.

Disclosures: D. Pantazis: None. **S. Qin:** None. **Y. Mohsenzadeh:** None. **Q. Li:** None. **R.M. Cichy:** None.

Nanosymposium

667. Representation of Features and Objects Along the Ventral Stream

Location: SDCC 1B

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Presentation Number: 667.07

Topic: D.06. Vision

Support: HHMI

Title: Perceptual untangling of natural image sequences

Authors: *O. J. HENAFF, R. L. T. GORIS, E. P. SIMONCELLI;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: The primate ventral stream is organized as a cascade of stages whose neural responses become successively more selective for particular image features and more tolerant of image transformations that preserve those features. This sequence of changes has been described as a means of "untangling" the trajectories corresponding to naturally-occurring transformations, ensuring that population responses lie within linear subspaces that are more readily decoded by downstream areas (DiCarlo & Cox, 2007). Some evidence for this hypothesis has been reported in the representation of simple geometric distortions such as translation and dilation in neural populations in macaque V4 and IT (Hung et. al., 2005).

Here, we propose an ecological and perceptual form of the untangling hypothesis: naturally occurring image sequences follow perceptually linear trajectories. We estimated the perceptual linearity of image sequences by asking observers to discriminate between all pairs of frames. We then used signal detection theory to estimate the perceptual distances between these frames, and combined these to yield an estimate of the perceptual length and curvature of the entire sequence. Consistent with our hypothesis, we find that a natural image sequence containing complex motion is nearly perceptually flat. That is, although the trajectory of the image pixels is highly nonlinear, the estimated perceptual curvature is not significantly different from zero. In contrast, an artificial sequence that follows a straight-line path between the end-frames of the same movie (i.e., "fading" from the first frame to the last) yields a perceptual path whose length is approximately twice the distance between the end frames, and thus highly curved. But not all sequences that follow straight-line paths in pixel space yield large perceptual curvature. For example, we find that an image sequence generated by gradually altering the contrast of the initial frame (as would occur when fog lifts) is also perceptually linear. Finally, consistent with previous physiological tests of untangling, we find that sequences generated by translating, dilating, or rotating an initial image produce minimal perceptual curvature.

These results provide direct evidence that image trajectories that occur in natural videos are linearized by the visual system, an ecological version of the "untangling" hypothesis. Moreover, these results also suggest the possibility that the transformations of the ventral stream arise

through an unsupervised learning process that aims to minimize the curvature of neural population response trajectories.

Disclosures: **O.J. Henaff:** None. **R.L.T. Goris:** None. **E.P. Simoncelli:** None.

Nanosymposium

667. Representation of Features and Objects Along the Ventral Stream

Location: SDCC 1B

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Presentation Number: 667.08

Topic: D.06. Vision

Support: ATTEND (Grandi progetti 2012)

Title: Decoding object shape and object category with MEG

Authors: ***D. PROKLOVA**, D. KAISER, M. PEELEN;
Cimec - Ctr. For Mind/Brain Sci., Rovereto, Italy

Abstract: Recent studies have shown that object category can be decoded from MEG sensor patterns, revealing the time course of object categorization with millisecond resolution. However, objects belonging to the same category often share characteristic shape features (e.g., most mammals have four legs), so that category decoding in these studies could have partially reflected shape similarity rather than categorical similarity. In the present study, we aimed to disentangle the contributions of object shape and object category to the MEG signal by using animate and inanimate objects that were closely matched for shape and low-level visual features (e.g., snake-rope). In a series of behavioral visual search experiments, different aspects of visual similarity of these objects were quantified (overall similarity, outline similarity, texture similarity). In a previous fMRI study using these stimuli, we found that both shape and category are represented in ventral temporal cortex. Following the analysis approach used in the fMRI study, neural dissimilarity of MEG sensor patterns was modeled using regression analysis, where visual dissimilarity (taken from the visual search experiments) and categorical dissimilarity served as predictors of neural dissimilarity. Preliminary results show that visual object properties are reflected in MEG patterns starting from 80 ms after stimulus onset, with a peak at 110 ms. Surprisingly, when regressing out the contribution of visual properties, no residual category information was present in MEG response patterns. These results suggest that MEG sensor patterns evoked by visually presented objects predominantly reflect visual object properties.

Disclosures: **D. Proklova:** None. **D. Kaiser:** None. **M. Peelen:** None.

Nanosymposium

667. Representation of Features and Objects Along the Ventral Stream

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Presentation Number: 667.09

Topic: D.06. Vision

Support: NSF GRFP

Google Faculty Research Award

NSF Collaborative Research in Computational Neuroscience Grant IIS-1309725

Title: A comparison of v4 and object recognition convolutional neural net response properties.

Authors: *D. A. POSPISIL, A. PASUPATHY, W. BAIR;
Biol. Structure, Univ. of Washington, Seattle, WA

Abstract: Convolutional neural nets (CNNs) are currently the highest performing general purpose image recognition computer algorithms. Their design is loosely inspired by the neural architecture of the ventral visual pathway in the primate brain, which is believed to underlie form perception and object recognition. Of interest is whether these hierarchical networks, following extensive supervised training, end up performing computations that are similar to those at various stages in the visual hierarchy. Early stage filters in CNNs are often Gabor-like, consistent with V1 physiology, so we wondered whether mid-level stages would show response properties like those found in area V4. Specifically, do CNN units encode a translation-invariant representation of boundary curvature in an object-centered coordinate system, as described by Pasupathy & Connor (2001)? To answer this question, we used an implementation of "AlexNet" (Krizhevsky et al., 2012) that was trained to classify the labeled images in the 2012 ImageNet challenge. From units in all layers of AlexNet, we recorded responses to the original Pasupathy & Connor shape stimuli (51 simple closed shapes at up to 8 rotations) presented at 100 spatial translations (1 pixel increments). For each unit, we fit the responses to the angular-position and curvature (APC) model simultaneously across all stimulus translations. We interpreted a high r-value ($r > 0.5$) between the best-fit APC model and the unit responses to indicate V4-like behavior: both good translation invariance and good boundary-curvature tuning. We found that only the later layers of AlexNet had a significant fraction of units well fit by the APC model over translation. The greatest fraction (320/1000) was found in the final, fully-connected layer of AlexNet where each unit represents an object category. It is surprising that a network that was neither built to replicate V4 responses, nor trained on the stimuli used to fit these responses, achieves state of the art performance in replicating V4-like boundary curvature tuning. It is also intriguing that only the final layer had more than 10% of V4-like units, given that this layer is associated with object categorization, whereas V4 is thought to be an earlier stage that represents

the local shape of object parts. Our results raise the exciting possibility that similar computations underlie object representation in both biological and artificial visual systems. Thus, deep networks may provide important insight for guiding neurophysiological investigations, and neurophysiology may offer hints for improving artificial vision systems.

Disclosures: **D.A. Pospisil:** None. **A. Pasupathy:** None. **W. Bair:** None.

Nanosymposium

667. Representation of Features and Objects Along the Ventral Stream

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Feodor Lynen Scholarship by the Alexander von Humboldt Foundation to RMC

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Title: Representation of visual features and categories across space and time in human, monkey, and convolutional neural networks

Authors: ***K. M. JOZWIK**[1], N. KRIEGESKORTE[2], R. M. CICHY[3], M. MUR[2];
[1]Cognition and Brain Sci. Unit, Free Univ., Berlin, Germany; [2]MRC Cognition and Brain Sci. Unit, Cambridge, United Kingdom; [3]Dept. of Educ. and Psychology, Free Univ. Berlin, Berlin, Germany

Abstract: Are visual features and category membership equally important for explaining object representations in inferior temporal (IT) cortex? They are: features and categories explain equal amounts of human IT representational variance, and combining them does not increase their explanatory power (Jozwik et al. 2015). Here, we ask the same question for monkey IT, and for layers of a deep convolutional neural network (CNN), the two most successful models of human IT. We further determine which features and categories are most important for explaining the IT representation, and when they contribute to explaining the representation.
Human observers (n=16) generated category labels (e.g. animal) and feature labels (e.g. eye) for a set of 92 real-world object images. Category labels included basic, intermediate and high-level (abstract) categories, whereas feature labels included object parts, colors, textures, and shapes. We fitted models derived from the labels to the brain representations, based on fMRI and MEG

(humans) and cell recordings (monkeys), and to the CNN representations, using non-negative least squares.

Both feature-based and categorical models explained the IT object representation significantly, and to a similar extent, in human and monkey. Object parts and high and intermediate-level categories were most important for the representation (Fig. 1A). Early CNN layers were dominated by features (color, shape and parts), whereas both categories and features dominated later layers. Feature-based and categorical models explained significant and similar amounts of MEG variance during the same time window after stimulus onset (110–449 ms; Fig. 1B).

Primate IT and a deep convolutional neural network use visual features – primarily object parts – that are associated with categories, especially of high and intermediate levels of abstraction, as a visual path toward semantics. The simultaneous appearance of feature and category information suggests that extracting category information might be synonymous to extracting object features indicative of category membership.

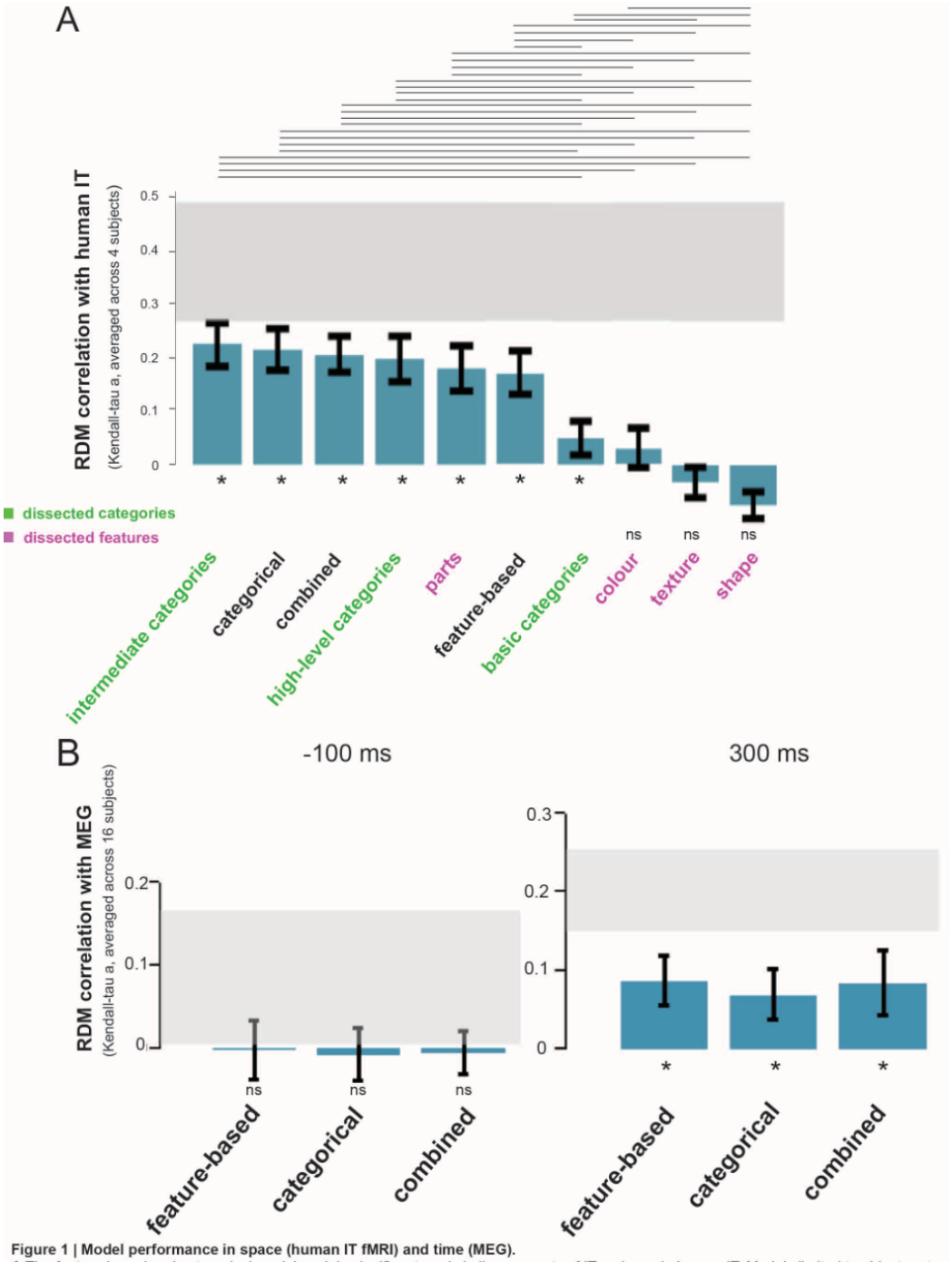

Figure 1 | Model performance in space (human IT fMRI) and time (MEG).
A The feature-based and categorical model explain significant, and similar, amounts of IT variance in human IT. Models limited to object parts or categories of high and intermediate levels of abstraction perform as well as the full models. The bar graphs show the correlation between the data representational dissimilarity matrix (RDM) and each of the model RDMs. **B** The feature-based and categorical models explain significant and similar amounts of MEG variance between 110 ms and 449 ms after stimulus onset. The bar graphs show the correlation between the data RDM and each of the model RDMs at 100 ms before stimulus onset and 300 ms after stimulus onset. For both panels, significant correlations between a model RDM and the data RDM are indicated by an asterisk (stimulus-label randomization test, $p < 0.05$ FDR corrected). Significant differences between models in how well they can account for the data are indicated by horizontal lines plotted above the bars (stimulus-bootstrap test, $p < 0.05$ corrected). Error bars show the standard error of the mean based on bootstrap resampling of the stimulus set. The thick gray bar represents the noise ceiling, which indicates the expected performance of the true model given the noise in the data.

Disclosures: **K.M. Jozwik:** None. **N. Kriegeskorte:** None. **R.M. Cichy:** None. **M. Mur:** None.

Nanosymposium

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Support: Intramural Research Program, NIH

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Title: Decoding the temporal evolution of task and stimulus-related brain signals

Authors: *M. N. HEBART[1], B. B. BANKSON[1], A. HAREL[3], R. M. CICHY[4], C. BAKER[2]; [1]Lab. of Brain & Cognition, [2]Natl. Inst. of Mental Hlth., Bethesda, MD; [3]Dept. of Psychology, Wright State Univ., Dayton, OH; [4]Dept. of Educ. and Psychology, Free Univ. of Berlin, Berlin, Germany

Abstract: The current goals of an observer determine what elements of visual stimuli are used as diagnostic information. Previous fMRI studies have demonstrated changes in visual representations according to task context. However, the dynamics of the interactions between task- and stimulus-related signals in humans are unclear given the limited temporal resolution of fMRI. Here we used MEG to investigate how the representation of task set and visual stimulus change over time.

Participants carried out four different categorization tasks on 8 different visually presented object classes. The task was changed on a trial-by-trial basis, and the task-relevant features were either physical (color: red / blue; rotation: counter clockwise / clockwise) or conceptual (content: man-made / natural; real-world size: large / small) properties of the images presented. Each trial was initiated by the presentation of a cue that indicated the task to be performed, followed after a short delay by the object stimulus, and then another delay before a response mapping screen that decoupled categorical responses from motor signals.

Using temporally-resolved multivariate decoding, we found that as expected visual categories were encoded shortly after stimulus presentation and their representation decayed to baseline within 1,500 ms. Category representations exhibited a more rapid decay of information for perceptual as compared to conceptual tasks. Interestingly, task was decoded at cue, but was not decoded before stimulus onset - as might have been expected - but then again gradually increased while category decoding decreased after stimulus presentation. Temporal cross-decoding demonstrated a difference between the nature of task-related signals after cue onset and those before behavioral responses. Together, our results demonstrate the dynamic interplay between task and stimulus decoding in the human brain.

Disclosures: **M.N. Hebart:** None. **B.B. Bankson:** None. **A. Harel:** None. **R.M. Cichy:** None. **C. Baker:** None.

Nanosymposium

667. Representation of Features and Objects Along the Ventral Stream

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Title: Task context transforms object representations in inferior temporal cortex but not V1

Authors: ***M. C. MUR**, J. DUNCAN;
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Abstract: Task context affects object responses in human ventral visual cortex (Cukur et al. 2013; Harel et al. 2014). Task modulations have not only been reported for inferior temporal cortex (IT), but also for regions at earlier stages of processing. V1, V2, and V3 are often analyzed together, despite clear functional differences between these areas. Are task effects present in each of these areas? Furthermore, what is the nature of the task effects?
Here, we address these questions using pattern analysis of functional magnetic resonance imaging (fMRI) data. We acquired whole-brain 3T fMRI measurements (voxel size: 3 mm^3) in 14 human subjects. Subjects viewed images of manmade objects, and written words referring to the same concept, while they performed multiple 2-way categorization tasks on the stimuli. We included a visual, a spatial, and a phonological task, and a control task at fixation, unrelated to the stimuli. We extracted stimulus activity patterns for each task and region of interest (V1, V2, V3v, V4, and IT) and tested whether stimulus information, as measured by the strength of image/word decoding, was modulated by task.
Visual inspection of the representational dissimilarity matrices (RDMs) suggests strong image/word decoding in all regions and tasks (Fig. 1AB), as confirmed by statistical inference ($p < .05$ corrected). The RDMs also suggest task effects in higher-level visual regions: images and words appear to elicit more similar response patterns within the fixation task than within the other, stimulus-related, tasks. This was indeed confirmed for V3v, V4, and IT (Fig. 1C; $p < .05$ corrected). The stimulus representation in V1 and V2 was not modulated by task (Fig. 1C).

Our findings show that task context transforms object representations in higher-level visual regions (V4, IT, and also V3v) by enhancing stimulus information during tasks that require active processing of the stimuli. The object representation in V1 and V2, however, is robust against changes in task context. These findings suggest a hierarchical structure of re-entrant feedback across the ventral stream.

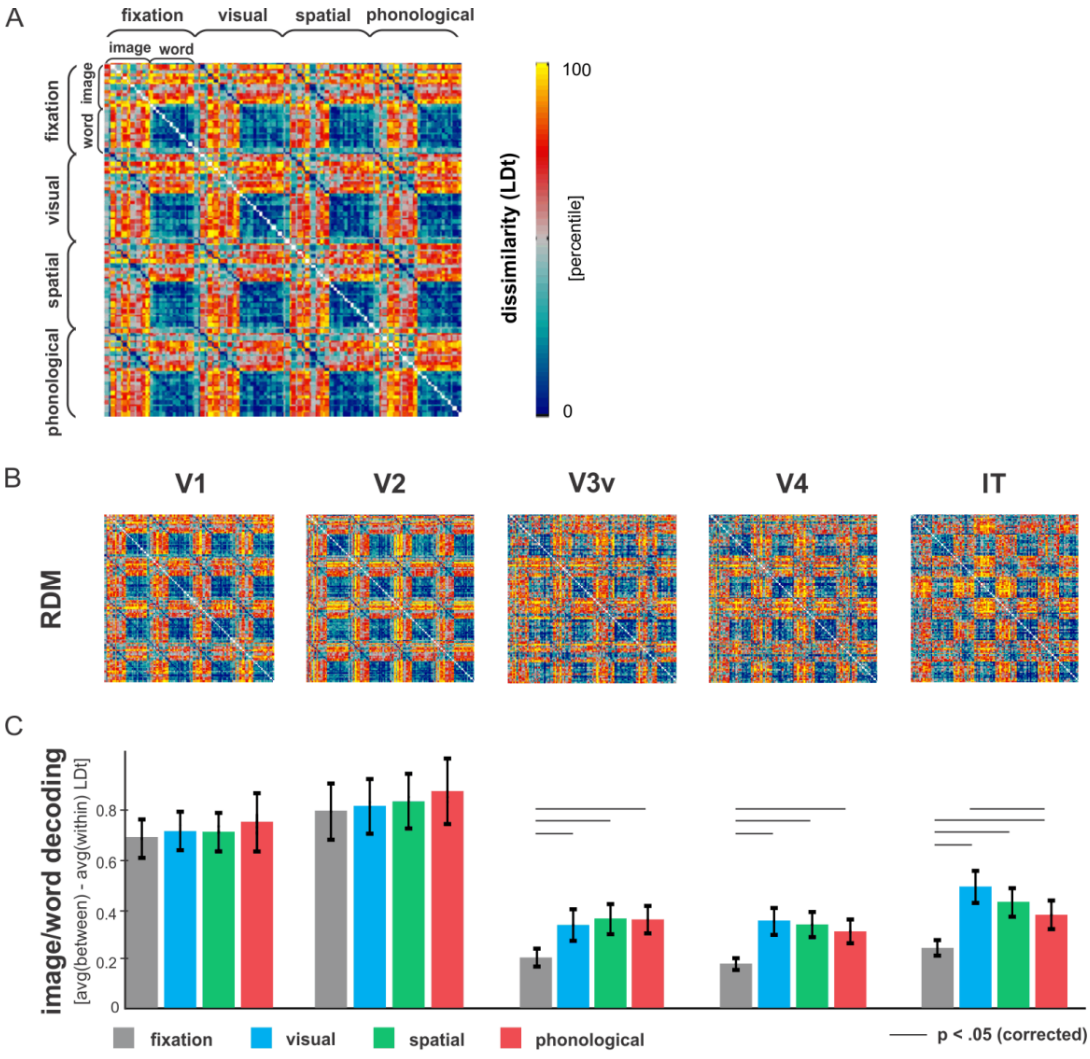

Figure 1 | Task context transforms object representations in higher-level visual cortex, but not in V1 and V2.

A An example representational dissimilarity matrix (RDM), showing the ordering of tasks and stimuli along the axes. We estimated response-pattern dissimilarities by computing linear discriminant t values. The RDMs are rank transformed and scaled into [0,1] for easier comparison between regions of interest (ROIs). **B** RDMs for ROIs along the human ventral visual stream: V1, V2, V3v, V4, and IT. ROIs were defined by selecting the 71 most visually-responsive voxels (voxel size: 3 mm^3) within anatomical (V1-4) and functional (IT) masks in each individual subject, using independent data. **C** All ROIs show significant image/word decoding across tasks (random-effects t tests across subjects; $p < .05$ corrected). However, in V3v, V4, and IT, the strength of image/word decoding is stronger in the stimulus-related tasks than in the fixation task (random-effects paired t tests across subjects; $p < .05$ corrected). In V1 and V2, no differences between tasks were detected.

Disclosures: M.C. Mur: None. **J. Duncan:** None.

Nanosymposium

667. Representation of Features and Objects Along the Ventral Stream

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ONR MURI N000141010934

Title: Framing scene perception in the brain

Authors: *E. M. AMINOFF, M. J. TARR;
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Abstract: Three brain regions selectively process scenes – the parahippocampal/lingual region (PPA), the retrosplenial complex (RSC), and the occipital place area (OPA/TOS). Here we attempt to disentangle the relative contributions of bottom-up processing – driven by the stimulus input – and top-down processing – driven by our experiences – across these brain regions. To address this issue, we presented images of outdoor scenes surrounded by either a window frame (WF) or a picture frame (PF) – the two surrounds being matched for low-level visual features. All participants viewed all scenes in both conditions during the experiment. WF connotes a natural scene viewed through an aperture, while PF connotes an image of a scene on a flat surface. Because the former is closer to real-world scene perception, we predicted that within scene-selective cortex, neural scene processing in WF would be more similar, and in PF less similar, to the typically used, decontextualized processing of natural scene images. Alternatively, if the top-down influence exerted by the two conditions is not relevant to processing in these regions, there should be no difference between conditions. Note, given that the scene stimuli were identical and presented using well-matched surrounds, we did not predict any differences in low-level visual regions irrespective as to our high-level predictions. Results revealed reliable effects between conditions within the OPA/TOS, with activity higher for WF as compared to PF. This advantage for WF was also observed beyond the OPA/TOS and included more extensive regions of the dorsal visual stream. Our findings suggest that OPA/TOS processing can be modulated by top-down interpretations of the visual input. In contrast, the PPA and RSC do not show reliable differences between the two conditions, indicating that these two regions encode a more abstract representation of the scene. This result is inconsistent with the proposal that the PPA is driven by visuospatial properties of the stimulus, which predicts a difference in PPA response across the two conditions. That is, to the extent that windows are perceived as more scene-like, these stimuli should engender better recovery of scene-relevant visuospatial properties (which are often 3D). In sum, we observed that the OPA/TOS was most sensitive to

the top-down manipulation of scene perception, whereas the PPA and RSC were not reliably affected, suggesting that the latter two regions encode more abstract and less stimulus-bound representations of scenes. As such, our results help to refine our understanding of the different functional roles subserved by different brain regions within the scene-selective network.

Disclosures: **E.M. Aminoff:** None. **M.J. Tarr:** None.

Nanosymposium

668. Representation of Faces and Bodies

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NEI 5 R01 EY021594-03

New York Stem Cell Foundation Robertson Investigator Award NYSCF-R-NI23

Title: Learning enables view-invariant prediction errors in macaque face patch ML

Authors: *C. M. SCHWIEDRZIK, W. A. FREIWALD;
Lab. of Neural Systems, Rockefeller Univ., New York, NY

Abstract: Extracting regularities from the environment allows the brain to make predictions about upcoming events. The brain is especially adept at detecting associations between co-occurring stimuli, as exemplified by statistical learning. However, it is currently unclear how these associations are represented and how they may affect sensory processing. We investigated whether learning to associate faces affects face processing at early, view-specific stage of the face processing hierarchy, in face patch ML. To this end, we exposed macaque monkeys to pairs of faces presented in fixed sequence so that the first image in the sequence would become predictive of the second image in the sequence. By pairing faces with particular views (e.g., left face A - right face B), we asked whether the face patch system learns specific-stimulus association (face pairs and their specific view configurations) or instead generalizes across views to learn abstract stimulus pairing at the level of identity (i.e., face A is followed by face B regardless of view). After several weeks of training, we used fMRI-guided single unit recordings

to test whether and how the associations between co-occurring faces affected face processing in ML. We found that neurons responded more strongly to facial identities that had not been associated during training relative to trained face pairs throughout the transient and the sustained phase of the response, reminiscent of a prediction error. This effect was not present for pairs in which identities had been associated during training but that were now tested with different views. Thus, learning enables view-tuned neurons in ML to signal view-invariant prediction errors about identity. This indicates that learned associations between faces are already represented at early, sensory stages of face processing and furthermore that they are stored in a generalized, view-invariant format.

Disclosures: **C.M. Schwiedrzik:** None. **W.A. Freiwald:** None.

Nanosymposium

668. Representation of Faces and Bodies

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Topic: D.06. Vision

Support: FWO

PF

IUAP

Title: Increased viewpoint-tolerant coding of body posture and identity in the anterior compared to the midSTS body patch.

Authors: ***R. VOGELS**, S. KUMAR;
KU Leuven, Leuven, Belgium

Abstract: FMRI studies in macaques show several body-category selective regions in the macaque inferior temporal cortex, of which at least two are located in the STS: the midSTS and the more anterior antSTS body patches. The latter is located in the lip of the anterior STS (A/P: 13-19 mm). Thus far, it is unclear whether these two body patches differ in how they represent images of bodies. To address this, we recorded in both fMRI-defined patches of the same monkey. In both patches, the average spiking response to bodies (of humans, monkeys, mammals and birds) was greater than the mean responses to faces, manmade objects and fruits/vegetables. We compared systematically the coding of body identity, posture and viewpoint between the two patches using a novel computer-generated stimulus set (n= 120 stimuli) depicting monkeys that differ in anthropometric features (identity; fat, normal and thin),

with 5 different postures (2 threat, 2 submissive and 1 neutral posture), rendered at 8 viewpoints (45 deg step; rotated around the vertical axis) - see Kumar & Vogels, SFN abstract, 2016. We employed linear SVM with cross-validation to decode viewpoint, posture and identity from the responses of the same number of neurons of the two patches. Identity and posture invariant viewpoint classification accuracy was significantly lower in the antSTS compared to the midSTS body patch. However, viewpoint-invariant identity and posture decoding significantly improved in the antSTS compared to the midSTS patch. Posture decoding was strongly viewpoint-dependent in the midSTS body patch, whereas training posture decoding at a single viewpoint and testing at other viewpoints showed above chance generalization of posture decoding to other viewpoints in the antSTS body patch. Training identity decoding at a single viewpoint and single posture produced a greater generalization of identity decoding across viewpoints and to a lesser extent across posture in the antSTS compared to the midSTS body patch. These data suggest that the antSTS body patch has more information about viewpoint-invariant body posture and identity than the midSTS body patch, while the midSTS body patch provides relatively more information about the 3D orientation of bodies.

Disclosures: **R. Vogels:** None. **S. Kumar:** None.

Nanosymposium

668. Representation of Faces and Bodies

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Topic: D.06. Vision

Support: DFG grant (TH 425/12-1)

Title: Neurons in the pSTS integrate information on the rule to follow gaze with information on the subsequent shift of attention prompted by the gaze cue

Authors: *H. RAMEZANPOUR, P. DICKE, P. THIER;
Dept. of Cognitive Neurol., Hertie Institue For Clin. Brain Res., Tuebingen, Germany

Abstract: Similar to humans, macaque monkeys are keenly interested in determining the direction of their conspecific's attention by following the other's gaze, allowing the observer to localize objects of common interest, objects of shared and eventually joint attention. Previous neuroimaging and electrophysiological studies have suggested that a well-defined cortical region (the gaze following patch, GFP) in the posterior superior temporal sulcus (pSTS) is a major node in a putative network subserving gaze following and joint attention in monkeys and man (Materna et al., J. Cogn. Neurosci., 2008; Marciniak et al., eLIFE 2014). However, how

individual pSTS neurons contribute to encoding the other's gaze direction and how they contribute to establishing joint attention is not clear yet. These questions motivated us to record from the GFP and neigboring parts of the pSTS of two macaque monkeys, deploying a set of experimental paradigms that allowed us to compare neuronal responses to shifts of spatial attention towards distinct targets guided by the other's gaze, alternatively to shifts of attention based on learned associations between distinct spatial targets and the other´s facial identity or learned associations between abstract symbols and distinct spatial targets. Finally, we also tested neurons for responses to the passive viewing of faces and a variety of biological and non-biological non-face stimuli in order to clarify if they were from one of the well-studied face patches in the STS, given the close proximity of the GFP and these face patches. We observed a huge variety of response types among more than 400 single units recorded hitherto from the right pSTS of the two monkeys, about 50% of them task-related. A preference for passive faces dominated the putative middle face patches. The putative GFP was dominated by units which were selective for the gaze following rule. Others were either activated by the identity following rule or by both. Here we report two new types of responses: One is characterized by activity evoked by shifts of attention to distinct spatial locations guided by gaze without being sensitive to covert or overt shifts of attention prompted by other spatial cues. The second one integrates sensitivity for the gaze following rule with spatially selective sensitivity to the subsequent shift of attention, exhibited by the former type of response. The properties of these neurons are in accordance with the notion that the GFP is instrumental in using the other's gaze to single out objects of joint attention in distinct spatial locations.

Disclosures: H. Ramezanpour: None. **P. Dicke:** None. **P. Thier:** None.

Nanosymposium

668. Representation of Faces and Bodies

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NIH Grant R90DA023420

Title: Neurodynamics of expression coding in the core face network

Authors: *Y. LI[1,2,3], M. J. WARD[3], W. J. LIPSKI[3], R. M. RICHARDSON[3,4], A. S. GHUMAN[3,4,2];
[1]Ctr. for the Neural Basis of Cognition, [2]Program in Neural Computation, Carnegie Mellon

Univ., Pittsburgh, PA; [3]Neurolog. Surgery, [4]Ctr. for the Neural Basis of Cognition, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Face processing is mediated by a network involving multiple distributed areas in the brain, with the occipital face area (OFA), fusiform face area (FFA), and posterior superior temporal sulcus (pSTS) considered the core nodes of the network. Results suggest that OFA is primarily involved in early perception of facial features, FFA is mainly involved in the processing of the static aspects of faces, and pSTS is mainly involved in the processing of the dynamic aspects of faces. Based on these results, the first models of the neural basis of face processing posited that pSTS codes for expression and FFA codes for identity. Recently, several neuroimaging studies have suggested that the FFA is involved in the processing of facial expressions and recent models have posited that the FFA is involved in structural encoding of face expression. To mediate between these hypotheses, we recorded intracranial electroencephalography (iEEG) data from 15 patients with electrodes in the OFA, FFA, and/or pSTS during face expression perception. Using pattern classification techniques, including naïve Bayes classifier, support vector machine, and linear discriminant analysis, statistical inference methods, including frequentist hypothesis testing and confidence intervals, and Bayes factors analysis, our results show that at the early stage of visual information processing (50-250 ms after stimulus onset), neural activity from FFA does not contain facial expression information. In contrast, facial expression information is seen in OFA and pSTS at this early stage of the process. However, in several patients facial expression information could be decoded from neural activity in FFA at a later stage (250-450 ms after stimulus onset). Given our previous results showing identity decoding in FFA in the same late time window, expression decoding at this time may be related to the integration of identity and expression information. Taken together, these results suggest that FFA activity is not intrinsically tuned to expression during early face processing and instead expression-related information may emerge through later, recurrent interactions across the face network.

Disclosures: Y. Li: None. **M.J. Ward:** None. **W.J. Lipski:** None. **R.M. Richardson:** None. **A.S. Ghuman:** None.

Nanosymposium

668. Representation of Faces and Bodies

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Presentation Number: 668.05

Topic: D.06. Vision

Support: EUROSTARS Grant 9 273

Title: Real-time mapping of the fusiform face area: An extension of functional brain mapping protocols

Authors: *C. KAPELLER[1], K. KAMADA[2], H. OGAWA[2], W. G. COON[3], C. GUGER[1]; [1]Guger Technologies OG, Schiedlberg, Austria; [2]Dept. of Neurosurg., Asahikawa Med. Univ., Asahikawa, Japan; [3]g.tec neurotechnology USA, Inc., Albany, NY

Abstract: Functional brain mapping prior to a brain surgery is an important step for optimizing the surgical outcome. Like electrical cortical stimulation (ECS) mapping, event-related activation in the high gamma frequency band (HGA) highlights functional regions of the cortex. This HGA also occurs during face recognition in the fusiform face area (FFA). Since ECS on the temporal base can be problematic for the patient, an alternative method using electrocorticography can overcome ECS related issues like pain or seizures.

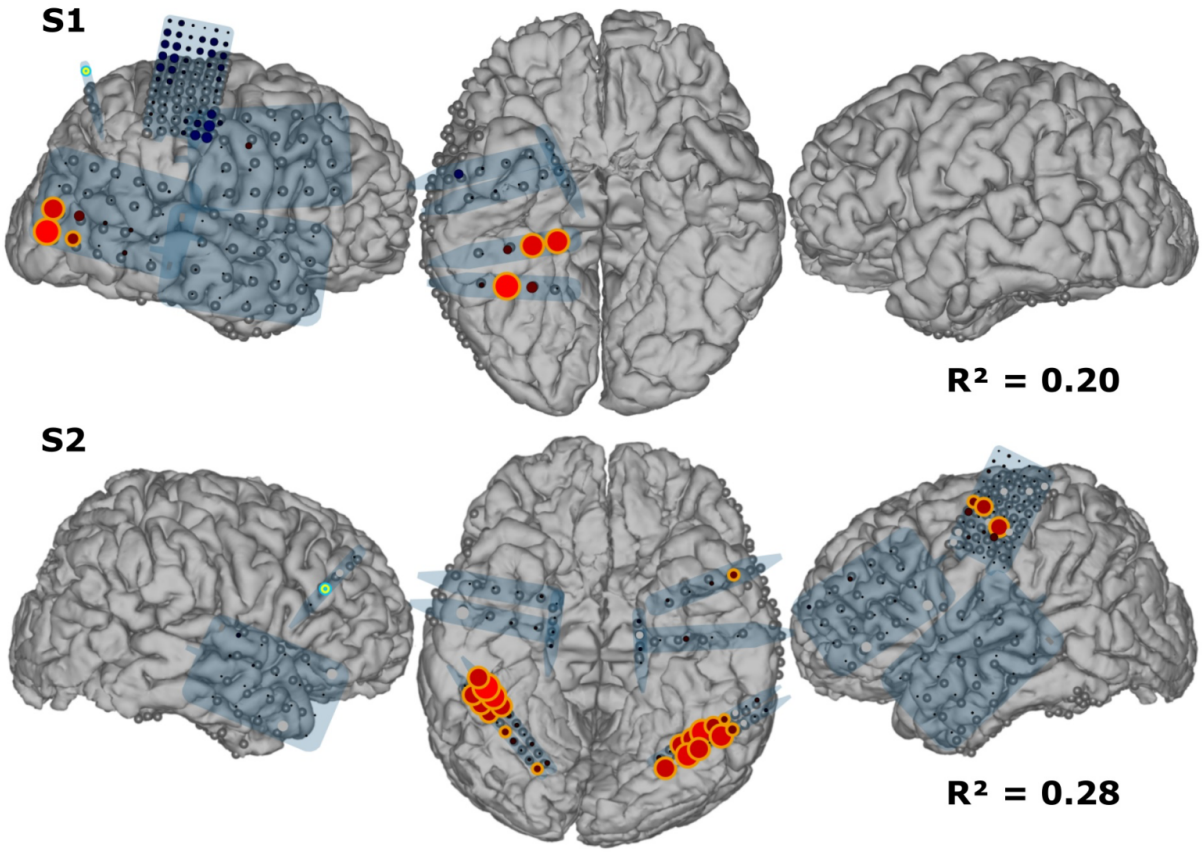
In this work we used a real-time mapping system (cortiQ, g.tec, Austria) to map the FFA. The system identifies activated cortical areas within minutes and brings the great advantage that the patient can perform tasks voluntarily, without the danger of stimulation elicited seizures.

The study included ECS and cortiQ mapping with two patients, who underwent neuro-monitoring prior to brain surgery. While for S1 144 electrodes were used with standard clinical electrodes over the right ventral stream (VS), S2 had 188 sites implanted with high-density grids across the VS of both hemispheres.

S1 looked at a black screen for 25.6 s, and then 32 times at a face and a black screen for 0.4 s each. S2 looked at scrambled faces for 25.6 s and then 32 times at a face and a scrambled face for 0.4 s each. Both subjects repeated the mapping three times, resulting in a recording time of 153.6 s.

Figure 1 shows face related significant HGA at specific electrode positions. While S1 showed activation on the dorsal stream (left, visual processing area) and VS (middle, face/symbol recognition area), S2 showed activation on the VS and tongue motor region. The validation with ECS gave a sensitivity/specificity of 75/97 % and 100/76 % for S1 and S2, respectively.

This study shows that FFA can be mapped within a short recording time, adding to a large battery of mapping procedures that can already be accomplished using cortiQ. These include localization of sensorimotor, receptive and expressive language, and auditory areas. In the future, localization of areas engaged during memory function will also be investigated.



Disclosures: **C. Kapeller:** None. **K. Kamada:** None. **H. Ogawa:** None. **W.G. Coon:** None. **C. Guger:** None.

Nanosymposium

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Topic: D.06. Vision

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ERC Grant facessvep 284025

Title: Visual adaptation reveals objective electrophysiological evidence of high-level facial identity discrimination

Authors: *T. L. RETTER[1,2], B. ROSSION[2];
[1]Univ. of Nevada, Reno, Reno, NV; [2]Univ. of Louvain, Louvain-la-Neuve, Belgium

Abstract: The ability to individualize conspecifics by their faces is a fundamental human brain function. Unfortunately, the neural response to different facial identities cannot readily be discriminated at a global level of brain organization. However, following a short period of visual adaptation to one facial identity, the suppressed neural response to this identity may be discriminated from the response to an unadapted facial identity and objectively identified with electroencephalographic (EEG) frequency-tagging (Retter & Rossion, 2016). Here, we investigate the potential contributions of low-level visual adaptation to this identity-specific discrimination response with 16 participants. In a first condition: 1) as in the original study, two facial identities are presented in alternation at a rate of six images per second (6 Hz; 3 Hz identity repetition rate) for a 20 s testing sequence, following adaptation to one of the facial identities for 10 s. Two novel conditions are added: 2) stimuli are inverted and 3) an identity not present in the upright testing sequence is adapted. In all three conditions, a significant response is present at 6 Hz in the frequency-domain of the EEG over medial-occipital channels, indicating a detection of image presentation; however, only after adapting to one of the upright facial identities of the testing sequence (condition 1) is a significant response present at 3 Hz over right occipito-temporal channels, indicating identity-specific discrimination. Thus, low-level visual features present in inverted or unrelated facial identities are not sufficient to produce the identity-specific adaptation effects found for upright facial stimuli, which appear to truly reflect high-level perceptual processes.

Disclosures: T.L. Retter: None. **B. Rossion:** None.

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NIH Grant 1R01EY02391501A1

Title: Development of neural sensitivity to face identity correlates with perceptual discriminability.

Authors: *V. S. NATU[1], M. BARNETT[1], J. HARTLEY[1], J. GOMEZ[1], A. STIGLIANI[1], K. GRILL-SPECTOR[1,2];
[1]Dept. of Psychology, Stanford University,, Stanford, CA; [2]Stanford Neurosciences Inst., Stanford Univ., Stanford, CA

Abstract: Face-selective regions in the human ventral stream undergo prolonged development from childhood to adulthood. However, the neural mechanisms underlying this development remain unknown. Here, we asked if development is associated with changes in neural sensitivity to face identity, changes in the overall level of response to faces, or both. Using fMRI, we measured brain responses in ventral face-selective regions (IOG-faces, pFus-faces, and mFus-faces) in children (ages 5-12, *N*=18) and adults (ages 22-34, *N*=12), when they viewed adult and child faces, which parametrically varied in the amount of their dissimilarity. Since similar faces generate lower responses than dissimilar faces due to fMRI-adaptation, this technique can be used to probe neural sensitivity across age groups. In both children and adults responses in ventral face-selective regions linearly increased with face dissimilarity (Fig 1a). We quantified neural sensitivity to face identity as the slope of this line and intercept as baseline responses. Results show that sensitivity to face identity was significantly higher in adults' face-selective regions than in children's, but there were no differences across age groups in object-selective regions (Fig. 1b). Additionally, baseline responses to faces were higher in adults' than in children's face- and object-selective regions (Fig. 1c). Notably, in both children and adults, perceptual sensitivity to face identity measured in an independent behavioral experiment was positively correlated with neural sensitivity of face-selective areas and not object-selective areas: participants with higher neural sensitivity in ventral face-selective regions had higher perceptual sensitivity (Fig. 1d). Our results suggest that developmental increases in neural sensitivity to face identity in face-selective regions improves perceptual discrimination of faces. These findings significantly advance understanding of neural mechanisms underlying the development of face perception and have implications for assessing both typical and atypical development.

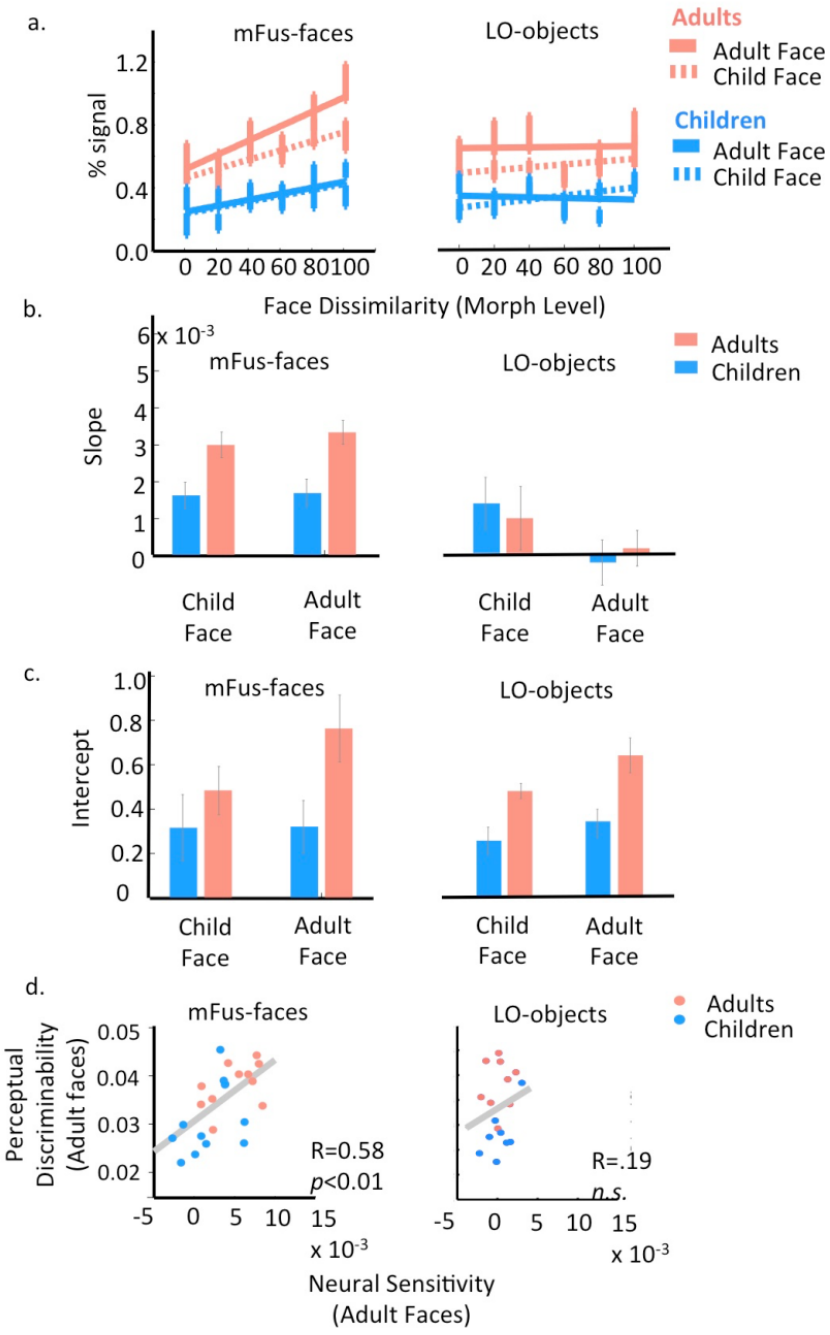

Figure 1. Development of neural sensitivity to face identity in face-selective region is coupled with behavior. a) *Left*: Mean percentage signal in face-selective mFus-faces in 12 adults (orange) and 18 children (blue) as a function of face dissimilarity. *Right*: Same in object-selective LO in 10 adults and 14 children. *Solid lines:* responses to adult faces; *Dashed lines:* responses to child faces. **b and c)** Slopes (indicating neural sensitivity to face identity) and intercepts (indicating baseline response level) of linear fits to responses to child and adult faces as a function of dissimilarity in mFus-faces (*Left*) and LO (*Right*). *Blue*: child participants. *Orange:* adult participants. *Error bars:* standard error of the mean, averaged across subjects of an age group. **d)** Correlation between perceptual discriminability *vs.* neural sensitivity in mFus-faces (*Left*) and LO (*Right*) for adult faces. Each dot is a subject. *Blue dots*: children. *Orange dots*: adults.

Disclosures: V.S. Natu: None. **M. Barnett:** None. **J. Hartley:** None. **J. Gomez:** None. **A. Stigliani:** None. **K. Grill-Spector:** None.

Nanosymposium

668. Representation of Faces and Bodies

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NIH R01 AT009036-01

Title: Stimulus based dynamic organization of the face network

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Abstract: There is a growing realization that face perception is accomplished via the coordinated activity of a neuronal face processing network. However, many fMRI studies still utilize a localized approach mostly focusing on "core brain regions" (e.g., fusiform face area - FFA, occipital face area – OFA), often neglecting the extended face regions and importantly the interaction between these different regions at the network level.
Using the face inversion effect and the face composite effect, which are behavioral hallmarks of face perception, we examined network mechanisms underlying face representation. Specifically we tracked fMRI dynamic functional connectivity within and between brain networks associated with face and non-face related regions. We developed a novel approach adapting the general linear model (GLM) framework classically used for univariate fMRI analysis to capture task-related fMRI dynamic connectivity of the face network. We show that under the face inversion manipulation, the face and non-face networks have complementary roles that are evident in their dynamic connectivity patterns as assessed by network decomposition into components or communities. Moreover, we show that the connectivity between these networks is associated with the behavioral face inversion effect. Consistently, applying the same methodology to the composite paradigm revealed wide spread network reorganization associated with the behavioral effect. Thus, we establish *a network-level signature* of the face inversion and composite effects and demonstrate how physical transformations of the face stimulus induces dramatic functional re-organization across related brain networks**.** We suggest that the dynamic GLM network analysis approach, developed here for the face network, provides a general framework for

modelling the dynamics of blocked task-based dynamic connectivity experimental designs and hence can be applied to a host of neuroimaging studies.

Disclosures: **G. Rosenthal:** None. **O. Sporns:** None. **G. Avidan:** None.

Nanosymposium

668. Representation of Faces and Bodies

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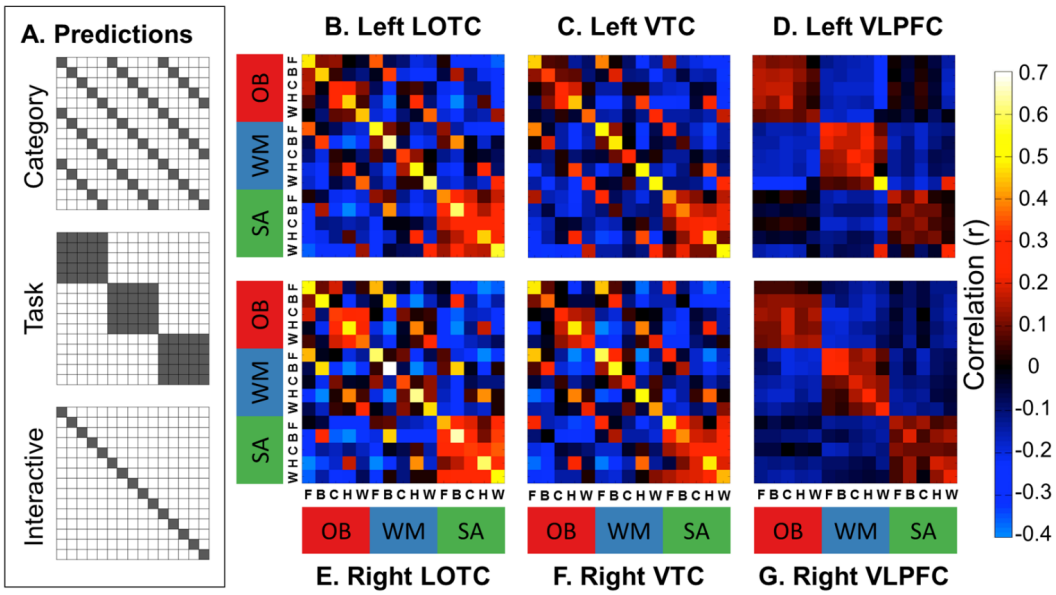
Stanford CNI seed grant

Title: Task warps category representations in prefrontal but not high-level visual cortex

Authors: ***L. BUGATUS**[1,2], K. S. WEINER[1,2], K. GRILL-SPECTOR[1,2];
[1]Psychology, Stanford Univ., Stanford, CA; [2]Stanford Neurosci. Inst., Stanford, CA

Abstract: It is unknown how distributed representations in lateral occipito-temporal (LOTC), ventral temporal (VTC), and ventrolateral prefrontal (VLPFC) cortices are affected by task and category. In this study, we considered three hypotheses: (1) task and category are independent, (2) task and category interact, and (3) effects of task and category vary across cortex. To test these predictions, we examined responses in LOTC, VTC, and VLPFC, as 12 subjects viewed images of faces, bodies, houses, cars, and pseudo-words under different cognitive tasks (oddball [OB], working memory [WM], and selective attention [SA]). Results show that distributed responses in LOTC and VTC are similar for the same category across tasks, but differ across categories irrespective of task ($F(2,132) = 41.47$, $p < 0.001$). Conversely, distributed responses in VLPFC are similar for the same task across categories, but differ for the same category across tasks ($F(2,132) = 6.44$, $p = 0.002$). A regression model showed no interaction, suggesting the effects are independent: in LOTC and VTC, category effect was twice as large as task ($\beta_{category} = 0.332 \pm 0.029$, $\beta_{task} = 0.17 \pm 0.017$), while in VLPFC task effect was five times as large as category ($\beta_{category} = 0.057 \pm 0.009$, $\beta_{task} = 0.281 \pm 0.04$). Additionally, category-selectivity maps in LOTC and VTC were reproducible across tasks, while in VLPFC category-selectivity was task-dependent and weakly reproducible ($F(2, 66) = 306.91$, $p < 0.001$). Implications of these findings are two-fold. First, distributed representations in LOTC and VTC are largely driven by category, whereas representations in VLPFC are driven by task. Second, the topography of distributed representations is task-independent in high-level visual cortex, but task-dependent in

VLPFC. Thus, the former provide stable representations of visual input, while the latter warp to accommodate task-relevant information.



Representational similarity matrices of distributed responses. A: Ideal matrices representing hypotheses. B-G: Average similarity across task and category conditions in examined regions.

Disclosures: L. Bugatus: None. **K.S. Weiner:** None. **K. Grill-Spector:** None.

Nanosymposium

668. Representation of Faces and Bodies

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Topic: D.06. Vision

Support: NSF Grant BCS-1354350

NSF Grant SBE-0542013

Title: Face processing selectively interferes with word processing during rapid serial visual presentation

Authors: *A. ROBINSON, D. PLAUT, M. BEHRMANN;
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Abstract: Words and faces have vastly different visual properties but increasing evidence suggests that word and face processing engage overlapping distributed networks. For instance, fMRI studies have shown overlapping activity for face and word processing in the fusiform gyrus despite well-characterized lateralization of these object types in the left and right hemispheres, respectively. Furthermore, acquisition of one stimulus class has consequences for the perception of the other class. To investigate whether perception of faces and words influences perception of the other stimulus class, we presented images using rapid serial visual presentations at 10Hz. Across three experiments, participants (N = 42, 20, 20) discriminated two face, word and glasses targets (T1 and T2) embedded in a stream of images. As expected, second target discrimination was impaired when it followed the first target by 200-300ms relative to longer inter-target intervals, the so-called "attentional blink". Interestingly, T2 identification was much lower at short inter-target intervals when a face target was followed by a word (face-word) compared with glasses-word and word-word combinations, indicating that face processing interfered with word perception. Faces did not cause a larger deficit for the other stimulus types. The reverse effect was not observed; that is, word-face discrimination was no different than the other object combinations. The same pattern of results was observed for different target identification tasks and relative image sizes. EEG results indicated face activity over left occipital electrodes was correlated with the word deficit for face-word, but not for other object combinations. Taken together, the results suggest face processing specifically interferes with word processing, providing evidence for overlapping neural mechanisms of these two object types. Furthermore, asymmetrical face-word interference points differences in the overlap of face and word representations in the left and right hemispheres.

Disclosures: **A. Robinson:** None. **D. Plaut:** None. **M. Behrmann:** None.

Nanosymposium

668. Representation of Faces and Bodies

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Time: Wednesday, November 16, 2016, 8:00 AM - 10:45 AM

Presentation Number: 668.11

Topic: D.06. Vision

Title: Developmental visual perception deficits in the presence of normal face perception but abnormal eye movements

Authors: *S. GILAIE-DOTAN[1], R. DORON[2];
[1]UCL, London, United Kingdom; [2]Optometry, Hadassah Academic Col., Jerusalem, Israel

Abstract: Visual categories are associated with eccentricity biases in high-order visual cortex: faces and reading with foveally-biased regions, while common objects and space with mid- and

peripherally-biased regions. As face perception and reading are among the most challenging human visual skills, and are often regarded as the peak achievements of a distributed neural network supporting common objects perception, it is unclear why objects are associated with mid- to peripheral bias rather than with a foveal bias. Here, we report on a 9 y.o. boy (BN) with developmental object and space perceptual deficits resembling object agnosia, but with seemingly normal face perception, and with acquired reading skills. Given BN's normal basic-level vision, but significant oculomotor impairments in pursuit and saccades, we raise the possibility that eye movements play a critical role in the development of normal object and space perception, but not as much in face perception or reading. Common objects and space prevail in mid to peripheral visual field, and thus may rely on eye movements to attract foveal vision to them.

Disclosures: S. Gilaie-Dotan: None. **R. Doron:** None.

Nanosymposium

669. Cortical Circuits for Grasping

Location: SDCC 24A

Time: Wednesday, November 16, 2016, 8:00 AM - 11:15 AM

Presentation Number: 669.01

Topic: E.04. Voluntary Movements

Support: German Research Foundation (DFG) SCHE 1575/3-1

Title: Continuous decoding of power and precision grips with a high dimensional brain computer interface

Authors: *A. AGUDELO-TORO, W.-A. SHENG, J. MICHAELS, H. SCHERBERGER; Neurobio., German Primate Ctr., Goettingen, Germany

Abstract: Past neuro-prosthetic studies have allowed non-human primates and patients to control artificial arms and hands, but these devices have only allowed simple hand closure commands. Recent studies have decoded individual finger movements from humans but how the brain produces these configurations and the brain dynamics to select a specific grip type are largely unknown. We report the successful implementation of a brain computer interface (BCI) for the continuous control of a high degree of freedom virtual limb by a macaque monkey. The BCI enables the generation of two grip types and can be used to study hand grasping dynamics among three brain areas.
A monkey was trained in a delayed grasping task in which it should reach a physical handle with either a precision (pinch) or a power grip (full hand). Simultaneously, a virtual arm and handle were presented while its own arm was concealed. Single- and multi-units were acquired from the

grasping related left anterior intra-parietal area, F5 area of the ventral premotor cortex, and primary motor cortex.
After successful training of the physical task, neural activity was used to predict the movement of the virtual limb at various levels of brain control (BC): from 0 (a pre-recorded grip) to 1 (full brain control). At other levels, a linear combination of the recorded grip and neural decoding was used. The BCI was trained following 3 control phases: C0 to C2. During C0 the monkey was required to watch the task at BC 0. During C1 the monkey was assisted by the pre-recording (BC 0.5). At the end of C0 and C1 a Kalman filter was regressed with the neural data and the linear combination. During C2 BC was manually increased up to 1 while trying to keep the monkey's motivation.
The training of the virtual task progressed over 9 recording weeks with the monkey reaching full control at the 7th week. The typical training session lasted 1.2 hours and after the 7th week the monkey remained at BC 1.0 during 38% of C2 (C2 on average ~50 minutes). In comparison to previous BCI studies were BC=1 at C1, it was observed that BC 0.5 at C1 showed the best combination between stability (lower BC produced a more unstable BCI) and dampness (higher BC produced a more damped BCI). No stereotypical movements of the real arm were observed during virtual grasping.
We present a method to train a high degree freedom arm BCI allowing continuous grasping. Besides advancing the state of the art in BCI training algorithms, our BCI provides an excellent tool to study grasping by separating motor planning and execution from physical movement of the arm. Having access to three areas of the cortical grasp circuit allows us to elucidate grip planning and execution dynamics.

Disclosures: **A. Agudelo-Toro:** None. **W. Sheng:** None. **J. Michaels:** None. **H. Scherberger:** None.

Nanosymposium

669. Cortical Circuits for Grasping

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Presentation Number: 669.02

Topic: E.04. Voluntary Movements

Support: R01 EY015545

Boswell Foundation

Della Martin Foundation

Title: Functional organization of human posterior parietal association cortex at the population level and implications for brain-machine interfaces

Authors: *C. Y. ZHANG[1], T. AFLALO[1], K. PEJSA[1], D. OUELLETTE[2], E. ROSARIO[2], N. POURATIAN[3], R. A. ANDERSEN[1];
[1]Caltech, Pasadena, CA; [2]Casa Colina Hosp. and Centers for Healthcare, Pomona, CA; [3]Univ. of California Los Angeles, Los Angeles, CA

Abstract: We tested how a diverse set of motor variables are coded within a 4 by 4 mm patch of the anterior intraparietal (AIP) area of the posterior parietal cortex (PPC) of a tetraplegic subject participating in a brain-machine interface clinical trial. Despite being traditionally thought of as a grasp region, we tested the cognitive strategies of imagined and attempted movements for both hands and both shoulders. Surprisingly, we found significant representations of both cognitive strategies for movements of either hand or shoulder. Single neurons responded to different combinations of these variables, indicating distributed and partially overlapping functional networks of neurons are recruited for each movement type. There was also considerable structure within the neural population, with greater overlap at the single neuron level for movements sharing similar traits (strategy, side, or body part). Despite mixed coding in individual neurons, each motor variable was linearly separable and decodable from the population. Given the different populations for attempted and imagined movements, we also tested which strategy was best for online brain control. Both cognitive strategies with both the left and right hand and shoulder were viable for control. Our results show that signals in the PPC are highly distributed, with a small patch of cortex representing many effectors and imagine and attempt strategies, but with structure within this distributed population. The organization of actions in the PPC is advantageous for prosthetic applications since intended movements of a large extent of the body can be sampled from a single recording array and, despite the distributed nature of the representation, many different motor variables can be decoded from the population activity.

Disclosures: **C.Y. Zhang:** None. **T. Aflalo:** None. **K. Pejsa:** None. **D. Ouellette:** None. **E. Rosario:** None. **N. Pouratian:** None. **R.A. Andersen:** None.

Nanosymposium

669. Cortical Circuits for Grasping

Location: SDCC 24A

Time: Wednesday, November 16, 2016, 8:00 AM - 11:15 AM

Presentation Number: 669.03

Topic: E.04. Voluntary Movements

Support: NIH Grant R01 NS079664

Title: Sequential encoding of location then object during reaching and grasping: implications for brain-machine interfaces

Authors: *A. G. ROUSE, M. H. SCHIEBER;
Neurol. and Neurosci., Univ. of Rochester, Rochester, NY

Abstract: The ability to manipulate objects in our surroundings requires both reaching to and grasping the desired object, involving motion both proximally and distally in the upper limb. Previously, reaching typically has been described as occurring with the arm, while grasping proceeds concurrently with the hand. Our recent results from two Rhesus monkeys performing a reach-grasp-manipulate task, however, have shown two distinct temporal phases of movement. In the first phase variation depends primarily on reach location, while in the second phase variation depends primarily on the object about to be grasped. Time-varying analysis of variance (ANOVA) demonstrates that joint kinematics, muscle activity, and neural firing rates in the caudal primary motor cortex (M1c), all vary sequentially, depending more on location early and object late. These sequential variations each occurred in joints, in muscles, and in M1c cortical representations (as assessed with intracortical microstimulation), both proximal and distal.
We also assessed the relationship of the neural recordings to kinematic variables. Two kinematic state spaces were defined related to reach location and hand grasp shape. The best prediction of location with a linear decoder occurred near the onset of movement while the best prediction of grasp shape occurred later, shortly before contact with the target object. The neural population of M1c showed little location versus object segregation: the same neurons that were best for decoding reach location also were best for decoding grasp shape. The larger shift occurred over time with the same neurons being first more related to location and then more related to object. Our results have key implications for improving brain-machine interfaces (BMI) that control upper-limb prostheses. Most current BMIs rely on continuous, linear decoders with constant and equal representation of all BMI output degrees of freedom. We propose sequential, non-linear decoders that better emulate the natural neural control of reaching and grasping will provide more natural movements of upper-limb prostheses. Furthermore, most current decoders have constant gains that provide similar speeds and accuracies at all times. We propose novel decoders that would allow the BMI user to shift attention from one subset of features for fast, efficient reaching movements, to another subset for precise grasping movements, depending on the phase of the task.

Disclosures: **A.G. Rouse:** None. **M.H. Schieber:** None.

Nanosymposium

669. Cortical Circuits for Grasping

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Presentation Number: 669.04

Topic: E.04. Voluntary Movements

Support: MIUR

FIRB 2013,RBFR132BKP

Title: The dorsomedial grasping area V6A of macaque monkey: influence of the visual feed-back.

Authors: *R. BREVEGLIERI, M. DE VITIS, A. BOSCO, C. GALLETTI, P. FATTORI; Pharm. and Biotech., Univ. Di Bologna, Bologna, Italy

Abstract: One of the major functions of vision is to enable an efficient and active interaction with the surrounding environment. The execution of complex movements, such as object grasping, requires the integration of multisensory inputs. Area V6A, located in the medial posterior parietal cortex of macaque brain, is involved in sensorimotor transformations and contains neurons responsive to visual stimuli as well as cells modulated by grasping movements (Fattori et al., 2015, Cerebral Cortex pii: bhv302). The present study aims at testing to what extent V6A grasping neurons are influenced by the visual feed-back. Two macaque monkeys were trained to grasp different three-dimensional objects in light and in dark. Five different objects were used. The animals employed five different grips to grasp the objects: whole-hand prehension, hook grip, finger prehension, primitive precision grip, and advanced precision grip. The spatial location of the object to be grasped was kept constant. We performed extracellular recordings from 100 V6A neurons, and quantified the neural activity during grasping preparation, execution, and object holding. The overwhealming majority of neurons (94%) resulted to be task-related (Student's t-test, $p<0.02$, corrected for multiple comparison). Most of task-related cells were found to be influenced by both visual background and grip type. Half of the neurons were excited by the visual input, half were inhibited. As to the grip sensitivity of task-related cells, it was poorly influenced by the visual feed-back in most cases. This result was confirmed by the demixed Principal Component Analysis, that indicated that the influence of visual information on the cell's discharge was lower than that of grip type (14% vs. 31%). This is quite surprising for a visual area, but is in line with the view that V6A integrates visual and motor-related signals in monitoring the prehension actions.

Disclosures: R. Breveglieri: None. **M. De Vitis:** None. **A. Bosco:** None. **C. Galletti:** None. **P. Fattori:** None.

Nanosymposium

669. Cortical Circuits for Grasping

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Time: Wednesday, November 16, 2016, 8:00 AM - 11:15 AM

Presentation Number: 669.05

Topic: E.04. Voluntary Movements

Support: CIHR MOP126158

Title: Functional coupling between the frontoparietal and occipitotemporal pathways during action, perception and rest

Authors: *J. P. GALLIVAN[1], R. M. HUTCHISON[2];
[1]Queen's Univ., Kingston, ON, Canada; [2]Dept. of Psychology, Harvard Univ., Cambridge, MA

Abstract: Several lines of evidence point to areas in the occipitotemporal pathway as being critical in the processes of visual perception and object recognition. Much less appreciated, however, is the role that this pathway plays in object-related processing for the purposes of visually guided action. Here, using functional MRI (fMRI) and functional connectivity measures, we examined interactions between areas in frontoparietal cortex (FPC) involved in grasping, reaching, eye movements and tool use and areas in occipitotemporal cortex (OTC) involved in object-, face-, scene-, body-, tool- and motion-related processing, both during the performance of sensorimotor and perceptual tasks, as well as passive fixation (rest). For the sensorimotor tasks, we analyzed data from 3 separate fMRI studies in which participants, in an event-related paradigm, were first instructed about which target-directed movement to make (i.e., either a grasp, reach, saccadic eye movement, or tool-related action) and then, following a delay period, executed the movement. For the perceptual tasks, we analyzed data from 2 separate fMRI studies in which participants, in a block-design paradigm, performed either a 1-back or detection task during the visual presentation of different stimulus categories (objects, faces, scenes, bodies and tools). Lastly, our analysis of functional connectivity between FPC and OTC regions during passive fixation was based on resting-state data acquired from the Brain Genomics Superstruct Project. Hierarchical cluster analysis of the timecourse data identified correspondence in the patterns of FPC and OTC connectivity during the perceptual tasks and rest, which both tended to parcellate areas along traditional FPC (dorsal) and OTC (ventral) pathway boundaries. During the sensorimotor tasks, however, cluster analysis revealed a notable separation in functional coupling between ventral-medial and ventral-lateral regions of OTC, with several of the latter areas being clustered together with sensorimotor areas in parietal cortex. This indicates that the functional coupling of ventral-lateral areas to parietal and ventral-medial structures is flexible and task-dependent, and suggests that regions in lateral occipital cortex, in particular, may play an important role in mediating interactions between the dorsal and ventral pathways during sensorimotor control.

Disclosures: **J.P. Gallivan:** None. **R.M. Hutchison:** None.

Nanosymposium

669. Cortical Circuits for Grasping

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Presentation Number: 669.06

Topic: E.04. Voluntary Movements

Support: CIHR Operating Grant MOP 130345

Title: Getting a grasp on real objects and pictures: grasping movements directed to real objects and pictures rely on dissociable neural representations

Authors: ***S. N. MACDONALD**[1], E. FREUD[2], J. CHEN[1], D. J. QUINLAN[1], M. A. GOODALE[1], J. C. CULHAM[1];
[1]Western Univ., London, ON, Canada; [2]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Although real objects and pictures of the same objects share a high degree of visual similarity, they differ fundamentally in the actions that can be performed on them. Indeed, previous behavioral studies have suggested that simulated grasping of pictures relies on different representations than actual grasping of real objects. Here we used functional magnetic resonance imaging (fMRI) to investigate how brain activation patterns differed for grasping and reaching actions toward real objects and pictures. To this end, we used Representational Similarity Analysis (RSA) on fMRI data acquired from participants who grasped or reached to touch real objects vs. visually similar pictures of the same objects. The analysis revealed that the anterior intraparietal sulcus (aIPS), a key region for visually guided grasping was differentially sensitive to the nature of both the format (real/picture) and the motor task (grasping/reaching). Interestingly, the sensitivity to the format of the object was stronger for the grasping movements compared to the reaching movements. Moreover, this sensitivity was evident even in the planning phase before movement initiation, and was not found in other cortical regions, including the motor and somatosensory cortices. This suggests that the dissociable representations in the aIPS were not based on haptic, motor or proprioceptive feedback. Together, these findings provide novel evidence that affordances conveyed by visual properties can modulate action representations in the human aIPS.

Disclosures: **S.N. Macdonald:** None. **E. Freud:** None. **J. Chen:** None. **D.J. Quinlan:** None. **M.A. Goodale:** None. **J.C. Culham:** None.

Nanosymposium

669. Cortical Circuits for Grasping

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Presentation Number: 669.07

Topic: E.04. Voluntary Movements

Support: NIH Grant NS011862

Title: Posterior parietal cortical representation of hand actions during prehension

Authors: *E. P. GARDNER[1], J. L. BAKER[2], D. GARDNER[3], K. P. PURPURA[2], J.-W. RYOU[2], J. C. VAN DAELE[4];
[1]Dept Physiology/Neuroscience, New York Univ. Sch. Med., New York, NY; [2]Brain and Mind Res. Inst., [3]Physiology/Biophysics, and Brain and Mind Res. Inst., Weill Cornell Med., New York, NY; [4]Dept Physiology/Neuroscience, New York Univ. Sch. of Med., New York, NY

Abstract: Bimanual movements that require coordinated and synergistic actions of the two hands may be coordinated by synchronous bilateral activation of parietal cortical areas in both hemispheres, by enhanced activation of individual neurons specialized for bimanual actions, or by both mechanisms. To investigate parietal cortical neural mechanisms that mediate unimanual and bimanual prehension, we compared actions of the left and right hands in a reach-to-grasp-and-pull instructed delay task. Neural activity was recorded bilaterally when the animal used the left, right, or both hands to manipulate objects that differed in shape and location in the workspace. Arrays of 32 independently moveable microelectrodes were implanted in the hand area of posterior parietal (PPC) and S1 cortex of macaques allowing us to measure and compare the relative timing, amplitude, and synchronization of cortical activity within and between hemispheres as the animal performed the task. Neurons in both hemispheres showed common task-related firing patterns that peaked during reach and/or grasp, but actions of the ipsilateral hand evoked weaker responses than those of the contralateral hand. When both hands moved in tandem, their actions were represented continuously in both hemispheres, leading to longer duration, higher amplitude responses. Bilateral population ensemble responses thereby provide an accurate depiction of hand actions during skilled tasks. Bimanual representation of hand actions may serve an important role in "motor equivalence" when the same movements are performed by either hand. These findings also demonstrate that somatosensory and posterior parietal cortical areas serve important cognitive and motor functions in skilled hand actions.

Disclosures: **E.P. Gardner:** None. **J.L. Baker:** None. **D. Gardner:** None. **K.P. Purpura:** None. **J. Ryou:** None. **J.C. Van Daele:** None.

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669. Cortical Circuits for Grasping

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Support: ERC Parietalaction

Title: Selectivity of single phaip neurons for observed hand actions.

Authors: *G. A. ORBAN[1], T. AFLALO[2], D. CORBO[1], N. POURATIAN[3], C. LIU[4], R. ANDERSEN[2];
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Abstract: The neural substrate of action observation is presently under debate. Cortical regions activated during the observation of action include the premotor cortex, lateral occipito-temporal cortex, and posterior parietal cortex (PPC). We used the exceptional opportunity to record single neurons from the PPC of two human subjects to investigate the selectivity of neurons for observed actions in the presumed human homolog of the anterior intraparietal cortex (phAIP) (Aflalo et al 2015). phAIP is an area of PPC thought to emphasize the planning of grasping and hand manipulative actions and has been shown previously to be involved in processing observed manipulative actions using functional imaging (Jastorff et al 2010, Abdollahi et al 2013 and Ferri et al 2015.) Observation selectivity was tested by having each subject watch pre-recorded movies of seven different actions (dropping, dragging, pushing, rolling, rotating, grasping, and squeezing) while varying fixation location, actor gender, and object type. We found that ~40% of recorded neurons were tuned to the action type but invariant to the gender of the actor and the type of object. Some neurons showed a high-degree of specificity, responding only to a single preferred action while other neurons showed equivalent activation for several actions. We used static and dynamic control stimuli to verify that selectivity was preferential for dynamic action movies. Although selectivity for all actions was recorded, certain actions were preferentially coded in the recorded population. However, the set of preferred actions differed between subjects suggesting that different subregions of phAIP may preferentially code different actions. Recordings were made using a 10x10 grid of electrodes spaced at 400 μm. We found a U-shaped topographic structure across the array; neighboring electrode locations had similar, correlated tuning properties, with the correlation decreasing, and then, surprisingly, increasing again for longer distances. This finding suggests a repeating lattice-like topography. These results represented unprecedented insight into the action-observation network at the level of individual neurons in humans. Further, when combined with functional imaging results using the same stimuli (Corbo et al. SFN 2016), these results provide one of the first direct comparisons between

neuroimaging and single unit recording in human cortex. Supported by NIH grants P50 MH942581, R01 EY015545, the Boswell Foundation and the Della Martin Foundation.

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Nanosymposium

669. Cortical Circuits for Grasping

Location: SDCC 24A

Time: Wednesday, November 16, 2016, 8:00 AM - 11:15 AM

Presentation Number: 669.09

Topic: E.04. Voluntary Movements

Support: ERC Parietalaction

Title: Selectivity of single putative human AIP voxels for manipulative hand actions

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Abstract: To complement the coding of observed action classes in posterior parietal cortex we searched for evidence of local exemplar coding. Since putative human AIP (phAIP) processes observed manipulative actions (Jastorff et al 2010, Abdollahi et al 2013, Ferri et al 2015), we investigated the specificity of single phAIP voxels for manipulation exemplars. We scanned 20 subjects who viewed video-clips showing a male or female actor, viewed from the side, performing seven hand actions: dragging, dropping, grasping, pushing, rolling, rotating, and squeezing a plumb or a mandarin. Ten runs including 8 blocks, corresponding to the 7 actions and a fixation baseline, repeated four times, were sampled in a single fMRI session. Each block included 4 variants (2 genders and 2 objects) of an exemplar. We assessed the selectivity of single voxels in left (n=419) and right (n=323) phAIP using the procedure of Serences et al (2009), whereby one run was used to determine the optimal action, and the other 9 to compute the responses of the voxel to the 8 conditions, a procedure repeated 10 times. In left and right phAIP 40-80% of the voxels were selective for one of the seven actions and remarkably the proportions in the two hemispheres were strongly correlated across subjects (R2=0.67). Comparison with voxels selective for fixation showed that the selectivity did not simply reflect the procedure. All actions were equally represented. This selectivity for a discontinuous variable (observed manipulative actions) differed from classical tuning curves in that for selective voxels any non-optimal action was equally non-optimal (average rank 3.5). Neighboring voxels had similar selectivity, a grouping that was stronger in left than right phAIP. Hence each left and right phAIP contained an action-topic map, with some degree of overlap

between individual subjects (a voxel being selective for the same action in up to 6 subjects). Further investigations showed that the maps contain local structure: voxels within 10 mm being selective for an action, and voxels further away for any of the other 6 actions, an organization we termed a flower arrangement, echoing the lack of structure in the responses to non-optimal actions. The maps also contain global features such as anterior-posterior gradients, or repetitions of clusters of voxels selective for a given action. These results provide strong evidence that single voxels are selective for exemplars of the class of hand manipulation, proving a new type of coding differing from any known so far. They also imply that single neurons in phAIP should be selective for these exemplars, a hypothesis tested in the companion abstract.
Supported by ERC Parietalaction.

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Nanosymposium

669. Cortical Circuits for Grasping

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Topic: E.04. Voluntary Movements

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Federal Ministry of Education and Research (BCCN II, DPZ-01GQ1005C)

Title: A recurrent neural network model of the visuomotor grasp generation circuit

Authors: *J. A. MICHAELS[1], B. DANN[1], R. W. INTVELD[1], A. AGUDELO-TORO[1], H. SCHERBERGER[1,2];
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Abstract: Grasping movements are essential for primates. In macaque monkeys, the core of the grasping circuit is formed by the interconnected anterior intraparietal area (AIP) in the parietal lobe, the hand area (F5) of the ventral premotor cortex, and the hand area of the motor cortex (M1). Yet, very few neural network models of this circuit exist. Generating appropriate delayed grasping movements involves many inter-related steps, from identification of visual target identity and spatial location, to the determination and working-memory of the appropriate hand shape, and finally the direct control of muscles during movement. We hypothesized that the grasping circuit could be effectively modeled by training a recurrent neural network implementing a dynamical system to produce muscle contraction rates as a linear combination of neural firing rates. The network was composed of three internally recurrent modules sparsely

connected to each other: an input module receiving visual information reciprocally connected with an intermediate module, which was reciprocally connected to an output module controlling muscles. In order to collect appropriate experimental data, we recorded neural populations from AIP and F5 using floating microelectrode arrays. During recording, a macaque monkey performed a delayed grasping task with two grip types and a memory component, in which the amount of preparation time was systematically varied using 12 discrete delays (0-1300 ms), plus a no-movement condition lasting 2 seconds. In addition, 27 degrees of freedom (DOF) were recorded in the joint angle space in a separate session using a tracking glove, which were further transformed into a 50 DOF muscle length space musculoskeletal model of the arm and hand. The model was successfully trained to produce the muscle contraction rates observed during grasping while withholding movement at all other times (normalized error: 3%). Importantly, biological regularizations were implemented to encourage simplistic solutions (firing rate penalty, complex state trajectory penalty). Interestingly, the similarity between the neural data and the model was high, as measured by canonical correlation (mean r = 0.77 over 5 dimensions of highest variability), while similarity to an unregularized (mean r = 0.54) or an untrained (mean r = 0.43) model was much lower. Crucially, the AIP data best matched the input module (mean r = 0.73), while the F5 data best matched the intermediate module (mean r = 0.83). Our model therefore provides a simplistic and accurate representation of the primate grasping circuit without the use of neural data nor assumptions of individual neuron tuning properties during training.

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Nanosymposium

669. Cortical Circuits for Grasping

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Presentation Number: 669.11

Topic: E.04. Voluntary Movements

Title: A subgroup of premotor cortex mirror neurons that may orchestrate the coordination of hand and eye movements

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Abstract: In the posterior bank of the arcuate sulcus saccade related responses have been reported, a finding that has suggested the existence of a premotor eye field. Further support for a premotor eye field comes from intracortical microstimulation (ICMS) experiments which have revealed spots in premotor cortex around the border between areas F4 and F5 whose stimulation

gave rise to centering eye movements. However, it has remained unclear if these spots, whose location seems to vary between monkeys, are parts of a continuous eye representation and whether they are congruent with the region exhibiting saccade-related signals. Finally, the relationship to a distinct group of neurons - mirror neurons (MN) - characterizing ventral premotor cortex/ F5 has remained elusive. MN are activated by the execution of goal-directed actions as well as by the observation of similar actions when carried out by others. In the current study we tried to clarify if the premotor eye movement representation is anatomically segregated and functionally independent of the parts of the premotor cortex accommodating mirror neurons. We deployed ICMS in 2 rhesus monkeys (Macaca mulatta) in order to identify eye movement related premotor cortex. We were able to delineate a small region, close to the spur of the arcuate sulcus , whose stimulation gave rise to centering eye movements. They were often associated with movements of the mouth, arm, trunk, or the pinnae, typically emphasizing the contralateral side of the body. If and to what extent evoked saccades were complemented by movements of other body parts depended on current thresholds, stimulation time and depth. In one of the 2 monkeys, we were able to subject neurons recorded from the eye patch to standard mirror neuron tests and additionally to a memory saccade paradigm. The MN tasks required the monkey to reach out to and to grasp a cued object from a set of three present on a table in front. In the action observation task, the animal was required to watch a 3 seconds movie showing a goal-directed arm/hand action to the same object. Some of the neurons isolated from the eye patch clearly qualified as MN. Surprisingly, a significant number also exhibited clear saccade related bursts in the memory saccade task, which were independent of target position. We hypothesize that eye patch MN may help to orchestrate a motor synergy involving the hand and the eyes evoked by the observation of others´ goal directed actions. Actually, the response pattern triggered by action observation may be even richer: this is suggested by the fact that microstimulation often evoked movements of additional body parts complementing the eye movements.

Disclosures: **S. Spadacenta:** None. **J.K. Pomper:** None. **P. Thier:** None.

Nanosymposium

669. Cortical Circuits for Grasping

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Support: Istituto Italiano di Tecnologia

European Union Grant "Cogsystem" FP7–250013

Title: Beyond the dorsolateral grasping network: comparative properties between area F6 and F5 neurons during vision and grasping of objects

Authors: *M. LANZILOTTO[1], M. MARANESI[2], A. LIVI[1], M. GERBELLA[1], L. FOGASSI[1], G. RIZZOLATTI[1], L. BONINI[2];
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Abstract: It is widely accepted that the visuomotor processing of graspable objects rely on a network of cortical areas lying on the dorsolateral part of the hemispheres (dorsolateral grasping network - DGN), while mesial premotor regions play a crucial role in sequencing and triggering motor plans encoded in the DGN. Nevertheless, there is no direct evidence on 1) the possible contribution of presupplementary (area F6) neurons in reaching-grasping actions, and 2) their possible relationships with ventral premotor area F5. To address these issues, here we investigated area F6 neuronal properties by using the same behavioral paradigm previously employed, in the same 2 animals, to study area F5 neurons (Bonini et al 2014).
All neurons were recorded by means of linear multielectrode silicon probes, and studied with a go/no-go visuomotor task in which each trial started when the monkey engaged fixation in complete darkness. A cue sound instructed it either to grasp or refrain from grasping the subsequently presented target. Then, a light switched on revealing one among three different graspable objects (target presentation). When the cue sound ceased (go/no-go signal), the monkey had to reach, grasp and pull the object either in the light or in the dark (go conditions), or to remain still (no-go condition).
We recorded 233 task-related neurons in area F6: 108 were purely motor, 103 visuomotor and 22 purely visual. Most purely motor neurons (n=75, 69.4%) discharged similarly during reaching-grasping in the light and in the dark and 32 (29.6%) also showed grip selectivity, similarly to those recorded in area F5. Among F6 neurons responding to target presentation (n=125), 28 (22.4%) also showed object visual selectivity. However, in contrast to F5 visuomotor neurons, the visual activity in area F6 was stronger during object presentation rather than action execution, even when considering visuomotor neurons alone, and exhibited a marked preference for the ring relative to other objects. This latter finding supports the idea that F6 neurons underlie visuomotor associations between observed objects and potential motor actions. In addition, we found that F6 neuronal activity peaks earlier than in F5, suggesting that area F6 plays a role in driving F5. Finally, F6 neurons were mostly characterized by phasic visual and motor activations, in contrast with the sustained visual-to-motor activity of area F5.
These findings demonstrate that area F6 plays a role in natural manipulative actions, and provide the first comparative account of the possible mutual roles of F6 and F5 in the organization and control of reaching-grasping actions.

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Nanosymposium

669. Cortical Circuits for Grasping

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Presentation Number: 669.13

Topic: D.03. Somatosensation: Touch

Support: PSC-CUNY 68854-00 46

Title: Temporary deafferentation evoked motor synergy attenuation in nature vs. non-nature manual tasks

Authors: *W. ZHANG[1], B. HAHN[2], C. BENSON[2];
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Abstract: Successfully learning and adapting a motor task requires afferent input to provide the reference information such as object properties (Zhang et. al., 2011), interactive force (Moerchen et. al., 2006), or digital position sense (Gentilucci et. al., 1997). In healthy individuals, the center nervous system is capable of processing an integration of the somatosensory and visual feedback with motor commands (Gordon et al., 1993), ensuring the development of motor synergies in a specific learned task. The motor synergy formulation has been suggested in a feed-forward control model (Simon et al., 2006). However, the contribution of the afferent input in the synergy development has not been well defined. Regional anesthesia will induce reversible deafferentation thus reducing the somatosensory input from the hand. The present study investigated the effect of temporary deafferentation on quantified multi-digit coordination patterns by performing a digital anesthesia procedure at selected digits. Thirty one (16M, 15F) healthy, right handed, young adults participated in this study. Four or five multi-dimensional force/torque sensors were installed horizontally on the table or vertically on a customized grip device to measure individual finger force/torque in isometric pressing or grasping-lifting tasks. The grasping-lifting tasks represent daily natural tasks. However, given that the force templates requirement and individual's performance were displayed on a computer monitor, the pressing tasks are non-natural, since neither force production is specified nor is explicit visual feedback provided in daily manual tasks. All tasks were performed before and during a digital anesthesia procedure on thumb, index and middle fingers. We analyzed subjects' digital maximal force, force sharing, enslaving effect, task performance, and motor synergies in anesthesia vs. control group. We found that subjects' maximal force ability was reduced at both deafferented and intact digits in both natural and non-natural tasks. Furthermore, digital deafferentation evoked significantly lower task-specific multi-digit synergy indices only in natural tasks, but not in non-natural tasks. These findings suggest that 1) an alternation at the central nervous level was induced by a temporary deafferentation thus leading to an across-digit information sharing

mechanism to attain the task performance; 2) afferent input from the digits reveals its contribution to the motor synergy development, indicating a feedback control scheme; 3) somatosensory feedback can be overridden by the visual signals and thus the potentially elicited motor deficits can be compensated.

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Nanosymposium

670. Neural Circuits for Learning and Decision Making

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Presentation Number: 670.01

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant MH85958

KAKENHI

Title: A neural correlate of Pavlovian conditioning in *Drosophila* brain

Authors: *M. YOSHIHARA[1], A. SAKURAI[1], J. T. LITTLETON[3], H. KOJIMA[2]; [1]Memory Neurobio. Project, [2]Bio ICT, NICT Kobe, Kobe, Japan; [3]The Picower Inst. for Learning and Memory, MIT, Cambridge, MA

Abstract: Classical conditioning demonstrated by Pavlov is a well-defined form of memory formation. Pavlov repeated auditory stimulation such as bell ringing (conditioned stimulus: CS) before feeding a dog (unconditioned stimulus: US), resulting in saliva secretion in response to the bell ringing only as a CS. To investigate potential neurophysiological mechanisms of Pavlovian conditioning, we need a simple neuronal network to correlate behavior with the underlying neuronal circuit dynamics. For this purpose, we recently identified a pair of command neurons for feeding behavior (Fdg neuron) in the *Drosophila* brain[1] following a screen of Gal4 lines established by the NP consortium[2]. The Fdg neuron is thought to function downstream of sensory and metabolic cues and at the top of the feeding motor programs. Therefore, it is expected that plastic changes in the Fdg neuron will be correlated to behavioral changes induced through Pavlovian conditioning. We have now established a novel conditioning protocol to associate somatosensory stimuli from a rod release (CS) with a feeding behavior (proboscis extension) induced by sucrose stimulation (US) in adult flies. After repeated pairing of the CS and US, flies extended their proboscis in response to release of the rod alone. By recording calcium signals while simultaneously observing feeding behavior[3], we have monitored activity of the Fdg neuron during the conditioning. Before the conditioning, the CS alone did not activate

the Fdg neurons. However, as the training is repeated, activation of the Fdg neuron by the conditioned stimulus alone progressively increased. Once activation of the Fdg neuron by CS alone reached a threshold, proboscis extension by CS alone was observed. These results suggest creation of a new connection from the neuronal circuit conveying the CS to the feeding circuit. Our results open an avenue for the systematic analyses of molecular and cellular basis of changes in circuit connections underlying memory formation at the single cell level using powerful *Drosophila* genetics. 1) Flood et. al. (2013) *Nature*, **499**, 83-87. 2) Yoshihara and Ito (2000) D. I. S. 83:199. 3) Yoshihara (2012) JoVE, 62, e3625.

Disclosures: **M. Yoshihara:** None. **A. Sakurai:** None. **J.T. Littleton:** None. **H. Kojima:** None.

Nanosymposium

670. Neural Circuits for Learning and Decision Making

Location: SDCC 5B

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Presentation Number: 670.02

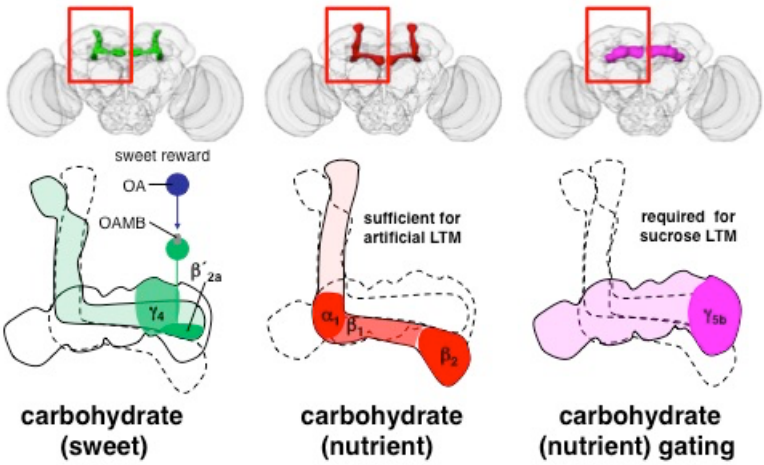
Topic: G.01. Appetitive and Aversive Learning

Title: Palatability and caloric value reinforce distinguishable memories via mushroom body dopamine neurons

Authors: *W. HUETTEROTH[1], E. PERISSE[2], S. LIN[2], M. KLAPPENBACH[2], C. BURKE[2], S. WADDELL[2];
[1]Univ. of Konstanz, Konstanz, Germany; [2]CNCB, Univ. of Oxford, Oxford, United Kingdom

Abstract: Recent studies in Drosophila unveiled a cluster of dopaminergic neurons that provide reward-learning signals like in mammals. All neurons of this cluster innervate distinct regions on the mushroom body, filling the gaps of previously identified dopaminergic neurons that convey aversive value. It appears as if quality and value of reinforcing stimuli are encoded in these different subregions of the mushroom body. But despite the fact that all neuronal cell types of the mushroom body have been identified by now, a complete assignment of reinforcing signals has not emerged yet. Previous work demonstrated that sugars can have two separable reinforcing properties: hedonic sweetness and nutritional value. Experimentally this can be addressed by using sweet sugars without any caloric value like arabinose, sweet and nutritious sucrose, or nutritious but tasteless sorbitol. Sweet reinforcement leads to short-lasting memory only, whereas long-lasting food memory requires a nutritional component. Subsequently we have shown that octopaminergic neurons convey exclusively hedonic sweetness, and act upon a subset of rewarding dopamine neurons through the alpha-adrenergic receptor OAMB. Here we were able to narrow down the short-term reinforcing effects of sweet taste to its responsible

dopaminergic neurons innervating the b'2 and g4 regions of the mushroom body lobes. The nutritional reward signal requires separable dopamine signaling in the g5b region, while artificial activation of dopamine neurons projecting to the b lobe and adjacent a1 region alone are sufficient to implant a long-lasting memory. Interestingly, this implanted long-term memory responds differently to the caloric state of the animal; while artificial implantation and expression of short-term memory is independent of satiety state, acquisition and retrieval of long-lasting food memory requires the flies to be hungry. Taken together, a more complete picture emerges how different reinforcing stimuli are represented, integrated and modulated in the fly on the cellular level.



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Nanosymposium

670. Neural Circuits for Learning and Decision Making

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Nudge-IT, (FP-7: 607310)

Title: Optogenetic inhibition of ventral tegmental dopamine neurons directly disrupts reward seeking behavior

Authors: *R. A. ADAN[1], R. VAN ZESSEN[1], J. FLORES[1], G. VAN DER PLASSE[1], G. STUBER[2], G. RAMAKERS[1];
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Abstract: The mesolimbic dopamine (DA) system plays a crucial role in reward processing and disturbances in its function are linked to multiple psychiatric disorders. Ventral tegmental area (VTA) DA neurons become phasically active during the presentation of sucrose, as well as cues predicting this reward. While activation of these neurons is inherently rewarding, the necessity of this activity for behavior remains unclear. We used optogenetic techniques to specifically inhibit VTA DA neurons in a time-locked fashion during presentation of sucrose-predicting cues and sucrose consumption. We find that inhibition of these neurons directly disrupts reward-seeking and consummatory behavior and in addition decreases performance on subsequent trials. Importantly, as locomotion is unaffected, this suggests that inhibition of VTA DA neurons causes an acute decrease in the potency of the cue and reward to generate an appetitive behavioral response. Moreover, these data indicate that the transient decrease of VTA DA activity results in an enduring reduction in the learned stimulus-reward association, most likely caused by a decrease in perceived reward value.

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Nanosymposium

670. Neural Circuits for Learning and Decision Making

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Topic: G.01. Appetitive and Aversive Learning

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MEXT/JSPS KAKENHI 23120011

MEXT/JSPS KAKENHI 15H04275

Title: Neurotransmission in dopamine D2 receptor expressing nucleus accumbens neurons is necessary for behavioral flexibility in an IntelliCage place discrimination task

Authors: *T. MACPHERSON[1], M. MORITA[1], Y. WANG[2], T. SASAOKA[3], A. SAWA[4], T. HIKIDA[1];
[1]Grad. Sch. of Med., Kyoto Univ., Kyoto-Shi, Japan; [2]Univ. of Illinois, Chicago, IL; [3]Niigata Univ., Niigata, Japan; [4]Johns Hopkins Univ., Baltimore, MD

Abstract: The nucleus accumbens (NAc) is widely accepted to play a critical role in the acquisition and flexibility of behavioral strategies. Accordingly, altered functioning of the NAc is known to contribute to the etiology of several neuropathologies associated with impaired behavioural control, including schizophrenia and drug addiction. Within the NAc, medium spiny neurons can be subdivided into those expressing dopamine D1- or D2-receptors (D1-/D2-MSNs). Our group has previously shown these distinct neuron classes to control reward and aversion learning, respectively; however, their role in controlling place learning is still unknown.
Here, we use a gene-manipulating technique, termed reversible neurotransmission blocking (RNB), to separately and reversibly block transmission of D1- or D2-MSNs in the NAc. Transgenic mice on a C57BL/6 background, in which expression of transmission-blocking tetanus toxin is driven by the interaction of tTA and TRE, were injected with a recombinant AAV virus into the NAc, which restricted expression of tTA specifically to neurons expressing Substance P or Enkephalin, which found in D1- and D2-MSNs, respectively. The effects of blocking transmission in NAc D1- or D2-MSNs on acquisition and reversal learning of a place discrimination task were explored in the IntelliCage, an automated group-housing experimental cage apparatus.
Blockade of activity in NAc D1- and D2-MSNs did not alter acquisition of the task, but suppression of activity in D2-MSNs impaired reversal learning and increased perseverative errors. Additionally, transgenic mice on a C57BL/6 background with a global knockout of the dopamine D2L receptor isoform also showed a similar behavioral phenotype to D2-MSN-blocked mice. Based on these findings, we suggest that D2L receptors and NAc D2-MSNs act to suppress the influence of previously correct behavioral strategies, allowing a shift of behavioral control to new strategies. D2L receptors and NAc D2-MSNs may provide efficacious therapeutic targets for the treatment of cognitive impairments associated with loss of behavioral control.

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Nanosymposium

670. Neural Circuits for Learning and Decision Making

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Title: Parafascicular-controlled neurons in the posterior dorsomedial striatum regulate internal state information to guide action selection

Authors: *L. A. BRADFIELD[1], B. BALLEINE[2];
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Abstract: We (Bradfield et al. 2013) have previously demonstrated that parafascicular-controlled cholinergic interneurons (CINs) in the posterior dorsomedial striatum (pDMS) are critical for interlacing the new and existing action-outcome contingencies that underlie goal-directed action. An adjoining commentary (Schoenbaum et al. 2013) suggested that animals with impaired CIN function might suffer a deficit in creating/retrieving internal contexts or 'states'. We investigated this suggestion. For all experiments we used ipsilateral vs. contralateral cytotoxic lesions of the parafascicular thalamic nucleus (PF) and pDMS. All rats were first trained to press a right and left lever for sucrose and pellet outcomes (respectively, counterbalanced), then these contingencies were reversed and rats were tested for performance in outcome devaluation and outcome-selective reinstatement. In Experiment 1 we examined whether altering the physical context during reversal could provide a substitute 'external state' for contralateral rats, and thus rescue performance. This manipulation did indeed rescue reinstatement performance, as contralateral rats performed similarly to ipsilateral rats (reinstated > nonreinstated). However, the deficit in outcome devaluation performance remained (ipsilateral rats: nondevalued > devalued, contralateral rats: nondevalued = devalued). This suggests that intact animals relied on internal state information when cues (i.e. outcomes) were absent during devaluation testing, but not when cues were present during reinstatement. Experiment 2 investigated whether extended training could reverse the deficit, as would be predicted on a state modulation account. First we replicated the deficits previously observed in both devaluation and reinstatement after 4 days of training on the reversed contingencies, but found that after an additional 6 days of training performance was intact on both tests. Experiment 3 demonstrated this was not simply a result of the alteration in temporal context because contralateral rats tested 6 days after reversal without additional training were impaired. Experiment 4 replicated this result when rats were tested 3 weeks following reversal training, however when given an additional week and then tested, all rats responded in accordance with the original contingencies (devalued > nondevalued), demonstrating that intact rats do indeed undergo state modulation to determine which set of contingencies are currently active. Together, these results are consistent with the suggestion that PF-controlled neurons in the pDMS are responsible for state modulation of contingencies for action selection.

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Nanosymposium

670. Neural Circuits for Learning and Decision Making

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Support: ARC Discovery Project

NHMRC Project Grant

Title: Projections from the prelimbic cortex to the dorsomedial striatum mediate the acquisition of goal-directed actions

Authors: *G. HART, B. BALLEINE;
Psychology, Univ. of New South Wales, Kensington, Australia

Abstract: Like humans, animals are capable of selecting actions according to the current value of their respective outcomes. In rats, the acquisition of this "goal-directed" action selection strategy has been demonstrated to rely heavily on the prelimbic region of the medial prefrontal cortex (PL) and its direct downstream glutamatergic target, the posterior part of the dorsomedial striatum (pDMS). We employed an instrumental training task to asses changes in cellular activity-related synaptic plasticity in this pathway within distinct layers of the PL. Rats were infused with the retrograde tracer, fluorogold (FG) into the pDMS, to retrogradely label corticostriatal projection neurons. Rats were then given a single session of instrumental training or yoked Pavlovian training, perfused either 5 minutes or 60 minutes later and brains were quantified using immunofluorescence for short-term (5 minute) and long-term (60 minute) changes in MAPK/ERK phosphorylation (pERK). We found that instrumental training produced heightened pERK expression in distinct populations at each time point; 5 minutes after training, projections from posterior PL Layers 5-6 showed heightened expression, and after 60 minutes, pERK expression was heightened in projections from anterior PL Layers 2-3. We next sought to assess whether this PL- pDMS pathway was functionally necessary for the acquisition of goal-directed actions. We used a two-virus strategy; retrograde AAV-cre ("retro-cre"; UPenn) was infused bilaterally into the pDMS, and cre-dependent hM4Di DREADDs (Armbruster et al., 2007) was infused bilaterally into the PL to specifically infect pDMS projecting PL neurons. Rats were trained with two different actions each giving distinct rewards. Prior to each training session, PL projection neurons were silenced in one group of rats with a systemic injection of CNO, whereas control rats received vehicle. On the next day, rats were tested for goal-directed action control in an outcome devaluation test under extinction; rats were pre-fed one of the previously earned outcomes to satiety before being returned to the instrumental chambers with each action available to them. Rats that had received vehicle showed a clear goal-directed preference for the action that had delivered the non-sated food, however rats that had received

CNO selected both actions equally, indicating that action control was habitual. Together, these results suggest that instrumental training triggers a signaling cascade in PL-pDMS projection neurons that underlies the long-term learning required for goal-directed action selection.

Disclosures: G. Hart: None. **B. Balleine:** None.

Nanosymposium

670. Neural Circuits for Learning and Decision Making

Location: SDCC 5B

Time: Wednesday, November 16, 2016, 8:00 AM - 11:00 AM

Presentation Number: 670.07

Topic: G.01. Appetitive and Aversive Learning

Support: DFG-FOR 1617

Title: Human sign- and goaltracking: Individual differences in striatal responses during Pavlovian conditioning

Authors: *D. J. SCHAD[1], M. A. RAPP[1], M. SEBOLD[2], N. KRÖMER[3], M. GARBUSOW[2], M. SMOLKA[3], F. SCHLAGENHAUF[4,5], A. HEINZ[4], P. DAYAN[6], Q. HUYS[7,8];
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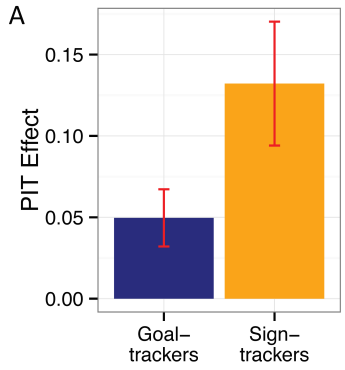
Abstract: Marked individual differences are apparent in appetitive Pavlovian conditioning. Over the course of learning, some subjects come to approach the CS (signtrackers; ST), while others learn to approach the location of later reward delivery (goaltrackers; GT). While ST and GT equally learn to predict the US from the CS, the CS acquires incentive salience (i.e., becoming attractive and wanted) only in ST. Animal findings suggest the classical dopaminergic reward prediction error (RPE) response in the Nucleus Accumbens (NAcc) during Pavlovian conditioning is present only in ST, but not in GT. However, whether human learning-related NAcc responses differ between sign- and goaltrackers is currently not clear. Here, we investigated human sign- and goal-trackers using fMRI and measuring eye position during Pavlovian conditioning. Sign- and goal-tracking was defined in terms of gaze distributions on the CS and US. Both groups were equally successful in Pavlovian and instrumental learning. However, sign-trackers exhibited stronger Pavlovian-Instrumental transfer than goal-trackers

(see Fig. 1). BOLD NAcc responses to CS and US diverged in signtrackers, but not in goaltrackers (p=.06); we are currently investigating how.



Disclosures: **D.J. Schad:** None. **M.A. Rapp:** None. **M. Sebold:** None. **N. Krömer:** None. **M. Garbusow:** None. **M. Smolka:** None. **F. Schlagenhauf:** None. **A. Heinz:** None. **P. Dayan:** None. **Q. Huys:** None.

Nanosymposium

670. Neural Circuits for Learning and Decision Making

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Title: Internally-generated action-selection bias in the posterior parietal cortex

Authors: *E. HWANG[1], J. DAHLEN[2], M. MUKUNDAN[1], T. KOMIYAMA[1];
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Abstract: When animals repeatedly encounter the same environmental stimuli, their response to the stimuli is not constant, but instead varies over time. Behavioral studies have suggested that a significant portion of the variability stems from the animals' internal bias that continuously changes with their past action-outcome experience. Neural activity in several brain areas has been shown to encode the past action and/or outcome information, the essential components of internal bias. However, it remains unknown where in the brain those history components are integrated to form internal bias that affects subsequent action selection. Here we combine behavioral modeling, two photon calcium imaging, and optogenetic perturbation in mice performing a two-alternative forced choice task and show that the posterior parietal cortex (PPC) is critically involved in transforming the action-outcome history into bias. In this task, mice were briefly presented with drifting gratings (forward or downward; 1sec) and were required to press the joystick in the remembered direction of the gratings after a 2-sec memory period. Despite the fixed stimulus-response rule, the choice of mice was highly variable. By fitting their behavioral variability with a regression model, we found that mice updated their internal bias on a trial-by-trial basis depending on their choice-reward history. The strategy of weighing different variables (e.g., choice history and reward history) changed from animal to animal and from session to session. In spite of such idiosyncratic and time-varying strategies, however, the trial-by-trial fluctuation of the internal bias was highly correlated with pre-stimulus activity of a subpopulation of PPC neurons which encode a mixture of previous trial reward, previous trial choice, and upcoming trial choice information during the inter-trial interval. This subpopulation was a distinct population from classically considered pre-motor neurons that become active during the peri-movement period. Furthermore, optogenetic perturbation of pre-stimulus activity in PPC, but not post-stimulus/pre-motor activity, altered the history-dependency of choice selectively in the perturbed trials. These findings suggest that PPC consists of distinct neural subpopulations, one producing history-dependent internal action-selection bias, and another reflecting pre-motor plans that are formed as a result of integrating both internal bias and external stimulus information.

Disclosures: **E. Hwang:** None. **J. Dahlen:** None. **M. Mukundan:** None. **T. Komiyama:** None.

Nanosymposium

670. Neural Circuits for Learning and Decision Making

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Fondation Bertarelli

Title: Different network dynamics encode information in auditory cortex and posterior parietal cortex.

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Abstract: Microcircuits are thought to represent information using populations of neurons organized into assemblies. However, it is not well understood if assemblies differ in their properties across brain regions. Also, assembly dynamics have not been fully compared for how sensory stimuli are encoded and how behavioral outputs are represented. Here, we performed calcium imaging in neuronal populations in auditory cortex (A1) and posterior parietal cortex (PPC) during a sound localization task. Sound stimuli were played from a spatial array of speakers surrounding the mouse's head. Mice reported decisions about the sound's location by running through a visual virtual reality T-maze, and were able to discriminate sound locations within 30 degrees in azimuth. We modeled activity in A1 and PPC neurons using a generalized linear model (GLM) that included task-related variables and the activity of other simultaneously imaged nearby neurons as predictors. Using a Bayesian decoder, we found that information about the sound stimulus' location was present in A1 neurons but was absent in the PPC. In contrast, information about the behavioral choice could be read out from activity in both areas. To investigate how information was represented at the population level, we analyzed the activity correlations in each area. In PPC, neuron-neuron noise correlations were markedly higher than in A1 and extended over long lags (> 1 second), despite transient activity in individual neurons. Furthermore, the inclusion of activity from simultaneously recorded neurons greatly improved the GLM predictions of individual PPC neuron activity time courses but provided much less enhancement in A1. Using the Bayesian decoder based on different sized time windows, we found that task-relevant information in A1 was largely independent from time point to time point, such that a read out functioning over long windows could accumulate different pieces of information. In contrast, in PPC information was mostly redundant across time points even though distinct sets of neurons were active at different points in a trial. Our results suggest that A1 functions moment-to-moment using a population of largely independent individual neurons. In contrast, the PPC may function as a correlated population, even across long timescales, forming a neuronal assembly as a trajectory through different population activity patterns. These results propose different network dynamics and mechanisms for cortical regions representing sensory stimuli compared to those accumulating sensory information to drive behavioral actions.

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Nanosymposium

670. Neural Circuits for Learning and Decision Making

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Title: Focused ultrasound applied to the dorsal striatum enhances sensorimotor decision-making in monkeys

Authors: *V. P. FERRERA[1], M. DOWNS[2], T. TEICHERT[4], M. E. KARAKATSANI[3], S.-Y. WU[3], A. BUCH[3], E. KONOFAGOU[2];
[1]Neuroscience, Psychiatry, Columbia Univ. Press, New York, NY; [2]Biomed. Engin., [3]Columbia Univ., New York, NY; [4]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Noninvasive brain stimulation using ultrasound has many potential applications as a research and clinical tool. Focused ultrasound (FUS) can be used for drug delivery and may have direct modulatory effects on neuronal activity or excitability. Here, we investigated the effect of FUS combined with intravenous microbubbles on visual-motor decision-making behavior in two rhesus monkeys. Monkeys were trained to discriminate random moving dot patterns with varying levels of motion strength that were presented on a touchscreen monitor. The reward magnitude, and the hand used to respond were also varied. Prior to half of the behavioral sessions, we injected intravenous microbubbles and targeted FUS (500 kHz, 400 kPa, 10 ms pulse length, 120 second duration) to the putamen in one hemisphere to open the blood-brain barrier, and then tested behavioral performance 3-4 hours later. On days when monkeys were treated with FUS, their decisions were more accurate than days without sonication; i.e. psychophysical thresholds for detecting motion direction were lower and response times were shorter. The enhanced performance cannot be accounted for by a speed-accuracy trade-off (criterion shift), but suggest that FUS treatment improved the quality of sensory evidence. The performance improvement was greater for responses made with the hand contralateral to the treated hemisphere than with the ipsilateral hand, suggesting that there is effector-specific evidence accumulation. A low dose of haloperidol (0.01 mg/kg) was given prior to some sessions. Haloperidol alone resulted in faster response times and lower accuracy compared to saline controls. The effect of haloperidol

was opposite that of increased reward magnitude (which yielded higher accuracy and longer response times), suggesting that haloperidol reduced motivation. The effects of haloperidol were modulated by sonication. The results suggest that a two-minute application of FUS can have a sustained impact on cognitive performance, and can modify the efficacy of psychoactive medications.

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Nanosymposium

670. Neural Circuits for Learning and Decision Making

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Title: Fear extinction without fear: Direct reinforcement of neural activity bypasses the need for conscious exposure

Authors: *A. KOIZUMI[1,2,3], K. AMANO[1], A. CORTESE[2,1,4,5], W. YOSHIDA[2,1,7], B. SEYMOUR[2,1,7], M. KAWATO[2,4], H. LAU[5,6];
[1]CiNet, NICT, Suita, Japan; [2]Dept. of Decoded Neurofeedback, ATR, Kyoto, Japan; [3]Psychology, Columbia Univ., New York, NY; [4]Grad. Sch. of Information Sci., Nara Inst. of Sci. and Technol., Nara, Japan; [5]Psychology, [6]Brain Res. Inst., UCLA, Los Angels, CA; [7]Engin., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Fear conditioning is a fundamentally important and preserved process across species. In humans it is linked to fear-related disorders such as phobias and post-traumatic stress disorder. One well-known procedure to overcome fear is to repeatedly pair encounterance of a fear conditioned stimulus (CS+) with a rewarding outcome (i.e., counter-conditioning). However, this procedure involves explicit presentations of CS+, which is itself aversive until fear extinguishes. Using real-time fMRI neurofeedback with multivariate decoding, we extinguished fear by directly counter-conditioning the activation patterns in visual cortex that resembled the decoded

features of visual CS+. We first constructed a decoder to distinguish two visual stimuli from their multivoxel activation patterns in the primary and secondary visual areas (V1/V2). Participants then went through Pavlovian conditioning, and acquired fear for the two visual stimuli (CS+) as they were paired with electric shocks. Afterwards, participants went through three days of neural reinforcement sessions during which they were rewarded on a trial-by-trial basis when the activation patterns in V1/V2 resembled the previously decoded patterns for one of the CS+ (Target) but not for the other (Control). Participants were not informed of the purpose of this procedure, and post-session tests confirmed that they were not consciously aware of the neural representation of Target CS+ during the neural reinforcement sessions. Nevertheless, participants showed reduced fear response for the Target CS+ relative to the Control CS+ when these stimuli were explicitly presented on a following day. Such reduction of fear was observed both in terms of skin conductance response as well as of amygdala hemodynamic activity. These results show that fear can be reduced without explicit reactivation of the fear memory, and this procedure may inspire new treatments for fear-related disorders such as phobia and post-traumatic stress disorder (PTSD), via unconscious processing.

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Nanosymposium

670. Neural Circuits for Learning and Decision Making

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Presentation Number: 670.12

Topic: H.01. Animal Cognition and Behavior

Title: PGO wave-triggered functional MRI: mapping the networks underlying synaptic consolidation

Authors: ***N. LOGOTHETIS**[1,2], Y. MURAYAMA[3], J. RAMIREZ-VILLEGAS[3,4], M. BESSERVE[3,5], H. EVRARD[3,6];
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Abstract: By combining concurrent electrophysiological recordings and fMRI, we recently demonstrated that the events known as hippocampal sharp wave-ripple complexes (SPW-R) are tightly associated with robust cortical activations that occur concurrently with a particularly

intriguing strong inhibition of large portions of subcortical brain structures that are closely involved in neural plasticity, such as the basal ganglia (BG), the pontine region (PONS) and the cerebellar cortex (Logothetis, Eschenko et al. 2012, Nature 491:547-53). Particularly intriguing was the strong inhibition of large portions of subcortical brain structures that are closely involved in neural plasticity, such as the basal ganglia (BG), the PONS and the cerebellar cortex. In primates, the negative BOLD in the pontine region was systematically associated with inhibition of the lateral geniculate nucleus (LGN) and foveal V1 activity, despite the overall positive fMRI responses in peripheral V1 and all other primary sensory and associational cortices. The deactivation of PONS may therefore be due to a temporary suppression of cholinergic sites involved in local plasticity and synaptic consolidation, such as those underlying the generation-propagation of theta rhythm, and so-called ponto-geniculo-occipital (PGO) waves. PGO waves have been often associated with the consolidation of procedural memory or synaptic consolidation in general. To examine this hypothesis and better understand the global regulation of brain activity during memory consolidation we set out to employ the methodology of Neural-Event-Triggered fMRI (NET-fMRI), combining simultaneous electrophysiological recordings in the region of the parabrachial nucleus (PBn) and MR imaging in monkeys under opioid anesthesia. First, we established a structural-MRI and angiography-based site-localization approach to access various brainstem regions with long electrodes without potential complications due to vasculature-injury. We subsequently physiologically identified PBn, LGN and the Hippocampal CA1/CA3 fields, and conducted concurrent, uninterrupted multi-site physiological and fMRI recordings in a 4.7T magnet. PGO events were considered to be the large field deflections, with various temporal and repetition profiles that typically co-occur in PBn and LGN. In sharp contrast to isolated LGN or PONS events, the PGO-like events - co-occurring in both pontine and thalamic structures - yielded a robust and striking pattern of up/down modulation, suggesting the PGO events correlated with upregulation of subcortical centers concurrently with inhibition of activity in neocortex.

Disclosures: **N. Logothetis:** None. **Y. Murayama:** None. **J. Ramirez-Villegas:** None. **M. Besserve:** None. **H. Evrard:** None.

Nanosymposium

671. Mechanisms of Cocaine Addiction

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Presentation Number: 671.01

Topic: G.08. Drugs of Abuse and Addiction

Title: Dopamine-induced inhibition of $GABA_A$-mediated currents in VTA dopamine neurons is input specific

Authors: *R. YAKA, M. WEITZ;
The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: Drugs of abuse increase dopamine (DA) levels in the reward system leading to euphoria and well-being. As a result, drug use is reinforced regardless of its many adverse effects, eventually resulting in addiction. Since the ventral tegmental area (VTA) is the major source for DA in the reward system and because initiation of drug addiction has demonstrated to occur in the VTA, its regulation is of great importance. The VTA receives excitatory and inhibitory inputs, both of which are modulated by acute and chronic exposure to drugs of abuse. As the excitatory inputs to the VTA are well studied, we focused on the GABAergic inhibitory inputs in this current study. Using rat midbrain slices we have previously shown that acute administration of DA or DA-increasing drugs inhibited $GABA_A$ receptor-mediated inhibitory post synaptic currents (IPSCs) in VTA DA neurons. Further, we found that DA-induced inhibition involves activation of DA D2-like receptors or $GABA_B$ receptors and has presynaptic locus as determined by measuring paired-pulse ratio and miniatures IPSCs. However, since electrical afferent stimulation was applied in those studies, the source of the GABAergic inputs to the VTA remained unknown. We hypothesized that the degree of DA-induced inhibition of $GABA_A$-mediated IPSCs in the VTA will differ in an afferent specific manner. Therefore, in this study optogenetic tools were used to discriminate between the different sources of inhibitory inputs innervating the VTA, emerging from the rostromedial tegmental area (RMTg), the lateral habenula (LHb), and the nucleus accumbens (NAc). We found that VTA DA neurons from rats injected with ChR2 in the RMTg presented strikingly greater DA-induced inhibition of light-evoked IPSCs than those recorded in response to afferent electrical stimulation (22% and 76% IPSCs inhibition in electric stimulation or optic stimulation of the RMTg, respectively). These results indeed suggest that region specificity plays a major role in determining the degree of DA-induced inhibition of IPSCs in VTA-DA neurons, eventually controlling their activity. Currently, we are assessing the inhibitory effect of DA application on IPSCs emerging from the LHb and NAc. Understanding the means by which DA modulates GABAergic inputs to the VTA exerted from different afferent pathways will expand our knowledge on how drugs of abuse act in the brain and become addictive.

Disclosures: R. Yaka: None. **M. Weitz:** None.

Nanosymposium

671. Mechanisms of Cocaine Addiction

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Tufts Collaborates Grant

Title: Functional identification of a CRF DA microcircuit in mice with relevance to drug abuse

Authors: *K. L. GOBROGGE[1], X. HAN[1], A. HOOPER[2], M. DAWES[1], J. DEBOLD[1], J. MAGUIRE[3], K. MICZEK[1];
[1]Psychology, Tufts Univ., Medford, MA; [2]Sackler Sch. of Grad. Biomed. Sci., Tufts Univ., Boston, MA; [3]Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Corticotropin releasing factor (CRF) signaling in the posterior ventral tegmental area (pVTA) mediates stress-induced psychostimulant self-administration. Recently, using a Cre-dependent tract-tracing approach with AAV-Flex-ChR2 in adult CRF-Cre male mice, we localized the source of pVTA-CRF to neurons projecting from the lateral hypothalamus (LH) and dorsal raphe nucleus (DRN) synapsing in the paranigral (PN) and parainterfascicular (PIF) sub-nuclei of the ventral posterior medial (VPM) sub-region of the pVTA. We observed that the DRN-, but not LH-, VPM-CRF circuit was activated after repeated, but not acute, social defeat stress. Furthermore, we found that repeated optical or chemo-genetic activation of CRF in the VPM was sufficient to engender amphetamine (AMPH)-induced-locomotor cross-sensitization and escalated cocaine intake. Specifically, we observed that male mice with a history of repeated CRF optical or DREADD stimulation in the VPM displayed a significant increase in distance traveled in an open field test after treatment with a low dose of AMPH (1.5 mg./kg.) compared to saline treated and Cre-/- littermate controls. Subsequent cocaine self-administration experiments demonstrated that repeated DREADD CRF activation in the VPM enhanced drug-seeking behavior. Here we build upon these data by focusing on the DRN-VPM CRF microcircuit by using a combination of techniques. Previously, we observed site-specific increases in CRF-ir in the PN/PIF but not the parabrachial pigmented area of the pVTA following stress. Thus, we bilaterally infused CRF-Cre male mice with the G(q) DREADD virus into the DRN and implanted a unilateral microdialysis probe aimed at the PN/PIF and sampled the VPM for CRF during saline and CNO treatment. Our data demonstrate enhanced CRF release in the VPM of DREADD G(q) infected DRN-CRF neurons, after i.p. CNO injection. These results suggest functional increases in released CRF in the VPM after activation of DRN-CRF neurons projecting to the pVTA. We will extend this work by measuring dopamine (DA) release in the nucleus accumbens shell of mice with a history of DRN-VPM CRF microcircuit activity. These latter experiments aim to ascertain mesocorticolimbic CRF-DA interactions and potential increases in rate of cocaine self-administration and reinstatement after abstinence. At present, our data reveal a site-specific CRF microcircuit exclusively projecting from the DRN to the VPM sub-region of the pVTA, its sensitivity to social defeat stress, and VPM-CRF release presumably altering DAergic output in the forebrain after repeated experiences with brief episodes of social stress.

Disclosures: **K.L. Gobrogge:** None. **X. Han:** None. **A. Hooper:** None. **M. Dawes:** None. **J. DeBold:** None. **J. Maguire:** None. **K. Miczek:** None.

Nanosymposium

671. Mechanisms of Cocaine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

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Title: Differential regulation of mood and motivation by separate nucleus accumbens shell outputs in cocaine self-administering rats

Authors: ***A. L. LORIAUX**, D. W. SELF;
Dept. of Psychiatry, UT Southwestern Med. Ctr., Dallas, TX

Abstract: Cocaine users often cite negative mood as a key factor behind relapse, but whether the same circuitry is implicated in both of these mood and motivational changes is unknown. The nucleus accumbens shell (NAcSh) is known for its ability to convert both rewarding and aversive affective information into appropriate motivational output. We previously reported that NAcSh projections to the lateral hypothalamus (LH) can affect both mood and motivation for cocaine in self-administering rats, suggesting some overlap in circuitry for these behaviors. The NAcSh also projects to the ventral pallidum (VP) and the ventral tegmental area (VTA) as part of the canonical indirect and direct striatal outputs. Recent work suggests differential regulation of reward and mood by these pathways in both dorsal and ventral striatal regions. However, the role of specific NAcSh projections to the VP and VTA in mediating mood and motivational changes in cocaine addiction has not been investigated. In the present study, we used a target-specific optogenetic approach to selectively activate NAcSh projections to either the VP or VTA in male Sprague-Dawley rats. Rats were bilaterally injected with either AAV2-hSyn-hChR2(H134)-EYFP or control virus into the NAcSh, and implanted with optic fibers in the terminal regions in the VTA or VP. Rats were trained to self-administer intravenous cocaine (0.5 mg/kg/infusion, i.v.) 4 h/day for 3 weeks. We measured the effect of laser stimulation of each pathway (30 min pretreatment, 10 sec/min, 20 Hz, 10-12 mW at fiber tip) on motivation for cocaine as assessed by 1) performance on a progressive ratio (PR) schedule of cocaine reinforcement, 2) drug-paired lever presses under extinction and 3) cocaine-primed reinstatement. We measured behavioral despair and anhedonia with the forced swim and sucrose preference tests, respectively.

Optogenetic stimulation of NAcSh-VP terminals, while having no effect on mood measures, significantly decreased the motivation for cocaine as indicated by a 44% drop in breakpoint and 64% drop in the number of days to extinguish. Conversely, NAcSh-VTA stimulation induced anhedonia, as indicated by 33% lower sucrose preference scores, while producing a 33% increase in cocaine seeking during reinstatement. Together with our previous work, these findings suggest that separate but overlapping pathways in the ventral striatal shell mediate distinct changes in mood and motivation accompanying cocaine addiction. Furthermore, while NAcSh projections to both LH and VTA may mediate these changes, selective targeting of the NAcSh-VP pathway may provide therapeutic benefits in treating cocaine craving without affecting mood.

Disclosures: **A.L. Loriaux:** None. **D.W. Self:** None.

Nanosymposium

671. Mechanisms of Cocaine Addiction

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Title: A shift in balance from frontal (and global) to sensory (and local) brain networks is modulated by withdrawal state in cocaine addicted individuals

Authors: ***A. ZILVERSTAND**, M. A. PARVAZ, S. J. MOELLER, R. Z. GOLDSTEIN; Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Previous studies in individuals with cocaine use disorder (iCUD) described localized changes in resting-state functional connectivity in active and past users. Research in nicotine smokers indicates that acute withdrawal modulates the functional connectivity of the reward and executive control network. Here, we used complex network analysis (graph theory) to investigate a state of withdrawal in iCUD and show interactions with chronicity of use. We estimated

functional integration of brain networks in iCUD during withdrawal [iCUD+ (urine positive)], in iCUD with non-recent drug use [iCUD- (urine negative)] and in race- and gender-matched controls. Measures of global efficiency, indexing functional integration across whole-brain networks, and local efficiency, indicating functional integration within brain regions, were computed.
Ten minute resting-state fMRI scans were acquired in iCUD+ (N=26, age 47±8 yrs), iCUD- (N=17, age 47±8 yrs) and controls (N=32; age 40±8 yrs; covarying for age). Participants' drug use histories and withdrawal symptoms were assessed by a structured interview and the Cocaine Selective Severity Assessment. The imaging data was analyzed with CONN (MIT, Cambridge). Each subject's connectivity matrix was derived using a 638 region anatomical template, whereby efficiency measures were computed.
The drug use assessment revealed higher withdrawal symptoms in iCUD+ compared to iCUD-. The resting-state analysis demonstrated linearly decreased global functional integration of the executive control network and subcortical brain regions implicated in reward processing (including putamen) as a function of recency of use (iCUD+<iCUD-<Controls). The local functional integration in frontal brain regions involved in value representation and the visual stream was linearly increased (iCUD+>iCUD->Controls). Global disintegration of the executive control network and increased local connectivity within visual networks were correlated (r=-0.61), indicating a shift in balance from frontal (and global) to sensory (and local) brain networks. This effect was strongest during withdrawal (in iCUD+), and less pronounced in iCUD-, indicating some recovery with less recent cocaine use. Correlations with lifetime use within iCUD+ indicated compensatory responses.
Overall, results demonstrate that measures of functional integration could provide a novel whole-brain tool for monitoring disease status in the addicted brain. Results also implicate the executive control network and the shift in global-to-local/frontal-to-sensory balance as possible targets for brain-based interventions in individuals with cocaine addiction.

Disclosures: A. Zilverstand: None. **M.A. Parvaz:** None. **S.J. Moeller:** None. **R.Z. Goldstein:** None.

Nanosymposium

671. Mechanisms of Cocaine Addiction

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Title: Adolescent cocaine experience differentially augments psychomotor sensitization in adulthood and alters dopamine receptor and epigenetic profiles in the nucleus accumbens of selectively bred high- and low-responder rats

Authors: *A. PARSEGIAN[1], J. GARCIA-FUSTER[3], S. J. WATSON, Jr.[1], S. B. FLAGEL[2], H. AKIL[1];
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Abstract: Both genetic predisposition and environmental factors are thought to contribute to addiction liability. Early initiation of drug use in adolescence reliably predicts the likelihood of addiction in adulthood, but the molecular mechanism by which this occurs and whether genetic predisposition is involved is not known. Here we used a unique genetic model of two selectively bred rat lines that are known to differ in addiction liability to examine the impact of adolescent cocaine experience on psychomotor sensitization in adulthood and accompanying neurobiological alterations. Relative to bred low-responder (bLR) rats, bred high-responders (bHR) are typically more sensitive to the psychomotor-activating effects of cocaine and reinstate drug-seeking more readily following a prolonged period of abstinence. Consistent with previous findings, we found that only bHRs given a 7-day sensitizing regimen of cocaine (15 mg/kg) during adolescence (PND 33-39) showed psychomotor sensitization on day 7. However, adolescent cocaine history shifted the bLR phenotype to express sensitization in adulthood. By contrast, bHRs with adolescent cocaine history sustained the same levels of sensitization in adulthood as in adolescence. To further examine gene-by-environment interactions, we focused on certain epigenetic factors (i.e., chromatin modifications) that have been shown to influence genes, downstream signaling pathways, and proteins that have also been implicated in addiction liability. We found that two such histone modifications, acetylation (ac) and tri-methylation (me3) on histone 3 lysine 9 (H3K9) are altered in the ventral striatum following adolescent cocaine sensitization. We also developed a novel technique combining immunohistochemistry, *in situ* hybridization, and unbiased stereololgy to quantify these epigenetic modifications specifically in subregions of the nucleus accumbens (i.e., core and shell). In bHRs, we found that, relative to saline controls, adolescent cocaine exposure reduced the repressive mark H3K9me3 in the core and increased it in the shell, and reduced the permissive mark acH3K9 in the core. In bLRs, adolescent cocaine increased acH3K9 expression in the core, but not the shell, relative to their saline controls. We are currently determining whether these epigenetic changes

occur more in certain striatal cell subtypes than others (e.g., D1 vs. D2). These results indicate that adolescent drug use can uniquely influence inborn genetic addiction liability via chromatin modifications in NAc dopamine neurons and promote subsequent drug sensitivity in adulthood.

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671. Mechanisms of Cocaine Addiction

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Title: Translational control by eIF2α phosphorylation regulates vulnerability to the synaptic and behavioral effects of cocaine

Authors: *S. KHATIWADA[1], W. HUANG[1], A. N. PLACZEK[1], G. V. DI PRISCO[1], C. SIDRAUSKI[2], K. KRNJEVIć[3], P. WALTER[2], J. A. DANI[4], M. COSTA-MATTIOLI[1]; [1]Baylor Col. of Med., Houston, TX; [2]Univ. of California, San Francisco, San Francisco, CA; [3]McGill Univ., Montreal, QC, Canada; [4]Mahoney Inst. for Neurosciences, Perelman Sch. of Med., Philadelphia, PA

Abstract: Drug addiction is a major global mental health problem and adolescents are especially prone to addiction; however, the underlying neurobiological mechanism remains largely unknown. We discovered that translational control by phosphorylation of the translation initiation factor eIF2α (p-eIF2α) accounts for adolescent hypersensitivity to cocaine.
In adolescent mice but not in adults, a low dose of cocaine reduced p-eIF2α in the ventral tegmental area (VTA)—a key reward area in the brain, potentiated synaptic inputs onto dopaminergic neurons in the VTA, and elicited drug-reinforced behavior. Like adolescents, adult mice with reduced p-eIF2α in the VTA were more susceptible to cocaine-induced synaptic potentiation and behavior. Conversely, like adults, adolescent mice with increased p-eIF2α in the VTA became more resistant to cocaine's effects.

Consistent with these findings, metabotropic glutamate receptor-mediated long-term depression—whose disruption is postulated to increase vulnerability to drug addiction—was impaired in the VTA of both adolescent mice as well as in adult mice with reduced p-eIF2α levels.
Our data suggest that cocaine hijacks p-eIF2α-mediated translational program, thus initiating synaptic potentiation in VTA dopaminergic neurons that contributes to addiction-related behavior. These insights may hold promise for new treatments for addiction.

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671. Mechanisms of Cocaine Addiction

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Title: Incubation of cocaine craving and the regulation of eIF2 in the nucleus accumbens

Authors: *M. T. STEFANIK[1], C. T. WERNER[2], M. MILOVANOVIC[1], M. E. WOLF[1]; [1]Neurosci., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL; [2]Dept. of Pharmacol. and Toxicology, The State Univ. of New York at Buffalo, Buffalo, NY

Abstract: The ability to learn from experiences, store information, and produce long-lasting changes in behavior requires synaptic modifications dependent on synthesis of new proteins. When protein synthesis is interfered with, profound alterations in synapse and circuit function can result. Recently, we discovered a novel role for protein synthesis in maintaining adaptations in the nucleus accumbens (NAc) that underlie incubation of cocaine craving after prolonged withdrawal.
The present study is part of an effort to understand the regulation of protein translation in the NAc under normal conditions and after incubation of cocaine craving. Here, we examined translation-related proteins from synaptoneurosomes obtained from NAc tissue of saline and cocaine self-administering rats that were killed either after 1 or >40 days of withdrawal (WD1, WD40), or on WD1 and WD40 *immediately following* a 30-min cue-induced seeking test (WD1-

test, WD40-test).
We discovered that the phosphorylation of a key regulator of protein synthesis, eukaryotic initiation factor 2α (eIF2α), was significantly decreased only in WD40-test cocaine rats. eIF2α did not change on WD1 in any group, on WD40 in saline or cocaine rats that did not undergo a test, or in WD40-test saline rats. Phosphorylation of eIF2α generally blocks translation, and thus the observed decrease potentially signals a transiently regulated environment in the NAc of cocaine animals during a seeking test that is permissive to protein synthesis and perhaps promotes drug-seeking behavior. Supporting this, pharmacologically preventing the decrease in phosphorylated eIF2α with microinjections of the small molecule phosphatase inhibitor SAL003 (20 μM/0.5 μL/hemisphere), directly into the NAc either 1 h or 15 min before a seeking test, significantly reduced cocaine seeking. We confirmed that SAL003 is acting to reduce protein synthesis by using fluorescent non-canonical amino acid tagging (FUNCAT) to visualize newly synthesized proteins in cultured NAc neurons. SAL003 significantly decreased translation in these cells.
Together, these results suggest that dephosphorylation of eIF2α and a resultant change in protein translation is necessary for the expression of "incubated" cue-induced cocaine seeking following prolonged withdrawal.

Disclosures: **M.T. Stefanik:** None. **C.T. Werner:** None. **M. Milovanovic:** None. **M.E. Wolf:** None.

Nanosymposium

671. Mechanisms of Cocaine Addiction

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T-32 DA007290

Title: Reversal of cocaine-induced dendritic spines through altered BDNF-TrkB signaling is dissociated from addiction-like changes in self-administration behavior.

Authors: ***E. M. ANDERSON**[1], A. WISSMAN[1], D. GUZMAN[1], C. COWAN[2], D. SELF[1]; [1]UT Southwestern, Dallas, TX; [2]McLean Hosp., Belmont, MA

Abstract: Chronic cocaine induces dendritic spine growth in accumbens shell (NACsh) neurons, but this has not been shown to directly enhance addictive behavior. Spine formation is

functionally linked to BDNF-TrkB signaling in other brain regions, but whether BDNF-TrkB signaling can alter cocaine-induced spines is unknown. We tested whether pathway-specific TrkB signaling can modulate spine formation induced by cocaine self-administration (CSA) and compared effects with modulation of CSA behavior.
Four Herpes Simplex Virus (HSV) vectors, all bicistronic for GFP, were constructed for this study. 1) HSV-TrkB-WT overexpresses the wild-type TrkB receptor, 2) HSV-TrkB-KD is a kinase-dead dominant negative mutant, 3) HSV-TrkB-515/SHC is a mutant that selectively blocks Src homology 2 domain-containing protein (SHC) docking complexes, but preserves TrkB signaling via phospholipase Cγ-1 (PLC), 4) HSV-TrkB-816/PLC selectively blocks signaling through PLC, while preserving TrkB signaling via SHC. HSV-GFP serves as a negative control. Rats with bilateral NACsh cannulae trained for fixed ratio CSA 3h/day for 3-4 weeks, then CSA dose-response was assessed before, during, and after transient HSV-mediated expression of TrkB mutants. A second HSV infusion was performed at least 2 weeks later to assess motivation for cocaine on a progressive ratio (PR) reinforcement schedule. Separate cohorts engaged in cocaine or saline SA for 3 weeks, and dendritic spine density was assessed 3d after HSV infusions and 1d after CSA. Spine densities were quantified in GFP-labeled neurons by confocal microscopy and Volocity 3D analysis.
The 816/PLC and KD TrkB vectors caused a transient leftward shift in the dose threshold necessary to maintain CSA compared with GFP controls, and increased breakpoints on the PR task. These results indicate increased sensitivity and motivation for cocaine reinforcement with loss of endogenous TrkB-PLC signaling. In contrast, WT and 515/SHC vectors did not alter CSA behaviors. CSA increased distal dendritic spine density in GFP-expressing NACsh neurons compared to saline. However, WT, 515/SHC and 816/PLC TrkB vectors all reversed cocaine-induced spine changes without affecting baseline levels, whereas the dominant negative KD TrkB vector failed to alter cocaine-induced spines. These findings indicate that gain of TrkB function (either SHC or PLC signaling) reverses cocaine-induce increases in dendritic spine density.
Thus, BDNF-TrkB activity after CSA triggers neuroplasticity that reverses spine formation, while loss of TrkB-PLC signaling enhances cocaine reinforcement, and these morphological and behavioral effects are entirely dissociable.

Disclosures: **E.M. Anderson:** None. **A. Wissman:** None. **D. Guzman:** None. **C. Cowan:** None. **D. Self:** None.

Nanosymposium

671. Mechanisms of Cocaine Addiction

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Title: β-arrestin2 in D2 receptor-containing neurons modulates the behavioral effects of drugs of abuse

Authors: *K. A. PORTER-STRANSKY[1], C. JEROME[1], N. M. URS[2], M. G. CARON[2], D. WEINSHENKER[1];
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Abstract: Psychostimulants and opioids increase dopamine neurotransmission, activating dopamine receptors. The protein β-arrestin2 (βArr2) is important in desensitizing and internalizing G protein-coupled receptors (GPCRs), including dopamine receptors, and can also initiate signaling cascades following GPCR activation. Previous work has shown that mice lacking βArr2 have altered responses to drugs of abuse, but the specific neurons mediating these effects are unknown. By crossing D1-Cre and D2-Cre transgenic mice with conditional knockout "floxed" βArr2 mice, we generated mice that lack βArr2 only in neurons containing D1 ($D1^{\beta Arr2}$) or D2 dopamine receptors ($D2^{\beta Arr2}$), and then examined drug-induced locomotion following multiple doses of D1- and D2-like agonists, cocaine, and morphine. A conditioned place preference paradigm was also used to test whether elimination of βArr2 in D1 or D2 neurons affects cocaine or morphine reward. While $D1^{\beta Arr2}$ mice had normal drug responses, $D2^{\beta Arr2}$ mice showed dose-dependent reductions in locomotor responses to cocaine, morphine, and the D2 agonist quinpirole, as well as a blunted place preference for cocaine. Together, these results show that βArr2 is necessary in D2 neurons for the rewarding and locomotor-activating effects of drugs of abuse.

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NARSAD Young Investigator Award

Title: Acute stress induces constitutive activation of kappa opioid receptors

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Abstract: Emerging evidence shows that dopaminergic neurons in the ventral tegmental area (VTA) are an important locus for the convergent effects of stress and drugs of abuse. We previously identified a long-term potentiation of GABAergic synapses on these neurons (LTP_{GABA}) that is blocked by acute stress through activation of kappa opioid receptors (KORs, Graziane et al, Neuron, 2013). Intra-VTA injection of a KOR antagonist also prevents reinstatement of cocaine seeking by acute stress, suggesting that KOR-mediated regulation of VTA inhibitory plasticity may play a role in stress-induced drug seeking. Our recent work shows that a single five minute cold water swim stress blocks LTP_{GABA} for at least five days. Surprisingly, blocking KORs with norBNI even well after stress restores LTP_{GABA} and prevents cocaine self-administration (Polter et al, Biological Psychiatry, 2014). In this study we examine the mechanism by which KORs are persistently activated by acute stress and the role of this activation in stress-induced reinstatement of drug seeking.
Here we show that the long-lasting block of LTP_{GABA} by stress is due to persistent changes in the KOR. While bath application of an inverse agonist (norBNI, 100 nM) rescues LTP_{GABA} in slices from stressed animals, a neutral antagonist (6-β-naltrexol, 10 μM) does not (LTP magnitude: norBNI after stress=144±18% of baseline, n=10; 6-β-naltrexol after stress=99±8% of baseline, n=10; $p<0.05$). These results suggest that LTP_{GABA} is blocked by constitutive activity of KORs rather than by persistently elevated dynorphin. In support of this, the ability of norBNI to reinstate LTP_{GABA} in the slice was blocked by pre-treatment of slices with the JNK inhibitor SP600125 (LTP magnitude in stressed animals: vehicle+norBNI=139±7% of baseline, n=6; SP600125+norBNI=105±9% of baseline n=11; $p<0.05$), suggesting that the effect of norBNI is non-competitive.
Simply activating KORs was sufficient to induce a lasting blockade of LTP_{GABA}, as U50488 (5 mg/kg i.p.), blocked LTP_{GABA} for 5 days (LTP: saline=140±10% of baseline, n=11; 1 day post U50488=108±5% of baseline, n=9; 5 days post U50488=99±9% of baseline, n=10; $p<0.05$). Our results show that a single exposure to acute stress or to a KOR agonist both cause long-lasting changes in plasticity of GABAergic synapses in the VTA through constitutive activity of KORs. These studies demonstrate a novel mechanism of KOR regulation and highlight a potential target for treatment of stress-induced drug seeking behavior.

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Nanosymposium

671. Mechanisms of Cocaine Addiction

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Title: Cocaine induced down-regulation of mir-125b modulates the expression of poly (adp-ribose) polymerase-1 (parp-1)

Authors: *S. DASH[1,5], M. BALASUBRAMANIAM[2], B. JONES[3], T. RANA[2], S. GOODWIN[2], F. VILLALTA[4], C. DASH[2], J. PANDHARE[4];
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Abstract: Acute as well as repeated exposure to cocaine activates persistent cellular and molecular changes in the brain reward regions that are mediated via alterations in neuronal gene expression profiles. Understanding the mechanisms responsible for cocaine induced gene expressions are critical for better characterization of reward mechanisms. MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression at the post-transcriptional level and are important in physiological and pathological processes. It is becoming clear that cellular miRNAs play important roles in cocaine addiction and reward. In this study, we investigated whether the cellular miRNA “miR-125b” is involved in cocaine-induced effects using differentiated neuronal cells. miR-125b is highly expressed in the brain and earlier work in our laboratory has demonstrated that cocaine modulates miR-125b expression in CD4+ T cells. Exposure of differentiated SH-SY5Y cells to varying concentrations of cocaine resulted in the downregulation of miR-125b expression. To better understand the biological significance of this phenotype, we identified Poly (ADP-ribose) polymerase-1 (PARP-1) as a potential target of miR-125b. Recently, it has been reported that cocaine treatment upregulates PARP-1 expression in mice brain. Accordingly, PARP-1 catalyzed poly-ADP ribosylation of histones has been shown to play essential role for cocaine-induced molecular, neural, and behavioral plasticity. However, the mechanism by which cocaine regulates PARP-1 remains largely unclear. Therefore, we tested whether miR-125b regulated PARP-1 expression in differentiated neuronal cells. Knockdown of miR-125b expression resulted in enhanced PARP-1 protein levels, while overexpression of miR-125b showed reduced levels of PARP-1 protein, suggesting a direct role of miR-125b in regulating PARP-1 expression. We further probed the direct binding of miR-125b to the PARP-1 mRNA 3'-untranslated region (3’ UTR) by employing a luciferase reporter assay system that demonstrated regulation of PARP-1 3’UTR activity by miR-125b. Finally,

using a dopamine transporter (DAT) blocker, we determined that cocaine-induced downregulation of miR-125b and upregulation of PARP-1 are dependent on cocaine binding to the DAT. Collectively, these results highlight an important role of miR-125b as a post-transcriptional regulator of PARP-1 expression in neuronal cells and suggest a novel mechanism for cocaine's rewarding effects.

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Nanosymposium

672. Perception and Imagery: Scene Perception and Spatial Navigation

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NSF GRFP

Title: Perceptual inputs to the cortical network for boundary-based navigation

Authors: ***J. B. JULIAN**, J. RYAN, R. H. HAMILTON, R. A. EPSTEIN;
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Abstract: Thirty years of research suggests that environmental boundaries—e.g. the walls of an experimental chamber—exert powerful influence on navigational behavior, often to the exclusion of other navigationally-relevant cues. Moreover, neurons that instantiate spatial memory in the hippocampus and surrounding structures exhibit firing fields that are strongly controlled by boundaries. Despite the clear importance of boundaries for spatial coding, however, little is known about how the brain mediates the perception of boundary information during navigation. We tested the idea that the Occipital Place Area (OPA), a scene-selective region located near the transverse occipital sulcus, might extract boundary information from visual scenes and thus provide critical perceptual input to the broader boundary-based navigation network. Repetitive theta-burst TMS was used to interrupt processing in the OPA during a navigation task that required participants to learn object locations relative to boundaries and non-boundary cues within a virtual arena. We found that TMS of the OPA impaired learning of locations relative to boundaries, but not relative to landmark objects. Moreover, this effect was only found when the boundary was defined by a wall, not when it was defined by a marking on

the ground. These results suggest that OPA may serve as the perceptual source of the boundary information that controls navigational behavior. To explore how OPA is integrated into the larger boundary-based navigation network, we examined resting-state fMRI functional connectivity between the OPA and brain regions known to be involved in spatial navigation. Preliminary results suggest two possible pathways connecting the OPA to the hippocampus, one through the posterior parietal cortex and another through parahippocampal cortex. Taken together, these findings elucidate how the OPA provides key perceptual inputs to the broader neural circuit for boundary-based navigation.

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Nanosymposium

672. Perception and Imagery: Scene Perception and Spatial Navigation

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Netherlands Organisation for Scientific Research NWO-Vidi 452-12-009

Title: Trigonometry predicts entorhinal processing of reference points

Authors: ***T. NAVARRO SCHROEDER**, C. F. DOELLER;
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Abstract: Entorhinal grid cells fire in spatial pattern of equilateral triangles, resembling triangulation networks used in cartography. The initial orientation of such networks is determined by the spatial layout of reference points in the environment, Here, we test the hypothesis that neural processing in entorhinal cortex relates to Euclidean triangulation. First, we employed computational modelling of navigation in different environments and demonstrate that paths perpendicular to environmental reference axes are optimal for triangulation. Next, in two fMRI studies with human participants freely navigating virtual environments, we show that preferred orientations of 6-fold rotational symmetry of entorhinal activity, consistent with grid-cell-like representations, followed optimal triangulation angles. Finally, we found that participants' distance estimates were more precise on paths along preferred orientations of entorhinal hexadirectional activity. Our results predict a central role of triangulation in the entorhinal grid-system, which provides a mechanistic explanation for its involvement in spatial cognition and has implications for autonomous wayfinding technology.

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Nanosymposium

672. Perception and Imagery: Scene Perception and Spatial Navigation

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Wallenberg Network Initiative on Culture, Brain, and Learning

University of Pennsylvania and the John Templeton Foundation Project on Prospection Psychology

Title: Mechanisms of prospective navigation in the human brain

Authors: ***T. I. BROWN**[1], K. F. LAROCQUE[1], V. A. CARR[1,3], S. E. FAVILA[4], A. M. GORDON[1], B. BOWLES[5], J. N. BAILENSON[2], A. D. WAGNER[1];
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Abstract: The mental representation of one's environment, including landmarks, scenes, and goal locations, is critical for goal-directed navigation. The hippocampus and neighboring medial temporal lobe (MTL) cortices are believed to play a critical role in spatial navigation through representation of spatial information (Johnson and Redish, 2007; Marchette et al., 2015; McKenzie et al., 2013), and by supporting goal-directed route planning (Brown et al., 2014; Hartley et al., 2003). Using whole-brain high-resolution functional magnetic resonance imaging (hr-fMRI), we examined whether the human MTL supports goal-directed navigation by representing future spatial goal states during planning. On day 1, seventeen healthy, right-handed young adults learned to navigate to hidden goal locations in a virtual circular track environment. Each location was uniquely associated with a distinct pair of fractal images. On day 2, participants repeatedly navigated to the goals during hr-fMRI scanning. Participants began each trial at a familiar location, after which the environment was hidden from view and participants were cued by of one of the fractals to plan navigation to its location. Participants subsequently navigated to this goal. Using multivoxel pattern analyses (MVPA), results demonstrate that patterns of activity in the hippocampus, along with a functionally linked neocortical network including MTL, orbitofrontal, and retrosplenial cortex, contain information during initial

planning that codes the spatial goal to which participants will subsequently navigate. Importantly, when navigating to a distal location, classifier evidence in the hippocampus was also greater for intervening locations along the route than for other non-goal locations. Our results also provide evidence for an association between prospective hippocampal representations and putative planning processes in frontopolar cortex. Collectively, these results suggest that the human hippocampus and related cortical structures support prospective, multi-featural representations of future goal states, and mental route simulation that could facilitate flexible planning of navigation behavior.

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Nanosymposium

672. Perception and Imagery: Scene Perception and Spatial Navigation

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Title: Grid-cell representations in mental simulation

Authors: *J. BELLMUND[1], L. DEUKER[1,2], T. NAVARRO SCHROEDER[1], C. F. DOELLER[1];
[1]Donders Institute, Radboud Univ., Nijmegen, Netherlands; [2]Ruhr Univ., Bochum, Germany

Abstract: Anticipating the future is a key motif of the brain, possibly supported by mental simulation of upcoming events. Rodent single-cell recordings suggest the ability of spatially tuned cells to represent subsequent locations. Grid-like representations have been observed in the human entorhinal cortex during virtual and imagined navigation. However, hitherto it remains unknown if grid-like representations contribute to mental simulation outside the domain of spatial navigation. Participants imagined directions between building locations in a large-scale virtual-reality city while undergoing fMRI without re-exposure to the environment. Using multi-voxel pattern analysis, we provide evidence for representations of absolute imagined direction at a resolution of 30° in the parahippocampal gyrus, consistent with the head-direction system. Furthermore, we capitalize on the six-fold rotational symmetry of grid-cell firing to demonstrate

a 60°-periodic pattern-similarity structure in the entorhinal cortex. Our findings imply a role of the entorhinal grid-system in mental simulation and future thinking beyond spatial navigation.

Disclosures: J. Bellmund: None. **L. Deuker:** None. **T. Navarro Schroeder:** None. **C.F. Doeller:** None.

Nanosymposium

672. Perception and Imagery: Scene Perception and Spatial Navigation

Location: SDCC 7B

Time: Wednesday, November 16, 2016, 8:00 AM - 10:30 AM

Presentation Number: 672.05

Topic: H.02. Human Cognition and Behavior

Support: Human frontiers science program

Starting Grant of the European Research Council (AGESPACE 335090)

Title: Representations of positional uncertainty in the human hippocampus and entorhinal cortex

Authors: *X. CHEN, P. VIEWEG, T. WOLBERS;
Aging and Cognition Res. Group, German Ctr. For Neurodegenerative Dis., Magdeburg, Germany

Abstract: Spatial information is usually contaminated by noises, leading to uncertainties in spatial knowledge of one's location. It is unknown how this uncertainty is implemented in neural circuits known to code positional information, e.g., the entorhinal-hippocampal system. The current experiment examined how reliability of a spatial cue affected uncertainty of position representation in the human entorhinal-hippocampal system. Twenty-two participants learned and memorized a target location along a linear track in a desktop virtual reality environment, while undergoing functional MRI at 7T. Participants were passively transported to one of 4 fixed test locations sampled around the previously learned target location and judged whether the target location was ahead of or behind them. The use of visual landmark cues and the use of self-motion cues were dissociated. Reliability was manipulated for each cue separately. Participants performed better with landmark cues compared to self-motion cues. For both cues, participants performed better when the cue was high in reliability compared to when it was low in reliability. For fMRI analysis, we applied multi-voxel pattern analysis to hippocampal subfields and the entorhinal cortex (ERC), which were manually segmented on high resolution T2 weighted images. Neural representational dissimilarity between the 4 test locations was quantified in terms of Euclidean distance and correlation distance. Location discrimination ability was defined as the correlation between inter-location distances and neural representation dissimilarity. The results

showed that CA3 encoded reliability information for landmark cues in terms of correlation distance. ERC encoded reliability information for landmark cues in terms of Euclidean distance and for self-motion cues in terms of both distance measurements. Implications of the results on possible neural coding strategies of positional uncertainty will be discussed.

Disclosures: **X. Chen:** None. **P. Vieweg:** None. **T. Wolbers:** None.

Nanosymposium

672. Perception and Imagery: Scene Perception and Spatial Navigation

Location: SDCC 7B

Time: Wednesday, November 16, 2016, 8:00 AM - 10:30 AM

Presentation Number: 672.06

Topic: H.02. Human Cognition and Behavior

Support: ONR MURI N00014-10-1-0936

Title: Unravelling retrosplenial cortex: Converging evidence for functional parcellation from meta-analyses and the Human Connectome Project

Authors: *E. R. CHRASTIL, S. M. TOBYNE, R. K. NAUER, A. E. CHANG, C. E. STERN; Dept. of Psychological & Brain Sci., Boston Univ., Boston, MA

Abstract: Interest in the retrosplenial cortex (RSC) has undergone a renaissance in recent years. The role of RSC in spatial navigation, scene perception, visual imagery, and episodic memory has sparked intense interest and debate regarding its contributions to cognition and behavior. The lack of consensus in anatomical boundaries for the RSC complicates research into its function. Previously reported anatomical and functional definitions of RSC encompass a large and diverse area, suggesting that the area could be functionally subdivided. Furthermore, gradients of functional specialization, similar to the spatial scaling observed in the hippocampus, may be present in RSC, particularly along the anterior-posterior or medial-lateral axes. We tested this hypothesis using a data-driven approach combining fMRI meta-analysis and resting state functional connectivity (rsFC) methods. First, we used NeuroSynth to obtain 101 previous studies that consistently reported RSC activation and linked those studies to terms we derived from textual analysis of the publications' titles and keywords: *navigation*, *scenes*, *imagery*, *memory*, *learning*, *vision*, and *emotion*. Next, we conducted meta-analyses using those studies to determine RSC sub-regions that were associated with one or more domains. Results showed that left hemisphere medial RSC was closely tied to episodic memory. In contrast, the parietal-occipital sulcus, often reported as part of the retrosplenial *complex*, was associated with both scene processing and navigation, although navigation was spread more diffusely. We further refined our results by conducting meta-analytic contrasts in NeuroSynth. This analysis revealed

that left medial RSC activation was significantly more associated with episodic memory and imagery than with either navigation or scenes. There were no differences within the entire RSC region between scenes and navigation, suggesting the RSC supports these functions similarly. Finally, we used the subregions derived from the meta-analysis to conduct whole brain rsFC analyses using 100 unrelated subjects from the Human Connectome Project. This analysis resulted in distinct connectivity patterns for the RSC subregions such that more anterior/medial regions connected to default mode network areas and more posterior/lateral regions connected to visual areas. This convergent evidence supports the conclusion that the RSC can be subdivided along anatomical and functional boundaries: the left medial anatomical RSC relates to episodic memory and imagery, and the parietal-occipital sulcus corresponding to the functional retrosplenial complex relates to scene perception and navigation.

Disclosures: E.R. Chrastil: None. **S.M. Tobyne:** None. **R.K. Nauer:** None. **A.E. Chang:** None. **C.E. Stern:** None.

Nanosymposium

672. Perception and Imagery: Scene Perception and Spatial Navigation

Location: SDCC 7B

Time: Wednesday, November 16, 2016, 8:00 AM - 10:30 AM

Presentation Number: 672.07

Topic: H.02. Human Cognition and Behavior

Support: ARUK grant

Title: Spatial navigation ability assessed in over 1 million people globally

Authors: *H. J. SPIERS[1], E. MANLEY[1], R. SILVA[1], R. CONROY DALTON[2], J. M. WIENER[3], C. HÖLSCHER[4], V. BOHBOT[5], M. HORNBERGER[6];
[1]Univ. Col. London, London, United Kingdom; [2]Northumbria Univ., Newcastle, United Kingdom; [3]Univ. of Bournemouth, Bournemouth, United Kingdom; [4]ETH Zurich, Zurich, Switzerland; [5]McGill Univ., Montreal, QC, Canada; [6]Univ. of East Anglia, Norwich, United Kingdom

Abstract: Spatial disorientation is one of the most common symptoms in Alzheimer's disease. However, detection of such symptoms is difficult as there are currently no global benchmarks of what constitutes healthy navigation behaviour on a mass population level. To address this we worked with a global telecommunications company (Deutsche Telecom) and a game development company (Glitchers) to develop the mobile video game "Sea Hero Quest", that tests spatial orientation on a mass population. Three different spatial tasks are examined in the game: way-finding, path integration and spatial working memory. The players navigated a boat through

waters in 5 different themed areas over 75 levels. Our way-finding task involved finding checkpoints that required players to learn where they were from a map or by discovering them in situ. In the path integration task the players were asked to shoot a flare back to starting location after traversing a curving route. The spatial working memory task was a virtual radial maze with 6 arms. We present preliminary results for how navigation ability changes over the adult life span from 18-99 for men and women, as well as how navigation ability differs across the different countries of the world.

Disclosures: **H.J. Spiers:** None. **E. Manley:** None. **R. Silva:** None. **R. Conroy Dalton:** None. **J.M. Wiener:** None. **C. Hölscher:** None. **V. Bohbot:** None. **M. Hornberger:** None.

Nanosymposium

672. Perception and Imagery: Scene Perception and Spatial Navigation

Location: SDCC 7B

Time: Wednesday, November 16, 2016, 8:00 AM - 10:30 AM

Presentation Number: 672.08

Topic: H.02. Human Cognition and Behavior

Support: Rubicon Grant from the Netherlands Organisation for Scientific Research

Title: Comparing computational, object and functional models of scene representation in the human brain

Authors: ***I. I. GROEN**[1], M. R. GREENE[2,3], C. A. BALDASSANO[4], D. M. BECK[5], L. FEI-FEI[3], C. I. BAKER[1];
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Abstract: The goal of the human visual system is to extract relevant information from complex visual environments to perform a multitude of tasks. Recent neuroimaging research has shown that complex scene perception is characterized by the activation of multiple regions in posterior cortex. So far, these regions have been mostly interpreted as representing visual characteristics of scenes, e.g. the type of environment they depict (“a kitchen”), the types of objects they contain (“an oven”), or the geometry of the environment (“a closed space”). Recent behavioral evidence, however, has suggested that the functions afforded by a scene (e.g. “could I prepare food here?”) play a central role in how scenes are categorized (Greene et al., 2016, JEP:General). Here, we studied to what extent the brain represents scene functions using a model-based approach. Healthy volunteers (n=20) viewed 120 individual images from 30 scene categories in the SUN database (Xiao et al., 2014, Int J Comp Vis) while their brain activity was recorded using an

ultra-high field 7T MRI scanner. Stimuli were carefully selected from a large set of scenes characterized in terms of their visual properties (derived computationally using a deep neural network), object occurrence, and scene function (derived using online behavioral crowd-sourcing experiments), such that these three types of information each predicted maximally distinct patterns of scene similarity. Using representational similarity analysis (RSA) in scene-selective regions (PPA, OPA and RSC) we found that fMRI similarity was best predicted by the visual model, with additional contribution from the functional but not the object model. Second, a whole brain analysis confirmed a strong contribution of the visual model throughout high-level visual cortex. The greatest correspondence with the functional model was observed in parts of medial parietal cortex and anterior ventral temporal cortex, overlapping with a network that is thought to be involved in memory retrieval. Overall, these results show that both computationally derived visual scene descriptions and functional properties predict pattern similarity in scene-selective regions, and that understanding real-world scenes may engage larger-scale networks beyond those revealed by simple visual activation experiments.

Disclosures: **I.I. Groen:** None. **M.R. Greene:** None. **C.A. Baldassano:** None. **D.M. Beck:** None. **L. Fei-Fei:** None. **C.I. Baker:** None.

Nanosymposium

672. Perception and Imagery: Scene Perception and Spatial Navigation

Location: SDCC 7B

Time: Wednesday, November 16, 2016, 8:00 AM - 10:30 AM

Presentation Number: 672.09

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant EY022350-03

Title: Neural coding of navigational affordances in visual scenes

Authors: ***M. F. BONNER**, R. A. EPSTEIN;
Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: An essential component of visually guided navigation is the ability to perceive features of the environment that afford or constrain movement. For example, in indoor environments, walls limit one's potential routes, while passageways facilitate movement. There is some evidence that scene-selective regions of the human visual system encode coarse aspects of the shape of local space, such as whether the expanse of a scene is wide or narrow; however, the hypothesis that these regions encode a more detailed representation of navigational affordances has not been tested. To address this issue, we performed two fMRI experiments. In the first study, subjects viewed images of artificially rendered rooms that had identical geometry

as defined by their walls, but different navigational affordances as defined by the number and position of visible exits. Multivoxel pattern analysis (MVPA) showed that the occipital place area (OPA), a scene-selective region near the transverse occipital sulcus, coded the layout of the exits even though the shape of the room was the same for all scenes; moreover, these layout representations were tolerant to differences in visual appearance. These findings reveal a region of the human visual system that is highly sensitive to the navigational structure of the local environment. However, given our tightly controlled, artificial stimuli, an important question was whether these conclusions would generalize to more complex, naturalistic scenes. We addressed this in the second study by using images of real-world indoor environments. To identify navigational affordances, we asked raters to indicate the paths they would take to walk through each scene. A separate set of subjects was then scanned while they viewed these scenes and performed an orthogonal category-judgement task that made no explicit reference to scene affordance or geometry. Once again, MVPA revealed that the OPA encoded the navigational layout of the scenes, thus demonstrating that this effect generalizes to naturalistic scenes with heterogeneous visual and semantic properties. These findings provide the first evidence for a region in the human visual system that encodes the navigational-affordance structure of spatial scenes.

Disclosures: **M.F. Bonner:** None. **R.A. Epstein:** None.

Nanosymposium

672. Perception and Imagery: Scene Perception and Spatial Navigation

Location: SDCC 7B

Time: Wednesday, November 16, 2016, 8:00 AM - 10:30 AM

Presentation Number: 672.10

Topic: H.02. Human Cognition and Behavior

Support: ERC 310809

McDonnell 220020284

Title: Visual and non-visual interactive virtual navigation, and the effect of brief vs. lifelong visual deprivation on the plasticity of this network in the human brain using fMRI and Sensory Substitution

Authors: *S. MAIDENBAUM[1,2], D. R. CHEBAT[3], A. AMEDI[4];
[1]Amedi Lab, ELSC & IMRIC, Hebrew Univ. of Jeru, Jerusalem, Israel; [2]Amedi Lab, ELSC & IMRIC, Hebrew Univ. of Jerusalem, Jerusalem, Israel, Jerusalem, Israel; [3]Ariel Univ., Ariel, Israel; [4]Amedi Lab, ELSC & IMRIC, Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: While human navigation is not considered to rely on vision or any other single sense, vision is still considered to be the dominant sense humans use for navigation and many of the navigation network's nodes lay in regions considered visual (such as PPA, OPA etc.). What happens when humans are deprived of vision, such as when blindfolded or after lifelong visual deprivation? Which nodes will be recruited? Will this happen selectively for navigation? Do these regions just process visual input for navigation or do they have a sensory modality independent navigation processing ability?
To explore these questions 3 groups of subjects interactively navigated virtual Hebb-Williams mazes during fMRI neuroimaging. Group 1 navigated visually, while groups 2 and 3 underwent a longitudinal auditory navigation training regimen and were scanned both before and after the training. The second group's members were congenitally blind, while the thirds were briefly blindfolded. To enable non-visual navigation subjects used the EyeCane Sensory Substitution Device developed in our lab. Between neuroimaging sessions the non-visually navigating subjects successfully completed these mazes both in the real-world and virtually.
We found that before training the blindfolded and congenitally blind subjects did not recruit the navigation nodes with the exception of weak dorsal visual stream recruitment (focused at the posterior precunues). After training these subjects recruited most of the network including early-visual retinotopic areas. These results, show that the human virtual navigation network is robust to both lifelong visual deprivation and to very brief temporary blindfolding and can be recruited for navigating via a novel sensory modality within hours, demonstrating the strength of cross-modal and task-specific plasticity, even in the earliest retinotopic areas.

Disclosures: **S. Maidenbaum:** None. **D.R. Chebat:** None. **A. Amedi:** None.

Nanosymposium

673. Molecular Techniques

Location: SDCC 4

Time: Wednesday, November 16, 2016, 8:00 AM - 10:45 AM

Presentation Number: 673.01

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Rational systematic peptide display on the AAV capsid allows for the development of cell type specific targeting and mapping of protein function *In vivo*

Authors: *T. BJORKLUND, M. DAVIDSSON, G. WANG, P. ALDRIN-KIRK, M. HARTNOR;
Mol. Neuromodulation, Wallenberg Neurosci., Lund, Sweden

Abstract: The wild-type capsid structure of the Adeno-associated viral (AAV) vector serotype 2 has been thoroughly characterized with regards to function and crystal structure. It has also been

found to be surprisingly malleable where specific domains of the capsid can be replaced with dramatic changes in cell type tropism and infectivity efficiency as a consequence. However, the predictability has been very low and most attempts have been based on serial *in vivo* screening using randomly modified capsid structures. In this study we have utilized a novel approach to rational, systematic peptide display on the AAV capsid surface using gene-chip array synthesis and molecular barcoding to generate a high functionality, replication deficient library for single generation *in vivo screening*. This approach has enabled us to generate 25 novel AAV serotypes with unique potential to transduce synaptic terminals of neuronal subtypes in the adult rodent brain with unprecedented retrograde transport potential. These vectors have been used to map forebrain circuitry and to allow for cell type specific modulation using chemogenetic receptors.

Disclosures: T. Bjorklund: None. **M. Davidsson:** None. **G. Wang:** None. **P. Aldrin-Kirk:** None. **M. Hartnor:** None.

Nanosymposium

673. Molecular Techniques

Location: SDCC 4

Time: Wednesday, November 16, 2016, 8:00 AM - 10:45 AM

Presentation Number: 673.02

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Novel barcode-based *In vivo* screening method for generating de novo AAV serotypes for CNS-directed gene therapy

Authors: *M. DAVIDSSON, G. WANG, P. ALDRIN-KIRK, M. HARTNOR, T. BJÖRKLUND;
Lund Univ., Lund, Sweden

Abstract: Capsid modification is a useful strategy to create adeno-associated virus (AAV) vectors with subtype specific neuronal targeting and enhanced retrograde transport. Incorporating known cell-specific binding ligands is a rational method, but the creation of vectors without prior knowledge has the potential to reveal novel targets. Directed evolution and phage display are broadly utilized high-throughput methods, but are inefficient due to displaying random peptides wherein the vast majority will be non-functional.
Here, we have developed a novel AAV library in which each virus particle display a peptide derived from known neuron-related proteins on the surface of an AAV2 capsid. The packaged viral genome encodes a unique barcode sequence to facilitate capsid identification. 92398 unique oligos encoding 14-amino-acid peptides derived from 135 proteins were synthesized using microarray. Four-fragment Gibson assembly and novel emulsion PCR was then used to generate a plasmid library by inserting oligos into the capsid gene and barcodes between the inverted

terminal repeats. This plasmid library was then used to assemble a diverse library of AAV capsids, such that particles were composed of only peptide-modified capsid proteins which package an expression cassette containing RNA expressed barcodes providing post hoc identification of the capsid structure.
In parallel, the plasmid library was sequences using Illumina paired-end sequencing to link the RNA expressed barcodes to the de novo capsid structures.
The successfully generated AAV library efficiently infected neurons and astrocytes *in vitro* and displayed a subset of peptides that had efficient retrograde transport ability in neurons *in vivo* (e.g., transported from striatum to substantia nigra). Functional
peptides, which successfully promoted neuronal infectivity or retrograde transport, were identified by Illumina sequencing of RNA expressed barcodes both *in vitro* and *in vivo* and efficacy modeled through barcode counting.
In conclusion, we developed a high-throughput combinatorial method to generate peptide-modified AAV libraries that are valuable for evaluation of receptor expression of neuronal populations and have the potential to generate novel vectors with unique properties for *in vivo* gene transfer in the CNS.

Disclosures: **M. Davidsson:** None. **G. Wang:** None. **P. Aldrin-Kirk:** None. **M. Hartnor:** None. **T. Björklund:** None.

Nanosymposium

673. Molecular Techniques

Location: SDCC 4

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Presentation Number: 673.03

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Howard Hughes Medical Institute

NIH R01 NS32405

NIH R01 NS092707

NIH K08 AI100699

NIH R21 AI114448

NIH F32 NS087708

NIH F31 AG041582

Title: A generalizable method for targeting cells based on intracellular product expression

Authors: ***C. TANG**[1], E. DROKHLYANSKY[2], B. ETEMAD[3], S. RUDOLPH[1], B. GUO[1], S. WANG[2], E. G. ELLIS[1], J. Z. LI[3], C. L. CEPKO[2];
[1]Harvard Med. Sch., Boston, MA; [2]Harvard Med. School, HHMI, Boston, MA; [3]Brigham and Women's Hospital, Harvard Med. Sch., Boston, MA

Abstract: Many biological applications would benefit from the ability to detect and/or manipulate specific cell populations based upon the presence of intracellular protein epitopes. In neuroscience, the ability to target specific cell types for manipulation or monitoring of neural activity would facilitate dissection of circuit function in animals. However, reagents that facilitate cell type specific targeting, such as cell-specific enhancers or transgenic Cre lines, are not always available. Protein binders such as nanobodies (Nbs) can target untagged proteins (antigens) in the intracellular environment. However, genetically expressed protein binders are stable regardless of antigen expression, complicating their use in applications requiring cell-specificity. Here, we created a conditional system in which the stability of an Nb depends upon an antigen of interest. We developed a strategy that can be used to rapidly create destabilized Nbs. Fusion of destabilized Nbs to various proteins enabled applications in living cells, such as optogenetic control of neural activity in specific cell types in the mouse brain, and detection of HIV-infected human cells by flow cytometry. These approaches are generalizable to other protein binders, and enable the rapid generation of single-polypeptide sensors and effectors active in cells expressing specific intracellular proteins.

Disclosures: **C. Tang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Harvard Medical School. **E. Drokhlyansky:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Harvard Medical School. **B. Etemad:** None. **S. Rudolph:** None. **B. Guo:** None. **S. Wang:** None. **E.G. Ellis:** None. **J.Z. Li:** None. **C.L. Cepko:** None.

Nanosymposium

673. Molecular Techniques

Location: SDCC 4

Time: Wednesday, November 16, 2016, 8:00 AM - 10:45 AM

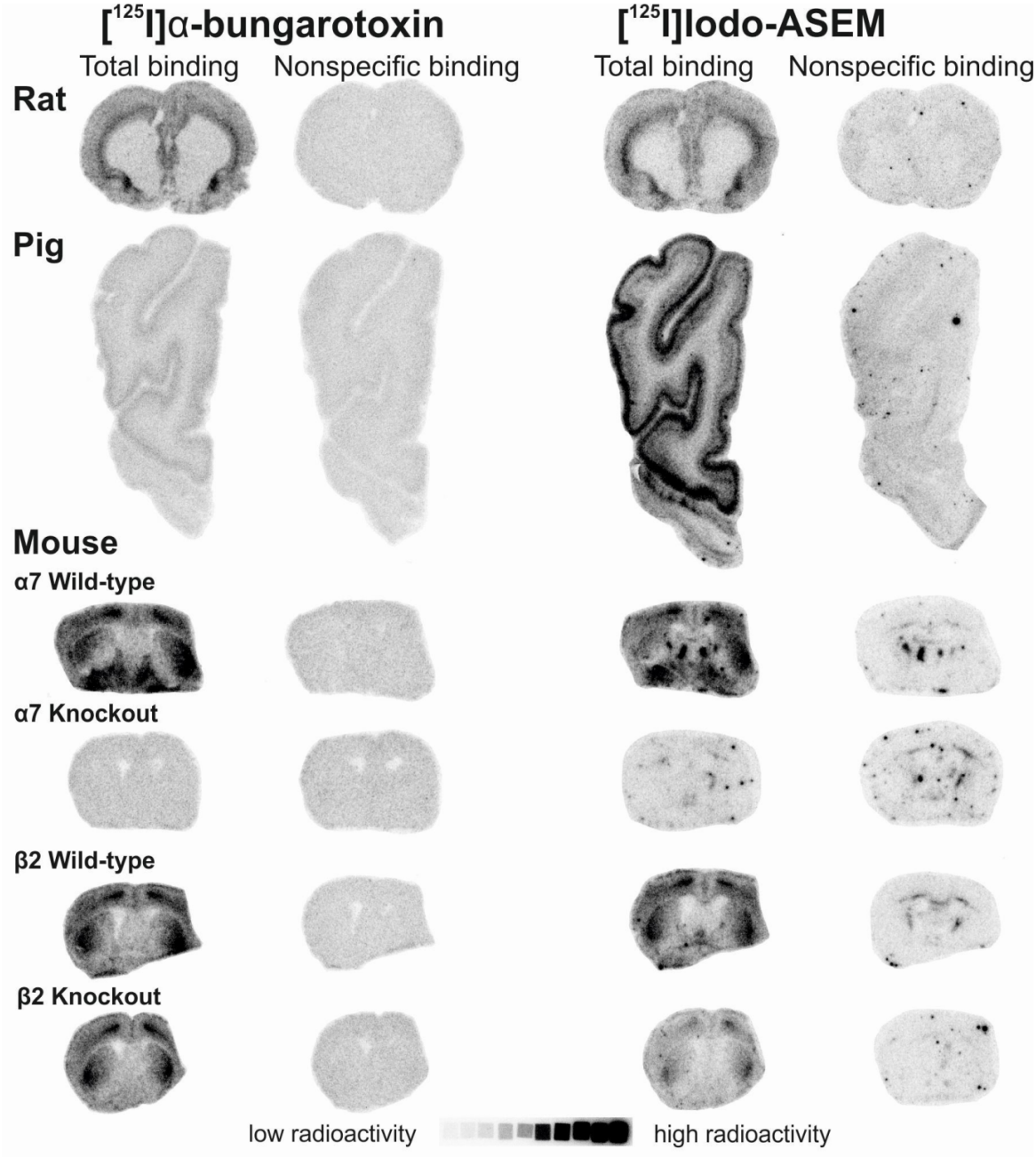
Presentation Number: 673.04

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: [^{125}I]Iodo-ASEM as a novel high-affinity radiotracer for detection of alpha7 nicotinic acetylcholine receptor binding sites

Authors: *C. K. DONAT[1,2], Y. GAO[3], R. C. MEASE[3], A. G. HORTI[3], H. H. HANSEN[2], M. G. POMPER[3], J. D. MIKKELSEN[2,4];
[1]Div. of Brain Sci., Imperial Col. London, London, United Kingdom; [2]Neurobio. Res. Unit, Copenhagen Univ. Hospital, Rigshospitalet, Copenhagen, Denmark; [3]Russell H. Morgan Dept. of Radiology and Radiological Sci., Johns Hopkins Sch. of Med., Baltimore, MD; [4]Bionomics Ltd, Adelaide, Australia

Abstract: The α7 nicotinic acetylcholine receptor (nAChR) is considered a potential drug target for cognitive dysfunction in schizophrenia and Alzheimer's disease. α7 nAChRs are found in brain regions involved in cognitive functions. With the α7 nAChR enhancing drugs being tested for therapeutic intervention, development of selective ligands for neuroimaging methods would greatly help the understanding of this receptor in relation to drug development and disease pathology. Iodo-ASEM, a derivative of ^{18}F-ASEM, is a potent α7 nAChR antagonist with subnanomolar affinity, high selectivity and in vivo binding applicability in the primate and the human brain. To further investigate ^{125}I-Iodo-ASEM as radiotracer for studying the α7 nAChR, we compared brain binding across several mammalian species, including the rat, mouse, pig and human with in vitro autoradiography. ^{125}I-Iodo-ASEM was found to show specific and displaceable binding in mouse, rat, and pig brain sections, while human tissue sections showed only very low specific binding. The binding pattern was in agreement with that of ^{125}I-α-bungarotoxin, and no specific binding was observed in brain sections from α7 nAChR knock-out mice. Surprisingly, radioligand binding to sections from β2 nAChR knock-out mice was more strongly reduced for ^{125}I-Iodo-ASEM (~50%) than ^{125}I-α-bungarotoxin (~20%) when compared to wild-type animals, suggesting different binding properties to α7β2 nAChR heteromers. K_d was found to be 1.5 nM in the pig and 1.1 nM in the rat cortex, respectively. B_{max} in the outer cortical layer of the pig cortex was 9.5 fmol/mg protein, similar to reported values using other α7 nAChR radioligands. In contrast, B_{max} in the rat cortex was considerably lower (0.7 fmol/mg protein). In the pig frontal cortex, ^{125}I-Iodo-ASEM specific binding was almost completely displaced by a range of α7 nAChR selective compounds (NS14492, TC-5619, EVP-6124, A582941, SSR-180711). Taken together, these findings indicate that ^{125}I-Iodo-ASEM is applicable for visualizing α7 nAChR binding in vitro, and that binding is different between species.



Disclosures: **C.K. Donat:** None. **Y. Gao:** None. **R.C. Mease:** None. **A.G. Horti:** None. **H.H. Hansen:** None. **M.G. Pomper:** None. **J.D. Mikkelsen:** None.

Nanosymposium

673. Molecular Techniques

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Presentation Number: 673.05

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH BRAIN Grant 2014/2015 U01 MH

Title: Directed evolution for GPCRs

Authors: *J. G. ENGLISH, B. L. ROTH;
Pharmacol., UNC Chapel Hill, Chapel Hill, NC

Abstract: Chemogenetics is routinely used to decipher the inner workings of the mammalian brain. DREADDs are fundamental chemogenetic tools derived from mutated G-protein coupled receptors (GPCRs). These mutations confer selective activation of the receptor by a previously inert chemical ligand while simultaneously inhibiting activation by endogenous ligands. The greatest barrier to designing additional DREADD receptors is in identifying the mutations necessary to confer a ligand-preference inversion. Here we will describe a new method for the rapid directed evolution of GPCRs in to DREADDs.

Disclosures: J.G. English: None. **B.L. Roth:** None.

Nanosymposium

673. Molecular Techniques

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Time: Wednesday, November 16, 2016, 8:00 AM - 10:45 AM

Presentation Number: 673.06

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Canadian Institutes of Health Research

Title: Phosphomimetic lipopeptides as new tools to investigate β-arrestin-mediated functions.

Authors: *É. BESSERER-OFFROY[1,2], C. E. MONA[1,2], P.-L. BOUDREAULT[1,2], J.-M. LONGPRÉ[1,2], R. LEDUC[1,2], É. MARSAULT[1,2], P. SARRET[1,2];

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Abstract: Arrestins play key regulatory roles in seven-transmembrane receptors (7TMRs) signal transduction and function. Over the last decade, β-arrestins' status changed from molecular adaptor proteins involved in 7TMR desensitization and internalization to scaffolds for MAP kinase cascades promoting ERK1/2, p38, and JNK activation. To date, no pharmacological inhibitor targeting selectively β-arrestin signaling has been developed. Thus, investigating the role played by β-arrestins in 7TMRs-mediated signaling relies mainly on the use of siRNA-mediated protein knockdown or on β-arrestin 1 or 2 knockout mice. In the present study, we designed and synthesized a phosphomimetic peptide based on the C-terminal tail of the vasopressin V2 receptor as it has been shown to interact with high affinity to both β-arrestins 1 and 2. To ensure intracellular delivery, we attached a palmitic acid residue at its N-terminal end which promotes plasma membrane anchorage and cell penetration by a passive flip-flop mechanism. We next investigated the *in-vitro* modulating efficacy of this N-terminal lipidated peptide on the β-arrestin recruitment at three distinct class A 7TMRs, namely vasopressin V2 (V2R), apelin (APJ) and chemokine receptor CXCR4. Using a BRET-based β-arrestin recruitment assay, we found that this lipopeptide was effective at inhibiting β-arrestin recruitment to class A 7TMRs. Indeed, kinetic assays revealed that the maximal inhibition occurred after 40 min of incubation and can completely abolish the recruitment of both β-arrestins 1 and 2 to the receptor, with no isoform selectivity. pA2 values were found to be receptor-dependent and ranged from 5.2 (6.3 μM) for CXCR4 and APJ to 4.2 (63 μM) for V2R. We further assessed whether this pepducin-like phosphomimetic peptide exerted agonistic activity on V2R as it mimics the C-terminal tail of this receptor. We did not observe any intrinsic activity of this lipidated peptide on β-arrestin- and G protein-dependent pathways, thus suggesting that it does not behave as an allosteric agonist at V2R. Finally, we examined whether this lipopeptide affected the ability of the endogenous ligands, vasopressin, apelin and SDF-1 to stimulate V2R-induced cAMP accumulation and APJ- or CXCR4-induced $G\alpha_i$ activation, respectively. Our results revealed that this lipidated peptide did not impact G protein-dependent activation, thus driving biased activity at 7TMRs. In conclusion, we report here a new strategy to selectively inhibit β-arrestin recruitment and to help in deciphering the contribution of β-arrestin-mediated signaling in 7TMR function.

Disclosures: É. Besserer-Offroy: None. **C.E. Mona:** None. **P. Boudreault:** None. **J. Longpré:** None. **R. Leduc:** None. **É. Marsault:** None. **P. Sarret:** None.

Nanosymposium

673. Molecular Techniques

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Topic: I.05. Biomarker and Drug Discovery

Support: Caltech Beckman Institute and the Arnold and Mabel Beckman Foundation

Hereditary Disease Foundation

Caltech–City of Hope Biomedical Initiative

National Institutes of Health (NIH) Director's New Innovator 1DP2NS087949

NIH/National Institute on Aging (NIA) 1R01AG047664

Title: Using CREATE to identify AAV variants for efficient and widespread gene transfer to the adult central nervous system

Authors: *B. E. DEVERMAN, Y. LUO, D. BROWN, T. DOBREVA, S. RAVINDRA KUMAR, K. CHAN, V. GRADINARU;
Caltech, Pasadena, CA

Abstract: Recombinant adeno-associated viruses (rAAVs) are commonly used vehicles for *in vivo* gene transfer. However, the tropism repertoire of naturally occurring AAVs is limited, prompting the development of novel AAV capsids with more desirable transduction characteristics. We recently described a capsid selection method, called Cre-recombination-based AAV targeted evolution (CREATE), that enables the identification of AAV capsids that more efficiently transduce defined cell populations *in vivo* (Deverman *et al., Nature Biotechnol* 2016). We generated AAV capsid libraries and used CREATE to identify variants that cross the blood brain barrier and efficiently and widely transduce cells in the mouse central nervous system (CNS) after intravenous injection in the adult. One variant, AAV-PHP.B, transduces the majority of astrocytes and neurons across multiple CNS regions. In my presentation, I will briefly discuss this published work and then describe our new efforts to use next generation sequencing (NGS) to more quickly and reliably identify capsid variants with desirable characteristics. We are using CREATE and NGS to evolve AAV-PHP.B for even more efficient gene transfer to the CNS and for more selective transduction of specific cell types.

Disclosures: B.E. Deverman: None. **Y. Luo:** None. **D. Brown:** None. **T. Dobreva:** None. **S. Ravindra Kumar:** None. **K. Chan:** None. **V. Gradinaru:** None.

Nanosymposium

673. Molecular Techniques

Location: SDCC 4

Time: Wednesday, November 16, 2016, 8:00 AM - 10:45 AM

Presentation Number: 673.08

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH

Title: Single cell molecular profiling in biopsied human olfactory neurons: a rapid and non-invasive method to study "state" and "trait" neuronal changes in mental disorders.

Authors: *Y. WU, Y. CHUNG, K. ISHIZUKA, A. SAWA;
Psychiatry and Behavioral Sci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Dynamic changes in symptom manifestation are prominent in psychiatric conditions, such as bipolar disorder and schizophrenia. Medication intervenes with these dynamic processes. However, the mechanisms of such processes including pathological “state” changes together with “trait” that continuously underlie the disease remain unclear.
Leading stem cell technologies, including induced pluripotent stem cells (iPSCs), allows us to generate human neuronal cells in a culture dish. A major drawback of this technique is the inability to recapitulate pathological “states” due to genetic reprogramming and prolonged culturing, but “trait” changes can be reasonably depicted. There is an urgent need for an experimental system that provides molecular snapshots of symptoms and treatment response at the time of biopsy.
Here, we introduce a new platform to study brain disorders using neurons derived from the olfactory epithelium (OE). We have established the nasal “brush” biopsy followed by “single cell” molecular profiling directly from biopsied samples. Multiple groups including ours have traditionally used punch biopsy via nasal endoscope which is still somewhat invasive. In contrast, the brush swab is a 3 mins procedure that is noninvasive and can be performed on children. The cells obtained by this procedure are processed for single cell capture, followed by single cell qPCR on a 96 well plate. Olfactory neurons were identified by their positive expression of neuronal markers, OMP and TUBB3, while negative for non-neuronal markers including HES1, ALDH1A3, and REG3G. We found that 40-70% of olfactory primary cells obtained by the brush swab were neurons and that the number of cells is sufficient for downstream molecular profiling at the single cell level.
To ascertain the utility and unique advantage of our experimental system, we studied molecular changes involving the Wnt pathway in patients with bipolar disorder compared with matched controls. Data from single cell analysis have been compared to the molecular profile data from olfactory neuronal cultures after the punch biopsy. We will present preliminary data supporting the idea that the nasal brush procedure combined with single cell analysis allow neuronal

selection which is predicted to detect greater and more specific abnormalities in living patients. Together, this approach is a new tool for the clinical setting and a powerful complement to the well-established iPSC technology in translational brain medicine.

Disclosures: **Y. Wu:** None. **Y. Chung:** None. **K. Ishizuka:** None. **A. Sawa:** None.

Nanosymposium

673. Molecular Techniques

Location: SDCC 4

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Presentation Number: 673.09

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: 1R01CA194697-01

5R00CA158066-05

Walther Cancer Foundation Advancing Basic Cancer Research Grant II

W81XWH-15-1-0021

Indiana CTSI core pilot fund

CCF-1217906

Title: An integrative platform for the three-dimensional quantitative analysis of spatially heterogeneous metastasis landscapes

Authors: ***I. H. GULDNER**, L. YANG, K. COWDRICK, Q. WANG, W. ALVAREZ BARRIOS, V. ZELLNER, Y. ZHANG, M. HOST, F. LIU, D. CHEN, S. ZHANG;
Univ. of Notre Dame, South Bend, IN

Abstract: Metastatic microenvironments are spatially and compositionally heterogeneous. This seemingly stochastic heterogeneity provides researchers great challenges in elucidating factors that determine metastatic outgrowth. Herein, we develop and implement an integrative platform that will enable researchers to obtain novel insights from intricate metastatic landscapes. Our two-segment platform begins with whole tissue clearing, staining, and imaging to globally delineate metastatic landscape heterogeneity with spatial and molecular resolution. The second segment of our platform applies our custom-developed SMART 3D (**S**patial filtering-based background removal and **M**ulti-ch**A**nnel forest classifiers-based 3D **R**econs**T**ruction), a multi-faceted image analysis pipeline, permitting quantitative interrogation of functional implications of heterogeneous metastatic landscape constituents, from subcellular features to multicellular

structures, within our large three-dimensional (3D) image datasets. Coupling whole tissue imaging of brain metastasis animal models with SMART 3D, we demonstrate the capability of our integrative pipeline to reveal and quantify volumetric and spatial aspects of brain metastasis landscapes, including diverse tumor morphology, heterogeneous proliferative indices, metastasis-associated astrogliosis, and vasculature spatial distribution. Collectively, our study demonstrates the utility of our novel integrative platform to reveal and quantify the global spatial and volumetric characteristics of the 3D metastatic landscape with unparalleled accuracy, opening new opportunities for unbiased investigation of novel biological phenomena *in situ*.

Disclosures: **I.H. Guldner:** None. **L. Yang:** None. **K. Cowdrick:** None. **Q. Wang:** None. **W. Alvarez Barrios:** None. **V. Zellner:** None. **Y. Zhang:** None. **M. Host:** None. **F. Liu:** None. **D. Chen:** None. **S. Zhang:** None.

Nanosymposium

673. Molecular Techniques

Location: SDCC 4

Time: Wednesday, November 16, 2016, 8:00 AM - 10:45 AM

Presentation Number: 673.10

Topic: I.05. Biomarker and Drug Discovery

Support: JPB Foundation

Title: Efficiency, safety and stability of non-invasive MRgFUS mediated gene therapy in the brain

Authors: ***M. A. STAVARACHE**, N. PETERSEN, M. G. KAPLITT;
Weill Cornell Med. Coll, New York, NY

Abstract: Recently, gene therapy became an important therapeutic alternative to neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease. However, an important limitation at the level of the CNS is its invasiveness since gene therapy vectors cannot efficiently cross the blood-brain-barrier (BBB), therefore requiring invasive implantation of infusion catheters into target regions. Microbubble-mediated magnetic resonance-guided focused ultrasound (MRgFUS) can be a useful tool for non-invasive gene therapy delivery to targeted areas in the brain by transiently permeabilizing the BBB to allow delivery of an adeno-associated virus (AAV) expressing various genes to specific areas in the brain. Here, we demonstrate that non invasive MRgFUS facilitated reporter gene green fluorescent protein (GFP) transduction in the CNS is efficient and stable over a long period of time, while FUS-induced local inflammatory response is transitory and without long-term effects. Sprague-Dawley rats (300-350 gr) were treated unilaterally at the level of striatum with FUS under MRI control, while a

mixture of viral vector (AAV2/1.GFP) and microbubbles was injected via a tail vein catheter. Gd-DTPA contrast agent diffusion confirmed the temporary opening of BBB on T1-weighted images post-sonication. Animals were sacrificed at different time points (3 hrs, 24 hrs, 2 weeks, 2 months, 6 months, 16 months) after receiving FUS, and the brains were harvested for histological analysis. First, we analyzed by immunohistochemistry the expression of GFP reporter gene in both sonicated and non-sonicated striatum to determine the distribution, efficacy and cell-specificity of transduced gene. The results showed efficient GFP transduction, limited mostly to neurons, in the area subjected to FUS treatment, persistent up to 16 months post-sonication. Next, we assessed the FUS-induced local inflammatory response by analyzing inflammatory markers. Our preliminary data showed that astrocytosis (GFAP - astrocyte marker), activation of microglia (defined by the change in morphology of IbaI), and local macrophage infiltration (MoMa-2) were present at the level of the targeted area in the first 3 hours following FUS treatment but not detected weeks and months later. CD68, a lysosomal protein associated, at high levels, with activated microglia followed the same pattern. No erythrocyte extravasation, edema and other FUS-induced changes in tissue structure were detected using Hematoxylin & Eosin staining. Overall, our results suggest that MRgFUS is a safe and efficient method for non-invasive long-term AAV-mediated gene therapy delivery to specific areas in the brain.

Disclosures: **M.A. Stavarache:** None. **N. Petersen:** None. **M.G. Kaplitt:** None.

Nanosymposium

673. Molecular Techniques

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Topic: I.04. Physiological Methods

Support: NIH P01 DA008227

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Howard Hughes Collaborative Innovator Award (HCIA)

Title: Brain-wide connectivity screen uncovers input- and dopamine subtype-specific control of drug-induced behaviors

Authors: *K. BEIER, C. KIM, B. HEIFETS, L. HUNG, S. NEUNER, K. DELOACH, K. DEISSEROTH, L. LUO, R. MALENKA;
Stanford Univ., Stanford, CA

Abstract: Dopamine (DA) neurons in the midbrain ventral tegmental area (VTA) integrate complex inputs to encode multiple signals, which influence motivated behaviors via diverse projections. We recently developed a novel technique, termed cTRIO, which combines axon-initiated viral transduction with rabies-mediated transsynaptic tracing and Cre-based cell type-specific targeting, to systematically map input–output relations of VTA-DA neurons. This whole-brain map permitted the unbiased linking of inputs to outputs of DA neuron subtypes, and revealed a novel top-down executive control circuit of DA neurons, the reinforcing function of which was demonstrated by optogenetic stimulation and classic behavioral pharmacology (Beier et al., *Cell* 162:622, 2015). Here, we extended this technology to develop an unbiased, whole brain screen for connectivity changes in response to a powerful experience, such as a single dose of a drug of abuse. Traditional methods for studying behavioral plasticity, such as slice electrophysiology, while powerful, lack the global and unbiased selection of cell types and synapses required for a comprehensive view of brain-wide experience-dependent circuit changes. We combined our transsynaptic tracing method with anatomy, *in vivo* calcium imaging, optogenetic, and chemogenetic circuit activation and inhibition to demonstrate the necessity of activity changes in specific brain regions identified from our unbiased screen for expression of drug-induced behaviors.

Disclosures: K. Beier: None. **C. Kim:** None. **B. Heifets:** None. **L. Hung:** None. **S. Neuner:** None. **K. DeLoach:** None. **K. Deisseroth:** None. **L. Luo:** None. **R. Malenka:** None.

Nanosymposium

764. Rett Syndrome

Location: SDCC 30B

Time: Wednesday, November 16, 2016, 1:00 PM - 4:30 PM

Presentation Number: 764.01

Topic: A.07. Developmental Disorders

Support: MH083911

AG045656

Stem Cell Fund from Pennsylvania State University

TR4-06747

DP2-OD006495-01

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R01MH103134

Title: KCC2 rescues functional deficits in human neurons derived from patients with Rett syndrome

Authors: *X. TANG[1], J. KIM[2], L. ZHOU[2], E. WENGERT[3], L. ZHANG[2], Z. WU[2], C. CARROMEU[4], A. R. MUOTRI[4], M. C. N. MARCHETTO[5], F. H. GAGE[5], G. CHEN[2]; [1]MIT, Whitehead Inst. For Biomed. Res., Cambridge, MA; [2]Dept. of Biology, Huck Inst. of Life Sciences, Pennsylvania State Univ., University Park, PA; [3]Bucknell Univ., Lewisburg, PA; [4]Dept. of Pediatrics/Rady Children's Hosp. San Diego and Dept. of Cell. & Mol. Medicine, Stem Cell Program, Sch. of Medicine, Univ. of California San Diego, La Jolla, CA; [5]Lab. of Genetics, Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Rett syndrome is a severe form of autism spectrum disorder, mainly caused by mutations of a single gene methyl CpG binding protein 2 (MeCP2) on the X chromosome. Patients with Rett syndrome exhibit a period of normal development followed by regression of brain function and the emergence of autistic behaviors. However, the mechanism behind the delayed onset of symptoms is largely unknown.

Here we demonstrate that neuron-specific K+-Cl− cotransporter2 (KCC2) is a critical downstream gene target of MeCP2. We found that human neurons differentiated from induced pluripotent stem cells from patients with Rett syndrome showed a significant deficit in KCC2 expression and consequently a delayed GABA functional switch from excitation to inhibition. Interestingly, overexpression of KCC2 in MeCP2-deficient neurons rescued GABA functional deficits, suggesting an important role of KCC2 in Rett syndrome. We further identified that RE1-silencing transcriptional factor, REST, a neuronal gene repressor, mediates the MeCP2 regulation of KCC2.

Because KCC2 is a slow onset molecule with expression level reaching maximum later in development, the functional deficit of KCC2 may offer an explanation for the delayed onset of Rett symptoms. Our studies suggest that restoring KCC2 function in Rett neurons may lead to a potential treatment for Rett syndrome.

Disclosures: **X. Tang:** None. **J. Kim:** None. **L. Zhou:** None. **E. Wengert:** None. **L. Zhang:** None. **Z. Wu:** None. **C. Carromeu:** None. **A.R. Muotri:** None. **M.C.N. Marchetto:** None. **F.H. Gage:** None. **G. Chen:** None.

Nanosymposium

764. Rett Syndrome

Location: SDCC 30B

Time: Wednesday, November 16, 2016, 1:00 PM - 4:30 PM

Presentation Number: 764.02

Topic: A.07. Developmental Disorders

Support: NINDS R01NS075062

International Rett Syndrome Foundation Basic Research Grant #2916

Civitan Emerging Research Scholar

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CFAR Core Grant AI027767

Title: RNA sequencing and novel proteomics approaches reveal multi-cellular deficits in the cortex of Rett syndrome mice

Authors: *N. L. PACHECO[1], D. K. CROSSMAN[2], M. R. HEAVEN[4], M. L. OLSEN[3]; [1]Univ. of Alabama At Birmingham, Birmingham, AL; [2]Dept. of Genet., [3]Cellular, Developmental and Integrative Biol., Univ. of Alabama at Birmingham, Birmingham, AL; [4]Proteomics, Vulcan Analytical, Birmingham, AL

Abstract: Rett syndrome (RTT) is an X-linked neurodevelopmental disorder caused by mutations in the transcriptional regulator MeCP2. RTT is characterized by having apparently normal development until 6-18 months, when a progressive decline in motor and language functions begins and breathing abnormalities and seizures present. Despite intense research, the molecular targets of MeCP2 and their contribution to the disease are unknown. Here we present the first comprehensive transcriptomic and proteomic analysis in a RTT mouse model. Examining whole cortex tissue in symptomatic males (*Mecp2*-null) with wildtype littermates, we have identified 414 genes (FDR < 0.05), 332 transcripts (FDR < 0.05), and 465 proteins ($p < 0.1$) considered to be significantly, differentially expressed. Surprisingly, we observed an overall poor correlation between global gene and protein expression (Pearson correlation 0.23). Pathway analysis in transcriptome data identified disrupted metabolism and cellular signaling, and proteome data identified cellular structure and maintenance disrupted pathways. Using a publicly available database containing CNS cell-type specific gene expression levels we identified that approximately 65% of the significant genes in our RNA-Seq data were globally expressed in all CNS cells. The remaining 35% were highly enriched in specific cell types. These data argue against the traditional paradigm that RTT is a neuronal-specific disease, highlighting the need to study individual cell populations in isolation. Future studies aim to examine cell-type specific gene expression changes in RTT animals. Through the identification of key groups of genes,

proteins and pathways, we can begin to identify new and much needed therapeutic targets for RTT patients.

Disclosures: **N.L. Pacheco:** None. **D.K. Crossman:** None. **M.R. Heaven:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Owns share in Vulcan Analytical. **M.L. Olsen:** None.

Nanosymposium

764. Rett Syndrome

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Time: Wednesday, November 16, 2016, 1:00 PM - 4:30 PM

Presentation Number: 764.03

Topic: A.07. Developmental Disorders

Support: AFM-Théléthon (Strategic pole MNH Decrypt)

Title: Benficial effect of gene therapy in a preclinical model of rett syndrome

Authors: ***V. MATAGNE**, L. VILLARD, J.-C. ROUX;
Aix Marseille Universite, INSERM, GMGF UMRS 910, Marseille Cedex 05, France

Abstract: Rett syndrome (RTT) is a severe X-linked neurodevelopmental disorder that is primarily caused by loss-of-function mutation in the methyl CpG binding protein 2 gene (MECP2) and is the second most prevalent cause of intellectual disability in girls. It is a disease affecting not only the CNS (profound cognitive and motor deficits) but also the motor and autonomic functions (including severe breathing abnormalities). Currently, treatments are aimed at alleviating symptoms and there is no cure for RTT.
Recently, two different research teams reported that gene therapy in the Mecp2-deficient RTT mouse model partially cured the disease (Gadalla et al 2013; Garg et al 2013). Although both studies reported some beneficial effects of gene therapy, they also showed that there was still room for improvement.
In order to try and improve vector delivery and expression, we designed a plasmid construct expressing a codon-optimized version of *Mecp2* that was used to generate a scAAV9 virus. Symptomatic mice, thirty day-old Mecp2 KO male (KO) and 5 month-old heterozygous (Hz) Mecp2 female mice, were injected with the virus through the tail vein ($2*10^{11}$vg/mouse). Despite a low percentage of Mecp2-expressing cells in the brain of AAV9-treated KO mice (10-24% of WT levels), we did find an improvement in spontaneous locomotor activity and sensorimotor coordination, as well as normalization of the number of apneas (145±68 in treated vs 4.5±3.2 in untreated KO, $p<0.001$) that are characteristic RTT symptoms. A significant decrease in the number of apneas was also observed in Hz female mice 4 weeks post-injection

(53.1±16 in treated vs 94.3±13 in untreated Hz Mecp2 mice, $p<0.05$).
Current studies are investigating the long-term effect of treatment in Mecp2 Hz females on various parameters including breathing function, behavioral deficits (locomotor activity, sensorimotor function and anxiety-related behavior) and potential toxic off-target effects. These preclinical data will provide us useful information that will hopefully be transferable to the treatment of RTT patients.

Disclosures: V. matagne: None. **L. Villard:** None. **J. Roux:** None.

Nanosymposium

764. Rett Syndrome

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Time: Wednesday, November 16, 2016, 1:00 PM - 4:30 PM

Presentation Number: 764.04

Topic: A.07. Developmental Disorders

Support: Lejeune Foundation Grant #1209

Title: Lack of Mecp2 interferes with mechanisms of cortical progenitors proliferation and differentiation

Authors: *F. BEDOGNI[1], C. COBOLLI GIGLI[1], L. SCARAMUZZA[1], D. POZZI[2], C. KILSTRUP-NIELSEN[3], M. MATTEOLI[2], N. LANDSBERGER[1,4];
[1]San Raffaele Res. Inst., Milan, Italy; [2]Humanitas Clin. and Res. Ctr., Milan, Italy; [3]Univ. dell'Insubria, Varese, Italy; [4]Univ. degli Studi di Milano, Milan, Italy

Abstract: Rett syndrome (RTT) is the most common genetic cause of intellectual disability in females. Classical RTT cases are linked to mutations in the X-linked Methyl-CpG-Binding Protein 2 (MeCP2) gene, encoding for a multi functional protein ubiquitously expressed from developmental stages to adulthood. RTT girls manifest overt symptoms after an apparently normal early post-natal development. Due to this reason, Mecp2 functions have in most cases been investigated during late postnatal stages or adulthood, ruling out its involvement in early development. However, many evidences now demonstrate that early signs of the pathology can be observed in both girls and RTT animal models even before the onset of overt symptoms. To highlight the possible roles played by Mecp2 during early stages of life, we focused on the development of the embryonic cerebral cortex of null mice. A preliminary transcriptional screening suggested that the mechanisms of embryonic corticogenesis are delayed in the cortex of *Mecp2* null mice. Based on this observation, we assessed the dynamics of cell cycle progression, exit and neuronal differentiation on embryonic and early postnatal cortical tissues. Our data remarkably show that the expression of markers that are typical of neuronal progenitors

is retained by null newborn neurons. On the contrary, the level of transcripts typically expressed by maturing or fully functional neurons are reduced in null cortices, including genes encoding for mediators of responsiveness to external stimuli such as glutamatergic and GABAergic receptors subunits and ionic channels components. Accordingly, calcium waves driven by stimuli are reduced in null neurons, a feature that is likely causative of the defective establishment of proper neuronal networks thoroughly described in *Mecp2* null adult tissues. Altogether, our data suggest that already during embryonic and early post-natal life lack of Mecp2 affects the acquirement of features that are necessary for proper cerebral cortex functions later in life. Moreover, we recently produced data showing that the top deregulated genes detected in the null embryonic cortex are accordingly deregulated in adult animals, indicating that part of the transcriptional derangement driven by lack of Mecp2 during early embryonic development persists in adulthood. This suggests the importance of the role Mecp2 plays during embryonic development and implies that the impairments displayed by RTT animal models can be considered the worsening of a condition that, at least to some extent, is generated during early stages of life.

Disclosures: **F. Bedogni:** None. **C. Cobolli Gigli:** None. **L. Scaramuzza:** None. **D. Pozzi:** None. **C. Kilstrup-Nielsen:** None. **M. Matteoli:** None. **N. Landsberger:** None.

Nanosymposium

764. Rett Syndrome

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Topic: A.07. Developmental Disorders

Support: NIH Grant 5R01NS057819-10

Howard Hughes Medical Institute

McNair Foundation

Baylor Research Advocates for Student Scientists

Title: Activity-dependent transcription in a mouse model of Rett syndrome

Authors: ***A. E. POHODICH**[1], A. RAMAN[2], H. K. YALAMANCHILI[3], Z. LIU[4], H. Y. ZOGHBI[3];
[1]Neurosci., [2]Structural and Computat. Biol. and Mol. Biophysics, [3]Mol. and Human Genet., [4]Pediatrics, Baylor Col. of Med., Houston, TX

Abstract: Rett syndrome (RTT), a neurological disorder characterized by seemingly normal development followed by loss of acquired speech, cognitive abilities, and motor coordination, is caused by mutations in the X-linked gene Methyl-CpG-Binding Protein 2 (*MECP2*). In patients, the delayed onset of disease coincides with the timing of peak synaptic reorganization, and abnormalities in neuronal plasticity have been observed in MeCP2-deficient mice. MeCP2 has been shown to be phosphorylated at multiple sites upon neuronal activation, and a handful of genes have been proposed to require MeCP2 for activity-induction, suggesting that an essential aspect of MeCP2's function in neurons may be regulation of activity-dependent transcriptional programs. To assess the necessity of MeCP2 for stimulus-induced gene expression, we adapted a deep brain stimulation (DBS) protocol to acutely elicit robust, *in vivo* activation of dentate gyrus (DG) neurons in 8-week-old wildtype (WT) and MeCP2-knockout (KO) male mice. We performed RNA-Sequencing on DG tissue from DBS and sham-treated mice and compared the expression patterns of WT and KO neurons at baseline and with activity. Surprisingly, we found: 1. neurons from fully symptomatic KO mice were capable of significant increases in activity-dependent gene expression; 2. the majority of the genes upregulated by DBS were the same for KO and WT mice; 3. the magnitude of the expression change was similar between genotypes for many genes, including for a number of genes previously thought to require MeCP2 for induction, such as brain derived neurotrophic factor (KO DBS: $\log_2$ fold change (L_2FC): 3.7, pAdj<0.001; WT DBS: L_2FC: 3.8, pAdj<0.001; WT DBS vs. KO DBS: pAdj=0.5) and neuron PAS domain protein 4 (KO DBS: L_2FC: 7.2, pAdj<0.001; WT DBS: L_2FC: 6.9, pAdj<0.001; WT DBS vs. KO DBS: pAdj=0.9). These findings suggest that MeCP2 is not required for induction of the majority of activity-dependent genes in the DG *in vivo*. However, we have identified 78 novel genes that may require MeCP2 for stimulus-induced expression. We are currently working to characterize these genes, as they may provide insight into the plasticity deficits observed in KO mice. Additionally, we are investigating the impact of activity on splicing in both WT and KO neurons. We found hundreds of activity-induced splicing changes in WT mice following DBS. Many of these splicing events have not been previously characterized with activity *in vivo*, and 170 involve transcripts of autism-associated genes. The results of these studies will further our understanding of the role of splicing with activity in neurons and yield clues as to how this process may contribute to neurologic disorders.

Disclosures: **A.E. Pohodich:** None. **A. Raman:** None. **H.K. Yalamanchili:** None. **Z. Liu:** None. **H.Y. Zoghbi:** None.

Nanosymposium

764. Rett Syndrome

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Presentation Number: 764.06

Topic: A.07. Developmental Disorders

Support: NIH Grant MH085802

Simons Center for the Social Brain/SFARI

IRSF Grant #3213

Title: Depolarizing GABA receptor causes cortical network deficits in Rett Syndrome

Authors: *K. LI[1], R. V. RIKHYE[2], A. BANERJEE[1], M. SUR[2,1,3];
[1]Picower Inst. for Learning and Memory, [2]Dept. of Brain and Cognitive Sci., [3]Simons Ctr. for the Social Brain, MIT, Cambridge, MA

Abstract: Rett Syndrome (RTT), a form of autism spectrum disorder, is mainly caused by mutations of a single gene, Methyl CpG binding protein 2 (MECP2). Symptoms of RTT include developmental regression of acquired motor and language skills, and severe cognitive impairment. The mechanism behind these symptoms are not yet clear, but in mouse RTT models the disruption of excitation/inhibition (E/I) balance in the brain has been observed. The E/I balance is crucial for the normal functioning of cortical networks. Even subtle E/I imbalance is reflected in changes in spike rate and timing, measured by signal-to-noise-ratio (SNR), and coding reliability and sparseness. Indeed, all these properties are decreased in RTT model mice. This E/I imbalance is moved in the direction of hyper-excitation in RTT, yet RTT also seems to feature hyper-connection of inhibitory neurons and reduced excitation.
Reduced efficacy of inhibitory GABAergic transmission following Mecp2 mutation is a likely mechanism for the above observations in RTT. At the synaptic level, Tang et al. (2015) found that K^+ /Cl^- cotransporter 2 (KCC2) was a critical downstream target of Mecp2, and KCC2 reduction following MECP2 mutation lowered intracellular Cl^- . Low intracellular Cl^- concentration caused depolarizing postsynatpic responses of $GABA_AR$, and weakened inhibitory transmission. However, it is not clear whether this synaptic mechanism can cause the observed E/I balance shift in cortical circuits *in vivo*.
To specifically rescue the abnormal intracellular Cl^- levels and observe its effect on cortical network behavior, we used bumetanide, a Na^+ K^+ Cl^- cotransporter 1 (NKCC1) antagonist that raises intracellular Cl^- concentration, and measured the network responses of V1 superficial layer neurons to a range of visual stimuli. In particular, we evaluated SNR, sparseness and reliability, in response to natural movies. After daily injections of bumetanide for a week, RTT model mice showed increased SNR and sparseness compared to sham-injected RTT model control, showing clear rescue of both properties. These results indicate that the altered intracellular Cl^- level, which leads to depolarizing GABAergic transmission, underlies a major portion of deficits observed in cortical network responses in RTT.

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Nanosymposium

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Presentation Number: 764.07

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Rettsyndrome.org

Rett Syndrome Research Trust

Title: Impaired intrinsic and synaptic properties of striatal medium spiny neurons in Mecp2 knockout mice

Authors: *W. LI, L. POZZO-MILLER;
Neurobio., Univ. of Alabama at Birmingham Dept. of Neurobio., Birmingham, AL

Abstract: Rett syndrome (RTT), a pervasive neurodevelopmental disorder, is caused by mutations in the X-linked methyl-CpG-binding protein 2 (*MECP2*) gene. Girls mosaic for the expression of the mutant allele survive and develop typically until 6-18 months of age, but then present with an array of neurological symptoms, including stereotypic hand movements and ambulatory difficulty. Motor disturbances strongly suggest dysfunction of the striatum, a brain region responsible for the control of voluntary motor movements. Previous brain imaging studies provide evidence of an association between reductions in striatal volume and neurological severity in RTT individuals. However, the cellular and network dysfunction underlying striatal neuropathology remains to be defined. In this study, we characterized the intrinsic membrane properties of striatal medium spiny neurons (MSNs), as well as the synaptic inputs they receive from the cortex, in the *Mecp2* knockout (KO) mouse model of RTT. We found that the *Mecp2* KO striatum has a higher density of smaller sized MSNs than WT controls. In addition, MSNs have smaller rectifying currents and longer action potential decay times, suggesting altered potassium channel activity in *Mecp2* KO mice. The properties of excitatory synapses between the motor cortex and the striatum are also altered in *Mecp2* KO mice. The input-output relationship of excitatory postsynaptic potentials (EPSPs) evoked by stimulation of the motor cortex, as well as the spatio-temporal spread of voltage-sensitive dye signals during single EPSPs, are both smaller in *Mecp2* KO slices. Moreover, the frequency of spontaneous miniature EPSCs is lower in *Mecp2* KO MSNs, without differences in their amplitude or kinetics. Considering that the paired-pulse ratio of evoked EPSPs is not altered, these observations suggest a lower number of excitatory synapses in striatal MSNs of *Mecp2* KO mice. Indeed, the

numerical density of dendritic spines in *Mecp2* KO MSNs is lower than WT mice. Lastly, we tested whether these synaptic deficits have a consequence for synaptic plasticity. Pairing theta-burst stimulation of cortical afferents with back-propagating action potentials in MSNs induced a stable long-term potentiation (LTP) of EPSPs in WT slices, but not in *Mecp2* KO slices. Collectively, these results demonstrate functional and morphological deficits in excitatory synapses between the motor cortex and MSNs in *Mecp2* KO mice. Current studies include testing whether D1 receptor- and D2 receptor-expressing MSNs are equally sensitive to *Mecp2* deletion by characterizing their intrinsic and synaptic properties in D1-tdTomato and D2-EGFP mice crossed with *Mecp2* KO mice.

Disclosures: W. Li: None. **L. Pozzo-Miller:** None.

Nanosymposium

764. Rett Syndrome

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Presentation Number: 764.08

Topic: A.07. Developmental Disorders

Support: RSRT

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Title: Vitamin D supplementation ameliorates Rett syndrome cortical phenotypes in Mecp2-null mice

Authors: *J. L. MACDONALD[1,2], M. C. RIBEIRO[1], S. M. MOORE[1], N. KISHI[2,3], J. D. MACKLIS[2];
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Abstract: Mutations in *Mecp2* cause the severe neurodevelopmental disorder Rett syndrome (RTT). We previously investigated genes that function downstream of MeCP2 in cerebral cortex

circuitry, and identified up-regulation of *Irak1*, a central component of the NF-κB pathway. Over-expression of *Irak1* mimics reduced dendritic complexity of *Mecp2*-null cortical callosal projection neurons (CPN), and NF-κB signaling is specifically up-regulated in the CNS with *Mecp2* loss-of-function. Strikingly, genetically reducing NF-κB signaling in *Mecp2*-null mice not only ameliorates CPN dendritic complexity, it substantially extends their lifespan, indicating broader roles for NF-κB signaling in RTT pathogenesis.
The amelioration of *Mecp2*-null phenotypes by genetic attenuation of NF-κB signaling raises the intriguing question of whether modulation of NF-κB signaling could provide a therapeutic avenue in RTT. Among the many known inhibitors of NF-κB signaling are vitamin D and its analogues, and, interestingly, there is a high prevalence of vitamin D deficiency among RTT patients. We analyzed vitamin D levels in 8 week old *Mecp2*-null mice, and found that they similarly have significantly reduced total serum 25(OH)D levels compared to wild-type littermates. To investigate whether vitamin D supplementation might reduce the aberrant NF-κB activity in *Mecp2*-null cortex, and might have therapeutic benefit, we treated *Mecp2*-null and wild-type littermates with vitamin D supplemented chow, beginning at 4 weeks of age (early symptomatic stage). We found that this simple, cost-effective dietary supplement ameliorates CPN dendritic morphology and soma size phenotypes in a dose-dependent manner, and modestly increases lifespan. These results provide new insight into both the fundamental neurobiology of RTT, and potential therapeutic strategies via NF-κB pathway modulation.

Disclosures: J.L. MacDonald: None. **M.C. Ribeiro:** None. **S.M. Moore:** None. **N. Kishi:** None. **J.D. Macklis:** None.

Nanosymposium

764. Rett Syndrome

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Presentation Number: 764.09

Topic: A.07. Developmental Disorders

Support: NINDS R01NS075062

Title: Astrocytes are a primary target of neuronal-derived BDNF: a novel mechanism for dysfunction in Rett Syndrome

Authors: *L. HOLT[1], M. L. OLSEN[2];
[2]Cell, Development, and Integrative Biol., [1]Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Mature astrocytes are one of the most morphologically complex cells in the central nervous system. This complexity is associated with several of the most well characterized

functions of this cell type, including neurotransmitter reuptake, K^+ homeostasis, and blood-brain barrier maintenance. While we know the developmental time window when astrocyte morphological maturation and refinement occurs, we know little else governing this process. Brain derived neurotrophic factor (BDNF) is a critical growth factor secreted largely by neurons and involved in the development and maturation of neurons, including neuronal growth and synapse refinement. Preliminary data demonstrates that astrocytes express high levels of the BDNF receptor TrkB when compared to neurons. In particular, the truncated version of TrkB, TrkB.T1, is the predominate receptor expressed. TrkB.T1 expression is highest in astrocytes during the critical period of astrocyte morphological refinement and maturation, a developmental time window which also coincides with the highest neuronal BDNF expression levels. Loss of BDNF expression is a hallmark of neurodevelopment disorder Rett Syndrome, and recent publications indicate that astrocytes have a significantly reduced morphological complexity and are dysfunctional in this disease. These findings have led us to hypothesize that BDNF/TrkB.T1 signaling is an important mediator of astrocyte morphological maturation and that reduced neuronal BDNF expression contributes to astrocyte dysfunction by modulating astrocyte morphology in Rett Syndrome.

Disclosures: L. Holt: None. **M.L. Olsen:** None.

Nanosymposium

764. Rett Syndrome

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Presentation Number: 764.10

Topic: A.07. Developmental Disorders

Support: International Rett Syndrome Foundation

Title: MeCP2 activates Cypin and regulates dendritic branching in Rett syndrome

Authors: *W. GOLD[1,2], E. BUSS[3], T. LACINA[3], L. CANTRILL[2,4], M. GRAHAM[5], J. CHRISTODOULOU[1,2,6,7];
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Abstract: Background: Rett syndrome (RTT) is a severe paediatric neurodevelopmental disorder, predominantly caused by mutations in the transcriptional regulator Methyl-CpG-binding protein 2 (*MECP2*) gene. Despite the genetic cause being known, the pathophysiology of the neurological phenotype is still largely unknown. A predominance of neuronal and synaptic dysfunction with abnormal dendritic spine morphology and density is an overarching feature of Rett syndrome. Recently, defective microtubule dynamics with aberrant trafficking of brain derived neurotrophic factor (BDNF) have been described in Rett syndrome. BDNF is a well-described target of MeCP2 and is downregulated in Rett syndrome patients and mouse models. Recently it has been demonstrated that BDNF increases the expression of the guanine deaminase Cypin, leading to increased dendritic branching in hippocampal cells. In addition to its ability to deaminase guanine, Cypin binds to tubulin and promotes microtubule polymerisation and is required for normal dendritic branching in neuronal cells. **Aims:** To investigate the role of Cypin and MeCP2 in dendritic branching in the *Mecp2*^{T158A} RTT mouse model. **Methods:** Microtubule stability was measured in RTT patient fibroblasts by treating cells with the depolymerizing agent nocodazole and measuring the rate of re-polymerisation. Cypin gene and protein expression was measured in the cortex of *Mecp2*^{T158A} mice. To tease out the relationship between MeCP2 and Cypin, we conducted affinity experiments using a GST-MeCP2 construct in brain fractions of wild type rats. **Results:** In our microtubule stability studies, RTT patient cells remained significantly depolymerised compared to control cells. As Cypin promotes microtubule polymerization we then investigated whether Cypin levels were reduced. As hypothesized, we observed a significant down-regulation in *Cypin* gene expression in the cortex of pre-symptomatic mice (5 weeks old) and symptomatic mice (21 weeks old) as well as a significant reduction in Cypin protein expression in symptomatic *Mecp2*^{T158A} male mice (13 weeks old). In addition, we observed a specific binding of Cypin to the GST-MeCP2 constructs suggesting that Cypin is a binding partner of MeCP2. **Conclusions:** As there are no binding motifs for MeCP2 on the promotor of the *Cypin* gene, these data suggest that MeCP2 is an activator of Cypin through a protein-protein interaction model. It is likely that the deficiency in Cypin in the *Mecp2*^{T158A} male mice leads to microtubule dysfunction and reduced dendritic branching in RTT.

Disclosures: W. Gold: None. **E. Buss:** None. **T. Lacina:** None. **L. Cantrill:** None. **M. Graham:** None. **J. Christodoulou:** None.

Nanosymposium

764. Rett Syndrome

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Topic: A.07. Developmental Disorders

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P30 NS47466

Title: MeCP2 deficiency results in robust rett-like behavioral and motor deficits in male and female rats

Authors: *K. PATTERSON[1], V. HAWKINS[2], K. ARPS[1], D. MULKEY[2], M. OLSEN[1]; [1]Univ. of Alabama At Birmingham, Birmingham, AL; [2]Univ. of Connecticut, Storrs, CT

Abstract: Since the identification of *MECP2* as the causative gene in the majority of Rett Syndrome (RTT) cases, transgenic mouse models have played a critical role in our understanding of this disease. The use of additional mammalian RTT models offers the promise of further elucidating critical early mechanisms of disease as well as providing new avenues for translational studies. We have identified significant abnormalities in growth as well as motor and behavioral function in a novel zinc-finger nuclease model of RTT utilizing both male and female rats throughout development. Male rats lacking *Mecp2* (*Mecp2*$^{ZFN\Delta/y}$) were noticeably symptomatic as early as postnatal day 21, with most dying by postnatal day 55, while females lacking one copy of *Mecp2* (*Mecp2*$^{ZFN\Delta/+}$) displayed a more protracted disease course. Brain weights of *Mecp2*$^{ZFN\Delta/y}$ and *Mecp2*$^{ZFN\Delta/+}$ rats were significantly reduced by postnatal day 14 and 21, respectively. Early motor and breathing abnormalities were apparent in *Mecp2*$^{ZFN\Delta/y}$ rats, whereas *Mecp2*$^{ZFN\Delta/+}$ rats displayed functional irregularities later in development. The large size of this species will provide profound advantages in the identification of early disease mechanisms and the development of appropriately timed therapeutics. The current study establishes a foundational basis for the continued utilization of this rat model in future RTT research.

Disclosures: **K. Patterson:** None. **V. Hawkins:** None. **K. Arps:** None. **D. Mulkey:** None. **M. Olsen:** None.

Nanosymposium

764. Rett Syndrome

Location: SDCC 30B

Time: Wednesday, November 16, 2016, 1:00 PM - 4:30 PM

Presentation Number: 764.12

Topic: A.07. Developmental Disorders

Title: Identification of novel CDKL5 substrates involved in cytoskeletal regulation

Authors: *L. BALTUSSEN, S. CLAXTON, M. MOESKOPS, V. CHRISTODOULOU, H. FLYNN, B. SNIJDERS, S. ULTANIR;
The Francis Crick Inst., London, United Kingdom

Abstract: Cyclin-Dependent Kinase-Like 5 (CDKL5) is a serine/threonine protein kinase important for neuronal development. Mutations in the CDKL5 gene have been found in patients diagnosed with atypical variants of Rett syndrome displaying a heterogeneous range of clinical phenotypes. CDKL5 is enriched throughout the brain during early postnatal development and is known to be involved in development of dendritic spines and synapses. Only few downstream effectors of CDKL5 or its molecular mechanisms of action are known.
We generated analogue-specific CDKL5 by mutating the gatekeeper residue in the ATP-binding pocket and introduced a second-site mutation to rescue kinase activity. Based on the utilization of bulky ATP analogues by analogue-specific CDKL5, we used an unbiased chemical genetic screen developed by the Shokat lab to identify new CDKL5 substrates and their phosphorylation sites. This led us to generate an initial list of potential CDKL5 substrates. We have validated some of these candidates by using phosphomutants for in vitro kinase assays and were able to confirm the CDKL5 consensus sequence (RPXpSX) described by Kameshita et al.
Currently phosphospecific antibodies are being developed to study In vivo phosphorylation levels of substrates in CDKL5 KO mice and neuronal cell cultures. Our newly identified CDKL5 substrates are all linked to cytoskeletal regulation. Therefore we are interested if phosphorylation by CDKL5 is crucial for exerting these functions, especially in relation to dendritic growth/ arborisation and dendritic spine formation. To further study CDKL5 and these novel substrates, we use constitutive and conditional KO mice to better understand the biological function of CDKL5 and its role in neurodevelopment.

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Nanosymposium

764. Rett Syndrome

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Topic: A.07. Developmental Disorders

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JPB Foundation

Title: Histone deacetylase 3 associates with MeCP2 to regulate FOXO and social behavior

Authors: *A. NOTT[1], J. CHENG[1], F. GAO[1], Y.-T. LIN[1], E. GJONESKA[1], T. KO[1], P. MINHAS[1], A. V. ZAMUDIO[1], J. MENG[1], F. ZHANG[2], P. JIN[2], L.-H. TSAI[1];
[1]MIT, Cambridge, MA; [2]Dept. of Human Genet., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Mutations in *MECP2* cause the neurodevelopmental disorder Rett syndrome (RTT). The RTT missense $MECP2^{R306C}$ mutation prevents MeCP2 interaction with NCoR/Histone deacetylase 3 (HDAC3); however, the neuronal function of HDAC3 is incompletely understood. We report that neuronal deletion of *Hdac3* in mice elicits abnormal locomotor coordination, sociability, and cognition. Transcriptional and chromatin profiling revealed HDAC3 positively regulates a subset of genes and is recruited to active gene promoters via MeCP2. HDAC3-associated promoters are enriched for the FOXO transcription factors, and FOXO acetylation is elevated in *Hdac3* KO and *Mecp2* KO neurons. Human RTT patient-derived $MECP2^{R306C}$ neural progenitor cells (NPCs) have deficits in HDAC3 and FOXO recruitment and gene expression. Gene editing of $MECP2^{R306C}$ cells to generate isogenic controls rescued HDAC3-FOXO-mediated impairments in gene expression. Our data suggests that HDAC3 interaction with MeCP2 positively regulates a subset of neuronal genes through FOXO deacetylation, and disruption of HDAC3 contributes to cognitive and social impairment.

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Nanosymposium

764. Rett Syndrome

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Topic: A.07. Developmental Disorders

Support: Autism Speaks Dennis Weatherstone fellowshipo

Title: Genetic rescue of brain morphometry in a mouse model of neurodevelopmental disorder

Authors: *R. ALLEMANG-GRAND[1,2], J. ELLEGOOD[2], L. SPENCER-NOAKES[2], B. J. NIEMAN[2], J. P. LERCH[2];
[1]Mouse Imaging Ctr., Toronto, ON, Canada; [2]Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Autism and other Neurodevelopmental disorders (NDD) are caused by genetic and environmental factors that disrupt brain maturation. Interestingly, recent studies using NDD mice have shown a rescue of many behavioural and cellular impairments, suggesting that the developmentally impaired brain can be treated. Although the findings are exciting, these studies quantified treatment success in a limited number of brain regions and restricted their analyses to a few time points. To fully understand the efficacy of the treatment effects, we longitudinally scanned a NDD mouseline with in vivo MRI before and after a genetic intervention to determine the spatial and temporal changes that occur across the brain.
For this study, we used the inducible Mecp2 mouse model of Rett syndrome. In these mice, the Mecp2 gene is silent from birth leading to behavioural and neuronal impairments. However at the time of the experimenters choosing, the functional Mecp2 gene can be reactivated. At P50, Mecp2 and WT litter mates were scanned in vivo with a Manganese-enhanced MRI protocol (T1-weigthed, 100um^3), followed by 4 weeks of tamoxifen (to reactivate Mecp2) or oil (leave Mecp2 silent). At P80, a follow up scan was conducted and a separate cohort was treated with tamoxifen in order to reactivate Mecp2 later in adult life. The collected images were aligned and analysed using deformation-based morphometry to determine baseline neuroanatomical differences between groups as well as differences in volumetric trajectory between time points. At P50, Mecp2 silent mice had a 11% decrease in brain volume compared to WT. Interestingly, Mecp2 reactivation increased total brain volume by 1.5% while the silent brain decreased by 3.8% ($p<0.01$). Furthermore, Mecp2 reactivation altered the growth of many regions (e.g. striatum, cerebellum and medulla), such that these areas acquired a trajectory similar to WT. However, these regions tend to atrophy within the group possessing a silent copy of Mecp2, leading to significant differences between these groups at P80 (10% FDR-corrected). Although many cortical regions atrophied regardless of Mecp2 status, the extent of atrophy was reduced in the reactivated relative to silent group. Interestingly, Mecp2 reactivation in late adulthood also leads to volumetric rescue within many of the same regions as early reactivation group.

However, the extent of volumetric growth is greater when reactivation occurs earlier in life. Our findings demonstrate that there are sensitive periods in which the trajectory of some regions of the developmentally impaired brain can be rescue by interventions that aim to restore Mecp2 function.

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Nanosymposium

765. Sigma-2/PGRMC1 Receptor Function in Disease and Therapeutics

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Presentation Number: 765.01

Topic: B.04. Ion Channels

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Title: Sigma-2/PGRMC1 antagonists as disease-modifying therapeutics for Alzheimer's disease.

Authors: ***N. J. IZZO**, K. MOZZONI, C. SILKY, C. REHAK, R. YURKO, G. LOOK, G. RISHTON, M. GRUNDMAN, H. SAFFERSTEIN, S. M. CATALANO;
Cognition Therapeut. Inc., Pittsburgh, PA

Abstract: CT1812 is a novel oligomer receptor antagonist that is the only drug candidate demonstrated to displace binding of Abeta oligomers to receptors on brain cells. CT1812 has high affinity and selectivity for sigma-2/PGRMC1 receptors (Ki=8.5nM, >100-fold separation from other receptors) and acts as an antagonist at this receptor. By stopping the initiating event in the Abeta oligomer cascade, this first-in-class drug candidate completely blocks downstream synaptotoxicity and restores memory to normal in aged transgenic mouse models of Alzheimer’s disease (AD) (Izzo et al., 2014a, b). CT1812 also dose-dependently displaces oligomers from neurons in vitro and from human Alzheimer’s patient brain tissue sections, suggesting that this drug will be effective at removing bound oligomers from Alzheimer’s patient’s brain. In two separate studies, CT1812 improved cognitive deficits in Y maze and Morris water maze

following 9-10 weeks of once daily administration in transgenic Thy1-hAPPLond/Swe+ male mice without affecting wt mouse behavior or causing adverse behavioral or histopathological events in any animal. Despite prior clinical experience with sigma-2 ligands, biomarkers of sigma-2/PGRMC1 antagonist functional target engagement have not been reported in the literature. In the presence of Abeta oligomers, GLP1-R receptor expression increases in neurons in vitro by 20%; close analogs of CT1812 restore expression to normal levels in the presence of oligomers. GLP-1R expression changes may thus constitute a potential clinical target engagement biomarker for sigma-2/PGRMC1 antagonists. In phase I clinical trials CT1812 was well-tolerated for 14 days of once daily administration at doses up to 840 mg in healthy young volunteers and 560 mg in healthy elderly volunteers (corresponding to >20x therapeutic dose). Plasma exposures in human subjects were dose-proportional across two orders of magnitude with no evidence of accumulation over 14 days. CSF analysis indicated that brain concentrations exceeded the minimum target expected to improve memory (>80% receptor occupancy). Further development will test the ability of CT1812 to improve cognitive ability in AD patients making CT1812 one of the first disease-modifying therapeutics to test the oligomer hypothesis of AD.

Disclosures: **N.J. Izzo:** A. Employment/Salary (full or part-time): Cognition Therapeutics, Inc. 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; 1R41AG052249-01. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognition Therapeutics, Inc. **K. Mozzoni:** A. Employment/Salary (full or part-time): Cognition Therapeutics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognition Therapeutics, Inc. **C. Silky:** A. Employment/Salary (full or part-time): Cognition Therapeutics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognition Therapeutics, Inc. **C. Rehak:** A. Employment/Salary (full or part-time): Cognition Therapeutics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognition Therapeutics, Inc. **R. Yurko:** A. Employment/Salary (full or part-time): Cognition Therapeutics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognition Therapeutics, Inc. **G. Look:** A. Employment/Salary (full or part-time): Cognition Therapeutics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognition Therapeutics, Inc. **G. Rishton:** A. Employment/Salary (full or part-time): Cognition Therapeutics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognition Therapeutics, Inc. **M. Grundman:** F. Consulting Fees (e.g., advisory boards); Cognition Therapeutics Inc. **H. Safferstein:** A. Employment/Salary (full or part-time): Cognition Therapeutics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognition Therapeutics, Inc. **S.M. Catalano:** A. Employment/Salary (full or part-time): Cognition Therapeutics, Inc. 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; U01AG047059. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognition Therapeutics, Inc.

Nanosymposium

765. Sigma-2/PGRMC1 Receptor Function in Disease and Therapeutics

Location: SDCC 23A

Time: Wednesday, November 16, 2016, 1:00 PM - 3:30 PM

Presentation Number: 765.02

Topic: B.04. Ion Channels

Title: Inhibition of Sig2R/PGRMC : A potential strategy for treating Alzheimer's disease

Authors: *B. YI[1], J. J. SAHN[2], P. MEMAR ARDESTANI[1], A. K. EVANS[1], L. SCOTT[3], J. Z. CHAN[2], J. PIERCE-SHIMOMURA[3], S. F. MARTIN[2], M. SHAMLOO[1];
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Abstract: Alzheimer's disease (AD) is a major health concern with grave consequences for both individuals and public health. Yet, AD still remains incurable without any medication that can prevent, delay, or cure it. The clear unmet medical need along with the huge health and social burden related to the disease call for the identification of a novel molecular target for the treatment of AD. Accumulating evidence suggests that Sig2R/PGRMC1 is involved in cellular processes relevant to disorders of the central nervous system, and that Sig2R/PGRMC1 can be modulated to provide therapeutic effects in a number of brain diseases. Herein, we report that Sig2R/PGRMC1 is involved in biological pathways associated with Alzheimer's disease (AD). We demonstrate that Sig2R/PGRMC1 modulates intracellular calcium concentrations, and the selective Sig2R/PGRMC1 antagonist SAS-0132 attenuates Sig2R/PGRMC1 agonist-induced increases in intracellular calcium. We also show that inhibition of Sig2R/PGRMC1 with the Sig2R/PGRMC1 antagonist SAS-0132 or genetic manipulation of Sig2R/PGRMC1 via knockout and RNAi knockdown experiments leads to neuroprotective effects in *C. elegans* model of neurodegeneration. Finally, we demonstrate that the Sig2R/PGRMC1 antagonist SAS-0132 rescues behavioral deficits and improves performance in learning and memory tests in the Thy-1 $hAPP^{Lond/Swe+}$ transgenic mouse model of AD as well as in wild-type mice. These data present a compelling case that Sig2R/PGRMC1 is a promising target for developing therapeutic agents to treat AD through a new mechanistic pathway. **Key words** : Sig2R/PGRMC1, Alzheimer's disease, Thy1-$hAPP_{Lond/Swe+}$ transgenic mouse model

Disclosures: **B. Yi:** None. **J.J. Sahn:** None. **P. Memar Ardestani:** None. **A.K. Evans:** None. **L. Scott:** None. **J.Z. Chan:** None. **J. Pierce-Shimomura:** None. **S.F. Martin:** None. **M. Shamloo:** F. Consulting Fees (e.g., advisory boards); Cognition Therapeutic.

Nanosymposium

765. Sigma-2/PGRMC1 Receptor Function in Disease and Therapeutics

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Presentation Number: 765.03

Topic: B.04. Ion Channels

Title: Shedding light on the controversial identity of the sigma-2 receptor with PGRMC1

Authors: *M. L. PATI, C. ABATE, M. NISO, F. BERARDI, N. A. COLABUFO;
Dept. di Farmacia-Scienze del Farmaco, Univ. Degli Studi Di Bari, Bari, Italy

Abstract: After four decades from their discovery, the enigma about sigma receptors is still unsolved. Despite the emerging controversies about the identification of the sigma-2 protein with the progesterone receptor membrane component 1 (PGRMC1), several literatures still refer to the PGRMC1/sigma-2 protein, and a misidentification of these proteins would seriously hamper their future therapeutic or diagnostic exploitation. A recent study highlighted the AD disease-modifying potentials of PGRMC1/sigma-2 proteins, demonstrating that a few sigma-2 receptor antagonists displace Abeta oligomers (which behave as 'regular' ligands at the PGRMC1/sigma-2 protein) *in vitro*, and reverse cognitive deficits in AD mice models *in vivo*. At the same time, both PGRMC1 and sigma-2 proteins represent promising targets for the therapy and diagnosis of tumors, because of their overexpression and proliferative effects in a wide variety of cancers. Therefore, it goes without saying that a clear identification of these proteins is mandatory. Our previous contribution towards this direction showed how the expression of sigma-2 receptor is independent of PGRMC1, and that the sigma-2 mediated activity is independent of the presence and amount of PGRMC1. Herein, we give further support to these findings by flow-cytometry studies in three cell lines (human breast adencarcinoma MCF7 cells) where sigma-2 receptors are constitutively present, and PGRMC1 is constitutively present or alternatively silenced or overexpressed. Three sigma-2 fluorescent tracers with different structures were used in the three cell lines, and were dose-dependently displaced by diverse sigma-2 ligands but not by AG205 - the putative PGRMC1 inhibitor. Additionally, localization experiments in the three cell lines were conducted by confocal microscopy in order to define the intracellular localization of both sigma-2 and PGRMC1 and their eventual co-localization. We feel that these results together with the very recent crystallographic characterization of the PGRMC1 and the delineation of its mechanism of action will be helpful for the clarification of the controversial relationship between

sigma-2 and PGRMC1, prompting future unbiased research for the development of AD-modifying agents. Within this context, sigma-2 antagonists with metal (copper or iron) chelating moiety may find place. These compounds, would preclude the Abeta oligomer binding to the neuronal sigma-2 receptor, while removing from the media copper and iron, whose impaired homeostasis is associated with AD. These combined actions would reinforce the AD-modifying properties of the sigma-2 antagonist.

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Nanosymposium

765. Sigma-2/PGRMC1 Receptor Function in Disease and Therapeutics

Location: SDCC 23A

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Presentation Number: 765.04

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Title: On the identity of sigma-2 binding sites and PGRMC1: evidence in neurons relating to Alzheimer's disease

Authors: K. MOZZONI, N. J. IZZO, R. YURKO, C. SILKY, C. REHAK, G. LOOK, G. RISHTON, H. SAFFERSTEIN, *S. M. CATALANO;
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Abstract: The publication by Xu, et al (2011) that the sigma-2 binding site is related to the PGRMC1 protein has ignited controversy in the field based on the persistence of DTG binding when the PGRMC1 receptor is knocked down in some cell types (Abate et al., 2015, Chu et al. 2015). Because there is little known about other family members of PGRMC1 (PGRMC2, neudesin and neuferricin) and where they are expressed, there remains the possibility that such studies may be dependent on cell and tissue type being studied. In neurons in the context of Alzheimer's disease biology (Abeta oligomer binding and synaptotoxicity), there is strong evidence that sigma-2 and PGRMC1 are closely associated functionally, if not identical. BINDING OF ABETA OLIGOMERS: Binding of oligomers to cultured neurons correlates

positively with the expression level of PGRMC1 protein in those neurons. Abeta binding to neurons can be decreased by knocking down the expression of PGRMC1 protein with siRNA and by using an antibody to PGRMC1 to compete for binding. Sigma-2 antagonists developed by Cognition Therapeutics (CogRx) compete for binding with [3H]-DTG and also displace binding of Abeta oligomers to neurons in a concentration dependent manner. The same antibody and compounds that displace Abeta oligomers in vitro also displace the endogenous Abeta oligomers from fresh frozen slices of post-mortem human AD donor brains (Izzo, et al 2014a,b).
UPREGULATION OF PGRMC1 AND SIGMA-2 BINDING SITES: In cultured neurons, treatment with Abeta oligomers progressively upregulates immunolabeled PGRMC1 by 13% ($p<0.05$) after 24 hours of exposure and by 28% ($p<0.05$) after 48 hours (Izzo 2014b). Cultured rat neurons (DIV21) were treated with Abeta oligomers, fixed with 4% paraformaldehyde and labeled with 125 nM SW120, a fluorescent agonist of the sigma-2 receptor that co-localizes with PGRMC1 in neurons (Zeng et al. 2015). Treatment with 0.5 uM Abeta oligomers for 24 hours resulted in an upregulation of SW120 binding by 16% ($p<0.05$), closely matching previous data for the upregulation of the PGRMC1 protein by Abeta oligomers. When a CogRx sigma-2 antagonist that displaces Abeta oligomers was co-incubated with SW120 on fixed neuronal cultures, SW120 labeling was decreased, demonstrating binding of the two compounds to the same site.
These studies provide evidence that the sigma-2 binding site and the PGRMC1 protein are functionally related in neurons in the context of Alzheimer's disease biology, and suggest they are closely associated with - if not identical to - each other.

Disclosures: **K. Mozzoni:** A. Employment/Salary (full or part-time): Cognition Therapeutics Inc. **N.J. Izzo:** A. Employment/Salary (full or part-time): Cognition Therapeutics Inc. **R. Yurko:** A. Employment/Salary (full or part-time): Cognition Therapeutics Inc. **C. Silky:** A. Employment/Salary (full or part-time): Cognition Therapeutics Inc. **C. Rehak:** A. Employment/Salary (full or part-time): Cognition Therapeutics Inc. **G. Look:** A. Employment/Salary (full or part-time): Cognition Therapeutics Inc. **G. Rishton:** A. Employment/Salary (full or part-time): Cognition Therapeutics Inc. **H. Safferstein:** A. Employment/Salary (full or part-time): Cognition Therapeutics Inc. **S.M. Catalano:** A. Employment/Salary (full or part-time): Cognition Therapeutics Inc..

Nanosymposium

765. Sigma-2/PGRMC1 Receptor Function in Disease and Therapeutics

Location: SDCC 23A

Time: Wednesday, November 16, 2016, 1:00 PM - 3:30 PM

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Topic: B.04. Ion Channels

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Upjohn Professorship in Pharmacology, Brown University

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Title: Divergent cytotoxic and metabolically stimulative functions of sigma-2 receptors: Structure-activity relationships in a series of SN79 analogs

Authors: H. NICHOLSON[1], W. ALSHARIF[2], C. R. MCCURDY[2], *W. D. BOWEN[1]; [1]Molec Pharmacol, Physiol, & Biotechnol, Brown Univ., Providence, RI; [2]Sch. of Pharm., Univ. of Mississippi, University, MS

Abstract: Sigma-2 receptors have been implicated in a number of disorders including cancer and Alzheimer's disease. A variety of structurally distinct ligands that can induce cell death in diverse cell types have been described, linking sigma-2 receptors to apoptotic pathways. Recently, we reported a novel metabolically stimulative function induced by sigma-2 receptor ligands (Nicholson et al., *JPET,* 2016). These ligands are able to stimulate hallmarks of glycolysis including rise in ATP level and increased HIF1α and VEGF levels, effects more consistent with a pro-survival function that could benefit cells through upregulation of the receptor. Interestingly, both cytotoxic and metabolically stimulative effects were observed with compounds related to the canonical sigma-2 antagonist SN79. Here, we investigate a series of SN79 analogs in order to assess the structural determinants governing these divergent effects. Substitutions on the heterocyclic ring of the core structure of SN79 resulted in high-affinity sigma-2 receptor ligands (Ki = 0.56 - 17.9 nM), with replacement of the heterocyclic oxygen with -NMe decreasing sigma-1 receptor affinity and a sulfur substitution at this position imparting high affinity at both subtypes and thus lowering subtype selectivity. Substitution of the methyl ketone moiety of these SN79 analogs with an isothiocyanate group resulted in ligands that irreversibly bound to the sigma-2 receptor and that also induced dose-dependent cytotoxicity in SK-N-SH neuroblastoma cells. The cytotoxic function of these ligands was irreversible, as comparable cytotoxicity was achieved after continuous 24-hour exposure to ligand or after 60 minute acute exposure followed by 24-hour recovery period without free ligand present. Non-sigma-2 receptor-targeting phenyl isothiocyanate had markedly less effect on cell viability, supporting a sigma-2 receptor-mediated effect. Cytotoxicity was observed to a much lesser extent with $-COCH_3$, $-NO_2$, $-NH_2$, or -F substitutions in this position, regardless of heterocycle type. These ligands tended to show the metabolic stimulative effect. All stimulative ligands demonstrated maximal increase in MTT reduction after 24 h incubation at 30 μM. Since the isothiocyanates were more cytotoxic, it appears that irreversible binding may play a role in the apoptotic mechanism with this structural series. These data further support a dual role for sigma-2 receptor activation, inducing both pro-survival and apoptotic pathways, and suggest that subtle changes in ligand structure can determine direction of the effect. These compounds will serve as tools for further study of this phenomenon.

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Nanosymposium

765. Sigma-2/PGRMC1 Receptor Function in Disease and Therapeutics

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Title: Targeting Sig2R/PGRMC1 for treating neurological disorders

Authors: J. J. SAHN[1], T. R. HODGES[1], M. D. WOOD[1], L. L. SCOTT[2], R. C. YEN[2], J. PIERCE-SHIMOMURA[2], A. A. PIEPER[3], T. C. YIN[3], T. PRICE[4], G. MEJIA[4], A. FERRAGUD[5], V. SABINO[5], P. M. ARDESTANI[6], M. SHAMLOO[7], *S. MARTIN[1]; [1]Chem., [2]Neurosci., Univ. of Texas at Austin, Austin, TX; [3]The Univ. of Iowa, Iowa City, IA; [4]The Univ. of Texas, Dallas, TX; [5]Boston Univ. Sch. of Med., Boston, MA; [6]Stanford Behavioral and Functional Neurosci. Lab., [7]Neurosurg., Stanford Univ., Stanford, CA

Abstract: Collectively, diseases and disorders of the central nervous system (CNS) are arguably among the most serious health problems facing modern society. Unfortunately, effective therapeutic approaches for many of these debilitating conditions are lacking, and new strategies are needed. For example, Alzheimer's disease (AD), traumatic brain injury (TBI), neuropathic pain, and alcohol addiction affect a substantial population worldwide, and available treatment options for these conditions are largely inadequate. In the context of developing new approaches to treat AD, we recently discovered that small molecule modulators of the membrane protein sigma 2 receptor/progesterone receptor membrane component 1 (Sig2R/PGRMC1) are promising lead compounds. We have extended those studies, and in a mouse model of TBI we found that animals treated post injury with Sig2R/PGRMC1 ligands exhibited cognitive performance similar to control animals. In another set of experiments, the spared nerve injury assay was used to assess the effects of Sig2R/PGRMC1 binding ligands on neuropathic pain in mice, leading to the identification of several novel compounds having an unprecedented onset of action and duration of analgesia. In a third investigation, ethanol-dependent rats were treated with a Sig2R/PGRMC1 binding ligand that was selected based on its efficacy in a *C. elegans*

model of alcohol withdrawal. Upon treatment with JVW-1034, alcohol consumption was reduced in a dose-dependent manner, whereas no changes in water consumption were observed in either the ethanol-dependent or non-dependent control group. Collectively, these exciting data suggest that Sig2R/PGRMC1 plays a critical role in a variety of neuropathological processes and that pharmacological intervention with compounds that bind to Sig2R/PGRMC1 represents a new paradigm for treating CNS disorders.

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Nanosymposium

765. Sigma-2/PGRMC1 Receptor Function in Disease and Therapeutics

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Topic: B.04. Ion Channels

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NIH T32 AG020494

Title: The role of mirna in regulating neuroprotective function of progesterone in ischemic brain

Authors: ***T. NGUYEN**, C. SU, M. SINGH;
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Abstract: Stroke is the fourth leading cause of death in the United States and one of the leading causes of adult disability. Although pre-menopausal women seem to be protected against stroke compared to men at comparable ages, postmenopausal women appear to be at greater risk of ischemic stroke and are likely to experience poorer outcomes. A considerable amount of research has supported that progesterone (P4) is a potent neuroprotectant and a promising therapeutic for stroke treatment. However, the underlying mechanism(s) remain unclear. Our laboratory has recently reported that Brain-derived neurotrophic factor (BDNF) is a critical mediator for P4's protective actions, and that P4 enhances BDNF release from glia, by acting via a novel membrane-associated progesterone receptor, Pgrmc1. Here, we investigated the role of let-7 microRNA (miRNA) as a potential negative regulator of Pgrmc1 and BDNF in glia, based on the existence of let7 binding sites on the transcripts of both Pgrmc1 and BDNF. Our data showed an

inverse correlation between the expression levels of this miRNA and the transcripts of Pgrmc1 and BDNF in post-ischemic mouse cerebral cortex. In addition, overexpression of this miRNA in primary astrocytes resulted in significant decreases in Pgrmc1 and BDNF at the mRNA and protein levels. These changes in Pgrmc1 and BDNF are consistent with our working model that in the ischemic brain, Let-7 miRNA negatively regulates Pgrmc1 and BDNF expressions, which disrupts P4-induced BDNF release from glia and ultimately leads to the attenuation of P4's beneficial effects. Strategies to inhibit the effect of Let-7 miRNA may therefore prove to be a useful strategy in mitigating the structural and functional deficits associated with stroke.

Disclosures: **T. Nguyen:** None. **C. Su:** None. **M. Singh:** None.

Nanosymposium

765. Sigma-2/PGRMC1 Receptor Function in Disease and Therapeutics

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Topic: B.04. Ion Channels

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Cure Alzheimer's Fund

Title: Reduction of sigma-2 receptor density in sleep deprived mice

Authors: ***J. XU**[1], M. LIM[3], D. HOLTZMAN[2], R. MACH[4];
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Abstract: *N*-[4-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1H)-*yl*)butyl]-2-methoxy-5-methyl-benzamide (**RHM-1**), a conformationally-flexible benzamide analogue, has been shown to have high affinity and selectivity for sigma-2 receptor versus sigma-1 receptor and the dopamine D_2 and D_3 receptors (*Bioorg. Med. Chem. Lett.* **2004**: 14; 195-202, *Eur. J. Pharmacol*. **2005**; 525: 8-17). Its high sigma-2 receptor affinity (K_i <10 nM) and selectivity (sigma-1: sigma-2 ratio > 300) indicates that it may be a useful radioligand to assess the density of sigma-2 receptors in the brain. Therefore, **RHM-1** was radiolabeled with tritium (specific activity = 80 Ci/mmol) and the binding of [^{3}H]**RHM-1** to sigma-2 receptors was assessed in the mouse brain in vitro. Because of the role of sigma-2 in cell proliferation, and the importance of stress and sleep in these cellular processes, we examined sigma-2 receptor density in mice that underwent 72 hours of total sleep deprivation compared to control mice. Compared to age-matched controls (n=4), sleep-deprived

mice (n=5) showed a statistically significant 23-24% decrease in sigma-2 receptor binding (116 +/- 12 vs. 151 +/- 12 fmol/mg tissue +/- SEM, $p<0.05$, t-test) in the piriform cortex, (110 +/- 9 vs. 145 +/- 13 fmol/mg tissue +/- SEM, $p<0.05$, t-test) in the auditory cortex, and (122 +/- 10 vs. 159 +/- 14 fmol/mg tissue +/- SEM, $p<0.05$, t-test) in the motor cortex. Sigma-2 receptor binding was not significantly different in mice subjected to 2 hours of acute restraint stress daily for 1 week compared to stress-free controls, suggesting that these changes are specific to sleep deprivation and not merely a byproduct of stress. Taken together, our data show that acute sleep deprivation differentially affects sigma-2 receptor density in the cortex. Further investigation of sigma-2 receptor and its regulation in the central nervous system may provide useful information on the role of this receptor in the cognitive and memory impairment related to sleep deprivation.

Disclosures: **J. Xu:** None. **M. Lim:** None. **D. Holtzman:** None. **R. Mach:** None.

Nanosymposium

765. Sigma-2/PGRMC1 Receptor Function in Disease and Therapeutics

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Topic: B.04. Ion Channels

Title: The sigma-2 receptor has different but overlapping binding sites for small molecules as measured by radioligand binding studies

Authors: ***R. H. MACH**, B. P. LIEBERMAN;
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Abstract: The sigma-2 receptor has been primarily characterized by in vitro binding studies using [3H]DTG as the radioligand. However, the high affinity of [3H]DTG for sigma-1 receptors and its modest affinity for sigma-2 sites (~20 nM) limits its utility for in vitro binding studies. [125I]RHM-4 was developed by our group as a high affinity sigma-2 receptor (Kd = 0.68 nM) ligand with low affinity for the sigma-1 receptor (>500 nM). The goal of the current study was to compare a panel of sigma-2 receptor ligands for their ability to displace [3H]DTG and [125I]RHM-4 binding from sigma-2 receptors. In both assays, unlabeled DTG was used to define nonspecific binding of the radioligand. The results of the in vitro binding studies indicate that sigma-2 receptor ligands fall into three different categories: 1) compounds having a similar affinity for [3H]DTG and [125I]RHM-4 (e.g., DTG, haloperidol, SM-21, SV-119, rimcazole); 2) compounds having ~10-50-fold higher affinity for [125I]RHM-4 versus [3H]DTG (e.g., SW-43, ISO-1, ifenprodil, RHM-4); and 3) compounds having a 100-8,000-fold higher affinity for [125I]RHM-4 versus [3H]DTG (PB-28, PX-II-116, PX-II-120). These data suggest that there are two different but overlapping binding sites for small molecules on the sigma-2 receptor: 1) a

binding site having complete overlap for the binding of both [3H]DTG and [125I]RHM-4; and 2) a secondary binding site having a high affinity for [125I]RHM-4 (and other sigma-2 receptor ligands) and a low affinity for [3H]DTG.

Disclosures: **R.H. Mach:** F. Consulting Fees (e.g., advisory boards); Cognition Therapeutics, Inc.. **B.P. Lieberman:** None.

Nanosymposium

765. Sigma-2/PGRMC1 Receptor Function in Disease and Therapeutics

Location: SDCC 23A

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Presentation Number: 765.10

Topic: B.04. Ion Channels

Support: JST ERATO

Title: PGRMC1/sigma-2 receptor: A novel carbon monoxide sensor to regulate cell function

Authors: ***M. SUEMATSU**, Y. KABE;
Biochem., Keio Univ. Sch. of Med., Tokyo, Japan

Abstract: Like liver, brain constitutes a major organ generating large amounts of carbon monoxide (CO), while its physiologic roles remained unknown. In the presence of molecular oxygen (O_2), CO is generated by heme oxygenase-2 in neurons and binds to cystathionine beta-synthase (CBS) that is expressed in astrocytes to suppress H_2S. Such gas-responsive relay systems result in regulation of neurovascular units to trigger hypoxic vasodilation in cortical microcirculation[1]. We challenged to explore novel unidentified CO receptors by using chemical biological approach with high-performance affinity nanobeads that were conjugated with heme, and successfully mined a novel CO-responsive protein progesterone receptor membrane component-1 (PGRMC1/Sigma-2 receptor). PGRMC1 is a heme-containing protein that interacts with epidermal growth factor receptor (EGFR) and cytochromes P450 to regulate cell functions. Crystallographic analyses of the PGRMC1 cytosolic domain at 1.95 Å resolution show that, in the presence of heme, it forms a stable dimer through stacking interactions of two protruding haem molecules[2]. The heme iron is five-coordinated by Tyr113, and the open surface of the heme mediates dimerization. Physiologically relevant concentrations of CO interfere with PGRMC1 dimerization by binding to the sixth coordination site of the heme. Heme-mediated PGRMC1 dimerization is required for interactions with EGFR and cytochromes P450, cancer proliferation and chemoresistance; these events are attenuated by either CO or heme deprivation in cells. Protein dimerization via heme-heme stacking has not been seen in eukaryotes, and provides insights into its functional significance in brain, where both PGRMC1 and heme

oxygenase-2 are abundantly expressed. Considering a broard spectrum of PGRMC1 expression in the body, the heme-stacking structure of PGRMC1 dimer shed light on putative binding sites of many small compounds and thus serves as a target for controlling diseases. **References** 1. Morikawa T, et al. Proc Natl Acad Sci USA 109(4):1293-8 (2012) 2. Kabe Y, et al. Nature Communications. 7:11030 doi: 10.1038/ncomms11030 (2016).

Disclosures: **M. Suematsu:** None. **Y. Kabe:** None.

Nanosymposium

766. Amyloid-Beta Toxicity

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Reduced amyloid-beta clearance in the hippocampus of Abca7 knockout mouse

Authors: ***W. S. KIM**[1,2], Y. FU[1,2], J.-H. HSIAO[1], G. PAXINOS[1], G. M. HALLIDAY[1]; [1]Neurosci. Res. Australia, Randwick, Australia; [2]Univ. of New South Wales, Sydney, Australia

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by dementia and abnormal deposits of aggregated amyloid-beta in the brain. Recent genome-wide association and sequencing studies revealed that ABCA7 is strongly associated with AD. Deletion of ABCA7 in a mouse model of AD exacerbates cerebral amyloid-beta plaque load. ABCA7 has also been implicated in the role of phagocytosis. However, the mechanism by which ABCA7 reduces amyloid-beta load in the brain is unknown. In this study we investigated whether ABCA7 mediates the clearance of amyloid-beta in the mouse brain. We performed intracranial injection of a dose of 200 pmol of amyloid-beta oligomers into the right side of the hippocampus of Abca7$^{-/-}$ and wild type mice. After six days, the mice were euthanized and brain sections prepared for immunohistochemical analysis. Iba1-positive microglia were present throughout the hippocampus with increased numbers in the vicinity of amyloid-beta injection site for both groups of mice. We analyzed the injected (right) side of the hippocampus as well as the un-injected (left) side, as a control, and found that activated microglia were increased in the injected side. We quantified the intensity of microglial signal and it was significantly reduced in the Abca7$^{-/-}$ hippocampus. Consistent with this result, the immunochemical signal to amyloid-beta was increased in the Abca7$^{-/-}$ hippocampus. Furthermore, the specific level of microglia relative

to amyloid-beta was reduced by ~50% in the Abca7$^{-/-}$ hippocampus. These results strongly indicate that ABCA7 plays a significant role in phagocytic clearance of amyloid-beta in the brain, and reveal a mechanism by which loss of function of ABCA7 increases the susceptibility to AD.

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Nanosymposium

766. Amyloid-Beta Toxicity

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Cure Alzheimer's Fund

The Helmsley Charitable Trust

Title: Antiviral activities of amyloid-ß (Aß) against the AD-linked virus HSV1

Authors: *W. EIMER, D. VIJAYA KUMAR, K. J. WASHICOSKY, X. O. BREAKEFIELD, D. Y. KIM, R. E. TANZI, R. D. MOIR;
Genet. and Aging Res. Unit, Massachusetts Gen. Hosp., Charlestown, MA

Abstract: INTRODUCTION; Alzheimer’s disease (AD) is a complex disease with unclear etiologies. Available data suggest genetic and environmental factors may play a role in Late-Onset AD (LOAD). Genetic studies have identified APOEε4 as a major risk factor for LOAD. Neuropathic pathogens have also emerged as likely involved in promoting or initiating LOAD pathology. Herpes simplex virus type 1 (HSV-1) has the strongest links to LOAD. Mounting evidence suggests HSV-1 may play a role in the increased risk of LOAD associated with inheritance of APOEε4. While strong epidemiological and histological data link HSV-1 infection to LOAD it has been unclear how the virus may promote AD pathology. We recently reported that the protein thought to drive AD pathology, the amyloid-β protein (Aβ), is an antimicrobial peptide (AMP). Aβ expression protects against bacterial and fungal infections in transgenic mice, *C. elegans*, and *Drosophila*, doubling host survival in some cases. Aβ fibrillization mediates the peptides protective antimicrobial activities. Our findings show soluble Aβ oligomers first bind microbial cell surfaces. Binding promotes Aβ fibrillization, leading to rapid fibril propagation from the microbe surface. Growing fibrils ensnare, agglutinate, and finally sequester cells in β-

amyloid/microbe aggregates. Here we present data consistent with a parallel model for Aβ-mediated HSV-1 inhibition. In this model, viral particles seed Aβ fibrillization, leading to HSV-1 sequestration in βamyloid deposits.
METHODS; Survival with high and low Aβ expression was characterized in genetically modified mice following HSV-1 infection. Pathways mediating anti-HSV-1 pathways were characterized using monolayer and 3-D cell culture models.
RESULTS; High Aβ expressing 5XFAD mice show increased survival compared to wild-type littermates following intracranial challenge with HSV-1. HSV-1 co-localize with extensive β-amyloid deposition in infected 5XFAD brain. In binding assays Aβ was found to target viral envelope surface glycoproteins. Protection from HSV-1 in cell culture is mediated by agglutination of viral particles and sequestration in fibril/virus aggregates.
CONCLUSION; Data show the protective AMP activities of Aβ extend to viruses as well as bacterial and fungal pathogens. Findings suggest Aβ fibrillization is involved in the normal protective anti-viral response of the innate immune system in brain. Our model of β-amyloid mediated anti-viral activity identifies, for the first time, a possible mechanism for direct induction of amyloidosis during HSV-1 brain infections.

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Nanosymposium

766. Amyloid-Beta Toxicity

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Antimicrobial and endotoxin neutralization activities of amyloid-β (Aβ) protein in animal and cell culture models

Authors: *D. VIJAYA KUMAR[1], S. CHOI[1], K. J. WASHICOSKY[1], W. A. EIMER[1], J. GHOFRANI[2], A. LEFKOWITZ[3], G. MCCOLL[4], L. E. GOLDSTEIN[2], A. LI[1], R. E. TANZI[1], R. D. MOIR[1];
[1]Genet. & Aging Res. Unit, Massachusetts Gen. Hosp. & Harvard Med. Sch., Charlestown, MA;

[2]Boston Univ., Boston, MA; [3]Albert einstein college of medicine, New York, NY; [4]The Florey Inst. of Neurosci. and mental health, Univ. of Melbourne, Victoria, Australia

Abstract: INTRODUCTION; We have previously reported *in vitro* evidence that amyloid-β protein (Aβ) is an antimicrobial peptide (AMP). AMPs are key effector proteins of innate immunity. High AMP concentrations disrupt membranes, mediating cytotoxic activities against microbes, and in some instances, host cells. Protective AMP pathways at sub-lethal peptide concentrations involve inhibition of microbe/host adhesion and microbe agglutination. AMP oligomerization mediates microbial agglutination and leads to sequestration of microbes within AMP/microbe aggregates. Amyloidogenic AMPs generate amyloid fibrils that help trap pathogens in agglutinates. AMP oligomerization also mediates neutralization of virulence factors, including lipopolysaccharides (LPS). Anti-adhesion, agglutination, and LPS neutralization activities target microbes and are not pathways that lead to host cell cytotoxicity. Here we present our findings on Aβ-mediated antimicrobial activities in animals and cultured cells. We propose a novel model for the protective *in vivo* activities of Aβ against pathogens and LPS.
METHODS; Mortality of mice, nematodes and Drosophila and cultured cell viability were followed post-infection. Experimental pathogens include the bacterium *Salmonella* Typhimurium and fungus *Candida albicans*. Tissues were analyzed for pathogen infiltration and β-amyloid deposition.
RESULTS; High Aβ expression was associated with protection against bacterial and fungal infections in transgenic mice (5XFAD), *C. elegans* (GMC101), Drosophila (GMR-Aβ42), and transformed cells, doubling host survival in some cases. In addition, APP-KO mice with low Aβ expression show attenuated encephalitis resistance. Aβ agglutinates microbial cells and LPS, leading to rapid seeding of beta-amyloid deposition in our infection models. As with classical AMPs, Aβ fibrillization appears to mediate protective activities, and leads to the entrapment and sequestration of pathogens and LPS.
CONCLUSION; Aβ expression is associated with increased bacterial and fungal resistance in five independent infection models. Aβ antimicrobial activities involve AMP-specific pathways targeting microbes and LPS. Thus, Abeta protection is not mediated by non-specific cytotoxic activities. Our findings show an unsuspected protective/damaging duality for Aβ oligomerization likely to help advance our understanding of amyloidosis in AD.

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Nanosymposium

766. Amyloid-Beta Toxicity

Location: SDCC 24A

Time: Wednesday, November 16, 2016, 1:00 PM - 4:30 PM

Presentation Number: 766.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant 5R01AI081990-02

Cure Alzheimer's Fund

The Helmsley Charitable Trust.

Title: Metal binding enhances the antimicrobial activity of the amyloid-β (Aβ) protein of Alzheimer's disease

Authors: *R. D. MOIR[1], D. K. VIJAYA KUMAR[1], K. J. WASHICOSKY[1], D. Y. KIM[1], M. JÄDICKE[2], W. A. EIMER[1], R. E. TANZI[1];
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Abstract: INTRODUCTION; Metal binding by the amyloid-β protein (Aβ) of Alzheimer's disease (AD) is widely characterized as a pathological pathway. However, we recently confirmed that Aβ is an antimicrobial peptide (AMP) and plays a protective role against infection *in vivo*. Aβ expression protects against bacterial and fungal infections in transgenic mice, *C. elegans*, and *Drosophila*, doubling host survival in some cases. AMPs are key effector proteins of the innate immune system and critically important for normal immunity. Among AMPs, metal binding mediates normal antimicrobial activities. AMPs target the anionic membranes and cell wall carbohydrates found on microbial surfaces. AMP/metal complexes have increased positive charge and show enhanced microbial targeting. AMPs bound to redox active metals also generate oxygen radicals (ROS) that can damage microbial membranes. Aβ is an anionic metallopeptide that specifically binds Zn^{++} and Cu^{++}. Metals modulate the physiochemistry of Aβ, including membrane binding. Aβ/Cu^{++} complexes also generate ROS. Our study investigated if, as with classical AMPs, Zn^{++} and Cu^{++} play a role in the antimicrobial activities of Aβ.
METHODS; Metal-mediated Aβ microbial binding and cytotoxicity were characterized in *in vitro* assays and a 3-D cell culture model of brain. Chelators and antioxidants were tested for modulation of Aβ AMP activities.
RESULTS; Aβ/Zn^{++} complexes show enhanced targeting of bacteria and viruses, with binding to some microbial species increased 40-fold over metal-poor peptide. Aβ/metal complexes also show attenuated host cell binding. Within Aβ/bacteria agglutinates microbial membranes accumulate extensive ROS mediated lipid peroxidation. ROS-mediated dityrosine cross-linking of Aβ within agglutinates increases fibril resistance to degradation from secreted bacterial

proteases.
CONCLUSIONS; Findings are consistent with enhanced antimicrobial activities for Aβ bound to Zn^{++} and Cu^{++}. We propose Aβ/metal complexes play a physiological role in innate immunity. Aβ/Zn^{++} complexes have increased specificity and affinity for anionic microbial surfaces. ROS generated by Aβ/Cu^{++} in β-amyloid/microbe agglutinates damage entrapped microbial cells. In addition, ROS generation impedes escape of sequestered microbes through dityrosine cross-linking of the confining Aβ fibril matrix.

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Nanosymposium

766. Amyloid-Beta Toxicity

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Presentation Number: 766.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Bright Focus

Cure Alzheimers Fund

NIH

Title: Characteristics of amyloid-beta oligomers from human Alzheimer's disease brain

Authors: *D. L. BRODY, T. ESPARZA, N. WILDBUGER, N. CAIRNS;
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Abstract: Background: Previous efforts to identify toxic amyloid-beta (Abeta) species involved in Alzheimer's disease (AD) utilized synthetic peptide preparations, over-expressing cell lines, and/or transgenic animal models. Studies that used human patient-derived material were performed using strongly dissociative conditions resulting in the extraction of both soluble and insoluble Abeta; thus preventing a clear identification of the potential soluble-mediators of toxicity. To our knowledge there has not been a successful isolation and characterization of the soluble Abeta oligomer species from AD post-mortem tissue.Methods: We developed a quantitative biochemical extraction protocol that permits scalable isolation and immunopurification of Abeta oligomer species. Cortical samples are dounce homogenized in PBS containing protease inhibitors and sub critical micelle concentrations of CHAPS detergent. Homogenates are further processed by differential ultracentrifugation which separates Abeta oligomers from monomers. Oligomers are further immuno-purified and subjected to mass

spectrometric characterization. Results: Using 2-3 grams of frontal or parietal cortex from pathologically confirmed CDR3 (severe) AD cases, we consistently isolated 10 ng or more of oligomeric Abeta . By using albumin blocking of every pipet tip and tube, we prevented non-specific loss and recovered >70% of the input oligomeric Abeta. Our method has provided sufficient purification for initial mass spectrometric and immuno-electron microscopic characterization of the soluble Abeta oligomers. Our method does not induce artificial oligomers when AD derived Abeta monomer is spiked into negative-pathology age-matched tissue and purified. Conclusions: We have successfully isolated and initially characterized AD patient-derived Abeta oligomers. This method will allow the future identification of oligomeric Abeta that arises presymptomatically (CDR0 + plaque pathology), and that may drive progression to dementia. Human brain Abeta oligomers may be logical targets for diagnostics, pharmacodynamic biomarkers, and therapeutic interventions.

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Nanosymposium

766. Amyloid-Beta Toxicity

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Time: Wednesday, November 16, 2016, 1:00 PM - 4:30 PM

Presentation Number: 766.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Owens Family Foundation

Title: Amyloid β oligomers inhibit nutrient-induced mitochondrial activity in neurons

Authors: *A. NORAMBUENA, H. WALLRABE, Z. SVINDRYCH, E. SWANSON, G. S. BLOOM;
Univ. of Virginia, Charlottesville, VA

Abstract: Altered mitochondrial behavior is thought to be a key contributor to Alzheimer's disease (AD) pathogenesis, but a comprehensive understanding of the relevant mechanisms remains to be established. In neurons, normally functioning mitochondria allow the proper delivery of nutrient-derived energy in the form of ATP, and provide timely clearance of reactive oxygen species and buffering of calcium. The limited available methods to track mitochondrial activity in live cells or tissues have hampered understanding of the molecular mechanisms that control mitochondria and how they go awry in disease. We have developed a two-photon fluorescence life time imaging assay that detects mitochondrial activity in live cultured cells by monitoring autofluorescence of the coenzyme(s), NAD(P)H, specifically in mitochondria. Increased fluorescence lifetime of NAD(P)H indicates increased engagement of coenzyme with

mitochondrial enzymes, and by extension, elevated mitochondrial activity. In cultures of primary mouse cortical neurons, nutrients (arginine plus leucine; or insulin) were found to induce rapid increases in the activity of perikaryal mitochondria. This effect was blocked by: 1) Torin1 inhibition of mTOR, the catalytic subunit of two multi-protein complexes, mTORC1 and mTORC2, that regulate intracellular behavior by sensing extracellular nutrients; 2) knocking down Raptor, an essential mTORC1 subunit; or 3) forcing mTORC1 to associate with the plasma membrane at the expense of lysosomes. In contrast, nutrient-stimulated mitochondrial activity did not require expression of: 1) Rictor, an essential mTORC2 subunit; 2) eIF4E, which mediates mTORC1-regulated mRNA translation; or 3) tau or α-synuclein, which are required for amyloid-β oligomer (AβO) induced, insulin-sensitive, neuronal cell cycle re-entry, a prelude to neuron death in AD (Seward, et al. J Cell Sci 126: 1278-1276; Norambuena et al, 2016, under revision; and Khan, Bloom, et al., manuscript in preparation). Mitochondrial stimulation by nutrients was found to occur independently of autophagy or fatty acid transport into mitochondria, but was strongly reduced by AβOs, which activate mTORC1 at the plasma mebrane, but not at lysosomes (Norambuena et al, 2016, under revision). The new collective data described here support the novel conclusions that: 1) nutrients induce mTORC1-dependent regulation of mitochondria; 2) mTORC1 coordinates the behavior of lysosomes and mitochondria in response to nutrients; and 3) in AD, AβOs block the normal response of mitochondria to nutrients by perturbing the functional link between mitochondria and lysosomes.

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Nanosymposium

766. Amyloid-Beta Toxicity

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Presentation Number: 766.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association NIRG-12-237751

Human Brain Project Neuroantibodies #604102

Title: Subcellular conformational-selective interference with endogenous Aβ Oligomers in primary fibroblasts and induced pluripotent stem cells from human Alzheimer’s Disease patients

Authors: *G. MELI[1], V. LA MARCA[1], C. SCOPA[1], A. MANCA[1], F. RUGGERI[1], R. SCARDIGLI[1,2], A. CATTANEO[1,3];

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Abstract: While Amyloidβ oligomers (AβOs) are considered the most relevant neurotoxic species in Alzheimer's Disease (AD), they are intrinsically complex and difficult to be traced and intercepted in living cells. This hampers their selective targeting for mechanistic studies and for therapy. To overcome this problem we successfully exploited the intrabody approach, based on the expression of intracellular antibodies (namely recombinant antibody fragments expressed as genes), in order to target toxic AβO conformations in subcellular compartments of living cells. In this way, we established a new experimental paradigm of subcellular-localized conformational-selective interference (CSI) in mammalian fAD cell lines (Meli et al., Nature Comm 2014). Here, we demonstrated the effectiveness of the intrabody-based CSI approach in different AD-relevant primary cells. The intrabodies were expressed by lentiviral vectors and targeted to the Endoplasmic Reticulum (ER) of: 1. primary neuronal stem cells (NSCs) derived from neurogenic niches of the adult brain of AD mouse model Tg2576; 2. primary human fibroblasts and induced pluripotent stem cells (iPSCs) derived from different AD patients. As for Tg2576 NSCs, mantained *in vitro* as neurospheres and differentiated in neurons or astrocytes, we demostrated that alterations in their growth and differentiation, representative of an impaired adult neurogenesis, are rescued by CSI. As for human AD primary fibroblasts, by CSI we were able to rescue mitochondrial dysfunctions and impaired cell homeostasis. Of note, intrabodies were successful expressed also in human iPSCs and derived neuronal precursors, and their actions are currently under investigation. Thus, the ER-localized CSI allows deciphering new pathways of actions of subcellular AβOs in primary cells investigated in this study. On this basis, we are proposing new mechanisms of AD pathogenesis occurring at subcellular levels, starting from the ER and influencing mitochondria and homeostatic pathways. In conclusion, the intrabody-based interference with subcellular AβOs is a targeting approach ideal for mechanistic studies of subcellular AD pathology and for new strategies of Aβ immunotherapy, useful also to identify new potential intracellular therapeutic targets.

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Nanosymposium

766. Amyloid-Beta Toxicity

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Exogenous amyloidogenic proteins function as seeds in Aβ aggregation

Authors: *K. ONO[1,2], R. TAKAHASHI[2], T. IKEDA[2], M. MIZUGUCHI[3], T. HAMAGUCHI[2], M. YAMADA[2];
[1]Showa Univ. Sch. of Med., Tokyo, Japan; [2]Kanazawa Univ. Grad. Sch. of Med. Sci., Kanazawa, Japan; [3]Toyama Univ., Toyama, Japan

Abstract: Background: Amyloid β-protein (Aβ) aggregation is considered to be a critical step in the neurodegeneration of Alzheimer's disease (AD). In addition to Aβ, many proteins aggregate into the amyloid state, in which they form elongated fibers with spines comprising stranded β-sheets. However, the cross-seeding effects of other protein aggregates on Aβ aggregation pathways are not completely clear. Methods: To investigate the cross-seeding effects of exogenous and human non-CNS amyloidogenic proteins on Aβ aggregation pathways, we examined whether and how sonicated fibrils of casein, fibroin, sericin, actin, and islet amyloid polypeptide affected Aβ40 and Aβ42 aggregation pathways using the thioflavin T assay and electron microscopy. Results: Interestingly, the fibrillar seeds of all amyloidogenic proteins functioned as seeds. The cross-seeding effect of actin was stronger but that of fibroin was weaker than that of other proteins. Furthermore, our nuclear magnetic resonance spectroscopic studies identified the binding sites of Aβ with the amyloidogenic proteins. Conclusions: Our results indicate that the amyloidogenic proteins, including those contained in foods and cosmetics, contribute to Aβ aggregation by binding to Aβ, suggesting their possible roles in the propagation of Aβ amyloidosis.

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Nanosymposium

766. Amyloid-Beta Toxicity

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Presentation Number: 766.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: intramural

Title: Evidence that exosome surface-associated amyloid beta peptide is highly neurotoxic

Authors: *E. EITAN[1], D. KAPOGIANNIS[2], M. MATTSON, 21224[2];
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Abstract: Alzheimer's Disease (AD) is an age-related neurodegenerative disorder in which aggregation-prone neurotoxic forms of amyloid β-peptide (Aβ) accumulates in the brain. Extracellular vesicles (EVs) are small 50-150 nanometer membrane vesicles that have recently been implicated in the prion-like spread of self-aggregating proteins, including Aβ. Here we provide evidence that Aβ is present on the surface of EVs, and that EVs with Aβ on their surface are potently neurotoxic. EVs were isolated by sequential centrifugation from the medium of presenilin 1 (PS1) mutant cells, from the plasma of APP/PS1 double mutant transgenic mice and 3xTgAD mice, and from the plasma and cerebrospinal fluid (CSF) of AD patients. The level of EV-associated Aβ was measured using a Mesoscale assay, and their neurotoxicity was evaluated using cultured rat cortical neurons. We found that EVs isolated from AD patient CSF and plasma, from the plasma of two AD mouse models, and from the medium of neural cells expressing familial AD PS1 mutations, destabilize neuronal Ca^{2+} homeostasis, impair mitochondrial function, and sensitize neurons to excitotoxicity. These adverse affects of the EVs were blocked by a specific Aβ antibody (6E10). EVs contain a relatively low amount of Aβ but have an increased Aβ42/Aβ40 ratio and the majority of Aβ appears to be located on the surface of the EVs. EV isolated from PS1 mutant cells contained higher levels of ubiquitinated proteins and cathepsin D, and we found that impairment of lysosome function results in increased generation EVs with elevated Aβ42 levels. Our findings suggest that EVs may mediate transcellular spread of pathogenic Aβ species and that impairs neuronal Ca^{2+} handling and mitochondrial function, and may thereby render neurons vulnerable to excitotoxicity. Supported by the Intramural Research Program of the National Institute on Aging.

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Nanosymposium

766. Amyloid-Beta Toxicity

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Presentation Number: 766.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG023084

Title: Endothelial-specific effects of picalm on amyloid-beta homeostasis.

Authors: *A. RAMANATHAN, Z. ZHAO, A. P. SAGARE, B. V. ZLOKOVIC;
USC, Los Angeles, CA

Abstract: PICALM, a gene encoding phosphatidylinositol-binding clathrin assembly protein, is a highly-validated genetic risk factor for Alzheimer's disease (AD). Although it is known that PICALM facilitates clathrin-mediated endocytosis, and regulates intracellular trafficking of endocytic vesicles, its direct role in AD pathogenesis is still being debated. PICALM is abundantly expressed in brain endothelial cells, a site of the blood-brain barrier (BBB) *in vivo*, which constitutes a major pathway for amyloid-beta clearance from brain into circulation. We have recently identified the role of PICALM in the transcytotic clearance of Abeta across the BBB by regulating LRP1-dependent efflux of Abeta from brain to blood across the BBB. Global *Picalm* deficiency in amyloid precursor protein (APP) overexpressing mice ($APP^{sw/0}$; $Picalm^{+/-}$) caused diminished Abeta clearance across the BBB, accelerated Abeta accumulation and worsened cognitive performance, indicating that PICALM can influence AD pathogenesis. However, models of global *Picalm* deficiency, where PICALM is deleted from multiple cell populations (for example, endothelia, neurons, microglia), are unable to delineate the specific role of each cell type in Abeta homeostasis including endothelial-specific role of PICALM. In order to parse out the function of PICALM in endothelia, and to eliminate contribution of other cell-types expressing PICALM in Abeta clearance, we generated endothelial-specific PICALM deficient mice. Endothelial-specific deficiency of PICALM caused increased cerebral retention of murine Abeta, suggesting its impaired brain-to-blood clearance. In summary, our data preliminarily suggest that endothelial PICALM plays a crucial role in the regulation of global Abeta homeostasis.

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Nanosymposium

766. Amyloid-Beta Toxicity

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Presentation Number: 766.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Medical Research Council/AstraZeneca CASE studentship

Title: The role of clusterin in the amyloid cascade in human neurons

Authors: *J. ROBBINS[1], R. KILLICK[2], E. M. RIBE[3], M. MARESCA[4], M. N. PANGALOS[5], S. LOVESTONE[3], J. PRICE[1];
[1]Dept. of Basic and Clin. Neurosci., [2]Old Age Psychiatry, King's Col. London, London, United Kingdom; [3]Dept. of Psychiatry, Univ. of Oxford, Oxford, United Kingdom; [4]Innovative Medicines, AstraZeneca, Gothenburg, Sweden; [5]Innovative Medicines, AstraZeneca, Cambridge, United Kingdom

Abstract: Background**:** Our understanding of the molecular processes underlying Alzheimer's disease (AD) is still limited, hindering the development of effective treatments and highlighting the need for human-specific models. The potential for human induced pluripotent stem cells (iPSCs) to provide a model for AD is an exciting advancement, and has great potential for investigating the mechanisms involved in neurodegeneration. Advances in identifying components of the amyloid cascade are progressing, including the role of the protein clusterin in mediating β-amyloid (Aβ) toxicity. This project aims to characterise the molecular cascade initiated by Aβ previously identified in rodent neurons. Aβ-mediated toxicity will be studied in cortical neurons differentiated from wildtype iPSCs and a clusterin-knockout cell line generated by precise genome editing.
Methods: Cortical neurons were differentiated for 35 days from iPSCs, obtained from a neurotypical male with an ε3/ε3 APOE genotype and treated with $A\beta_{1-42}$ oligomers. The downstream effects of the Aβ exposure on the cells was measured by using a high-throughput imaging assay, optimised to identify changes in neuronal morphology. Western blotting and qPCR were used to assess changes in protein and gene expression downstream of Aβ. CRISPR cas9-mediated gene editing generated a CLU-/- iPSC line. The morphological and transcriptomic effects of the CLU deletion were then investigated using the imaging assay and transcriptomics in the wildtype and CLU knockout neurons.
Results: In wildtype cells imaging indicated that neuronal processes degenerate following Aβ treatment, in a dose dependent manner. We also observed that intracellular levels of clusterin are increased following Aβ treatment, as occurs in rodent neurons. However, in CLU knockout neurons these morphological effects of Aβ neurotoxicity were absent, suggesting that clusterin is an important component of the amyloid cascade. Transcriptomic data from RNAseq have been obtained and are currently being analysed to identify mechanisms underlying this clusterin-mediated effect.
Conclusions: We have established an iPSC based isogenic model of sporadic AD with iPSC lines of different genotypes. Determining the role of these risk alleles on established measures of Aβ-neurotoxicity using high throughput assays will be of great value to obtaining a better understanding of the underlying disease mechanisms and for the evaluation of compounds able to modulate these pathways.

Disclosures: J. Robbins: A. Employment/Salary (full or part-time): AstraZeneca. **R. Killick:** None. **E.M. Ribe:** None. **M. Maresca:** A. Employment/Salary (full or part-time): AstraZeneca. **M.N. Pangalos:** A. Employment/Salary (full or part-time): AstraZeneca. **S. Lovestone:** None. **J. Price:** None.

Nanosymposium

766. Amyloid-Beta Toxicity

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JPB Foundation

NINDS P01 NS080675

Title: The effects of peripheral and central high insulin on brain insulin signaling and amyloid-beta in young and old PS1/APP mice

Authors: *M. STANLEY, S. L. MACAULEY, E. E. CAESAR, L. J. KOSCAL, W. R. MORITZ, G. O. ROBINSON, J. ROH, H. JIANG, D. M. HOLTZMAN;
Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Hyperinsulinemia is a risk factor for late-onset Alzheimer's disease (AD) and population studies report that individuals with AD tend to have higher blood insulin than healthy controls. In vitro studies suggest that high insulin could be causal to AD by directly increasing extracellular amyloid-beta (Aβ), but high concentrations of Aβ oligomers could be leading to neuronal insulin resistance. Due to the latter, intranasal insulin is being tested as an AD therapeutic to increase insulin signaling in the brain to enhance memory. While in vitro experiments have found potential connections between insulin, insulin signaling, and Aβ, in vivo experiments are needed to validate these relationships under physiological conditions. First, we performed acute hyperinsulinemic-euglycemic clamps with hippocampal microdialysis in young $PS1_{dE9}/APP_{swe}$ mice to increase blood insulin while continuously collecting interstitial fluid (ISF) from the hippocampus in awake, behaving animals. We found that both a postprandial and supraphysiological insulin clamp modestly but significantly increased ISF Aβ (9-10%) compared to controls. Blood insulin has been shown to cross the blood brain barrier by a process that is saturated at physiological levels. We could detect no increase in brain, ISF, or cerebrospinal fluid insulin or brain insulin signaling in response to peripheral hyperinsulinemia, despite detecting increased insulin signaling in the muscle. Next, we acutely delivered insulin directly into the hippocampus of young $PS1_{dE9}/APP_{swe}$ mice via reverse microdialysis, then immediately collected

hippocampal tissue around to probe to measure tissue insulin and insulin signaling. Tissue insulin was increased dose-dependently, yet insulin signaling was only significantly increased at a high dose. ISF Aβ was unchanged by CNS insulin administration. Results in young mice demonstrate that peripherally high insulin can modestly increase ISF Aβ, likely through an indirect mechanism. Finally, to determine if peripheral and central high insulin has differential effects in the presence of significant amyloid pathology, we repeated these experiments in aged $PS1_{dE9}/APP_{swe}$ mice. Postprandial insulin clamps had no effect on ISF Aβ and direct delivery of insulin significantly increased tissue insulin and insulin signaling, but had no effect on Aβ in old mice. Results suggest that in the presence of Aβ pathology, the brain is still responsive to insulin but increased insulin signaling does not acutely elevate Aβ in vivo. These results have implications for understanding the role of insulin in the brain and hyperinsulinemia as a risk factor for AD.

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Nanosymposium

766. Amyloid-Beta Toxicity

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: IL17A modulates brain amyloid beta and cerebral amyloid angiopathy by upregulating endothelial ABCA1 via ERK activation

Authors: *J. YANG[1], J. KOU[1], R. LALONDE[2], K.-I. FUKUCHI[1];
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Abstract: Neuroinflammation is a characteristic of Alzheimer’s disease (AD) and is caused by activation of microglia and/or infiltrating immune cells. T helper 17 (Th17) cells and their signature cytokine, interleukin-17A (IL-17A), play important roles in inflammatory diseases such as multiple sclerosis. Recent studies indicate that activities of Th17 cells and IL-17A increase in patients with AD. However, the role of IL-17A in AD pathogenesis remains to be

elucidated. In the current study, we overexpressed IL-17A in the brains of TgAPP/PS1 mice, an AD mouse model, via recombinant adeno-associated virus serotype 5 (rAAV5)-mediated gene delivery. IL-17A overexpression improved glucose metabolism and reduced Aβ levels in the cerebrospinal fluid, hippocampus and cerebral blood vessels (cerebral amyloid angiopathy) without exacerbating neuroinflammation and cognitive deficits in TgAPP/PS1 mice. In line with in vivo study, endothelial cell culture study suggests that IL-17A may decrease Aβ in the brain by upregulation of ATP-binding cassette transporter A1 (ABCA1) through the ERK signaling pathway.

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Nanosymposium

766. Amyloid-Beta Toxicity

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Cure Alzheimer's Fund

Title: Alpha-synuclein enhances amyloid-beta-induced ectopic cell cycle re-entry in primary cortical neurons and hAPPJ20 mice.

Authors: ***S. S. KHAN**[1], M. LACROIX[3], S. E. LESNÉ[3], G. S. BLOOM[2];
[2]Biol., [1]Univ. of Virginia, Charlottesville, VA; [3]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: The accumulation and aggregation of α-synuclein into Lewy body inclusions are hallmarks of several neurodegenerative disorders, most notably Parkinson's disease. Recent work has implicated α-synuclein in the etiology of Alzheimer's disease (AD). For instance, increased levels of soluble α-synuclein strongly correlate with AD symptom severity. Furthermore, in mice, simultaneously overexpressed human α-synuclein, APP, and tau mutants synergistically promote neurodegeneration. However, whether endogenous α-synuclein is required for AD pathogenesis is unknown. Specifically, the effect of α-synuclein on ectopic neuronal cell-cycle re-entry (CCR), whereby normally post-mitotic neurons re-enter, but cannot

complete the cell cycle and subsequently die, has not been tested. Hence, we reduced α-synuclein expression in primary mouse cortical neurons by lentiviral delivery of shRNA before neurotoxic amyloid-β oligomer (AβO) treatment. We report that α-synuclein depletion attenuates AβO-induced CCR in primary cortical neurons. α-Synuclein knockdown also reduced AβO-induced tau phosphorylation at serine 409, a site required for CCR. Next, we crossed TgI2.2 mice, which overexpress wild type human α-synuclein with hAPPJ20 AD model mice, then measured CCR *in vivo*. hAPPJ20/TgI2.2 mice have enhanced CCR in comparison to the hAPPJ20 parental line. Importantly, overexpression of hWT α-synuclein alone, as in the TgI2.2 strain, was insufficient to induce CCR *in vivo* or with lentiviral-mediated overexpression *in vitro*. However, overexpression of A53T mutant α-synuclein in primary cortical neurons was sufficient to induce CCR. To test whether CCR driven by A53T α-synuclein is tau-dependent, A53T α-synuclein was overexpressed in tau knockout (KO) neurons. Interestingly, neither A53T nor WT α-synuclein variants were sufficient to induce CCR in tau KO neurons, even when their overexpression was combined with AβO treatment. Taken together, these results indicate that AβOs promote CCR through an α-synuclein and phospho-tau dependent mechanism. Therapeutic targeting of α-synuclein may therefore be beneficial not only for canonical synucleinopathies, but also for AD.

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Nanosymposium

767. Alzheimer's Disease: Animal Models

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Alzheimer's Association IIRG-12-241042

Title: The Tau-Fyn interaction affects memory in a Tau-Fyn double knockout mouse

Authors: ***G. M. LIU**, R. J. TAUGHER, J. A. WEMMIE, G. LEE;
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Abstract: Since Tau's discovery as a microtubule binding protein, our laboratory has shown that Tau interacts with Fyn and potentiates Fyn activity. We have also found that Fyn phosphorylates Tau at tyrosine and that tyrosine phosphorylated Tau occurs in Alzheimer's disease. However, the functions of this Tau-Fyn interaction are still not very well understood. Tau has been shown to bring Fyn to the synapse where it phosphorylates NMDA receptor subunit 2B (NR2B).

However, it is highly probable that additional functions are affected by the Tau-Fyn interaction. To investigate, we created a Tau/Fyn double knockout (DKO) mouse and compared its behaviors to those of wild type and single KO mice. Using the pole test, we found that DKO mice had the same motor deficits as Tau KO mice while Fyn KO and WT mice had no deficits, suggesting that Fyn was not involved in the motor pathway. We also induced seizures in the DKO mice with pentylenetetrazole (PTZ) and found that they had significant protection against seizures, with the level of protection being identical to that shown by Tau KO mice. Fyn KO mice had an intermediate level of protection, being more protected than WT mice but less protected than Tau KO mice. Because DKO and Tau KO mice behaved similarly, tau might affect seizures independently of Fyn and more severely. Memory was tested by the novel object recognition test and contextual fear conditioning. In both tests, DKO mice had significantly more memory deficits when compared to Fyn KO mice. In turn, Fyn KO mice had more memory deficits when compared to WT mice, with WT and Tau KO mice performing similarly. In addition, we prepared and analyzed postsynaptic density protein 95 (PSD-95) fractions from cortex and hippocampus. In the hippocampal fraction, we found that relative to WT, Tau KO had less tyrosine phosphorylated NR2B (PY-NR2B), Fyn KO had even less, and DKO had the least PY-NR2B. Total NR2B was unchanged in all genotypes. In the cortex PSD-95 fraction, DKO and Fyn KO had similar PY-NR2B levels while Tau KO and WT had higher but similar PY-NR2B levels. Also, Tau KO had less Fyn than WT in hippocampal PSD-95 fraction whereas in the cortex PSD-95 fraction, Tau KO and WT had similar Fyn levels. This indicated that Tau's ability to bring Fyn to the synapse may be brain region dependent. Together, these findings suggest that while the Tau-Fyn interaction may not influence seizure response to PTZ, the interaction does play a role in memory deficits seen in DKO mice. The memory deficits are correlated with the level of PY-NR2B in the hippocampal PSD-95 fraction.

Disclosures: **G.M. Liu:** None. **R.J. Taugher:** None. **J.A. Wemmie:** None. **G. Lee:** None.

Nanosymposium

767. Alzheimer's Disease: Animal Models

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CREST from JST.

Title: Accumulation of phosphorylated tau in neurons in FUS-silenced mice

Authors: *S. ISHIGAKI[1], Y. FUJIOKA[1], D. HONDA[1], S. YOKOI[1], K. ENDO[1], H. OKADO[2], H. WATANABE[1], M. KATSUNO[1], A. TAKASHIMA[3], G. SOBUE[1];
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Abstract: Fused in sarcoma (FUS) is genetically and clinicopathologically linked to frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). We reported that FUS regulates alternative splicing of Mapt gene at exon10 which generates two pathogenic isoforms of neural microtubule-associated protein tau (Tau) protein. Silencing of FUS resulted in the increased ratio of 4-repeat tau (4R-tau)/ 3-repeat tau (3R-tau). Mice with hippocampus specific FUS-knockdown exhibited abnormal behaviors mimicking FTLD-like behavioral impairments in the early disease-stage. FUS-silenced mice at 12 months post injection or later showed accumulation of phosphorylated tau in the residual neurons. This phosphorylated tau deposition was rescued by normalization of the 4R-tau/ 3R-tau ratio using shRNA against 4R-tau. The presence of phosphorylated tau deposition in FUS-silenced mice without genetic tau intervention is impactful. Thus, our findings suggest a novel pathophysiological link between FUS and tau in FTLD and 4-R tauopathies through the regulation of 4R-tau/ 3R-tau isoforms.

Disclosures: S. Ishigaki: None. **Y. Fujioka:** None. **D. Honda:** None. **S. Yokoi:** None. **K. Endo:** None. **H. Okado:** None. **H. Watanabe:** None. **M. Katsuno:** None. **A. Takashima:** None. **G. Sobue:** None.

Nanosymposium

767. Alzheimer's Disease: Animal Models

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Title: *In vivo* multimodal imaging of tauopathy revealed a rapid turnover of pathological tau inclusions in a tauopathy mouse model

Authors: *N. SAHARA, H. TAKUWA, A. ISHIKAWA, T. URUSHIHATA, T. MINAMIHISAMATSU, M. TOKUNAGA, M. SHIMOJO, S. UCHIDA, I. MATSUMOTO, M.-R. ZHANG, T. SUHARA, M. HIGUCHI;
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Abstract: Accumulation of intracellular neurofibrillary tangles (NFTs) consisting of microtubule-associated protein tau is a major hallmark of tauopathy. The PBB3 ligand selectively binding to tau inclusions was currently developed for diagnosis of tauopathy. This ligand is useful for not only positron emission tomography (PET) imaging but also fluorescence imaging. Taking advantage of multimodality of PBB3 ligand, in vivo monitoring of NFT formation has been examined in a tauopathy mouse model, rTg4510 mice. This model typically exhibits forebrain atrophy and intraneuronal tau accumulation by 6 months of age. In this study, we performed in vivo two-photon microscopic imaging to investigate the progression of tau pathology at cellular levels. A chronic cranial window onto the bone of the skull enabled to examine longitudinal monitoring up to two months. In parallel, volumetric magnetic resonance imaging and [^{11}C]PBB3-PET were conducted for diagnosing neurodegeneration. As results, visualization of PBB3-positive inclusions by two-photon imaging was succeeded as early as 4 months of age while forebrain atrophy and [^{11}C]PBB3 signal became noticeable at 6 months of age. PBB3 signals of both PET and two-photon imagings reached a plateau by 6-7 months of age. Interestingly, two-photon imaging revealed that PBB3-positive inclusions were continuously produced whereas subpopulations of PBB3-positive inclusions were disappeared within a few weeks. These results suggest the existing of a rapid turnover of PBB3-positive inclusions. In addition, rates of generation and disappearance were significantly reduced by the suppression of human P301L tau indicating that regulating human P301L tau expression enables to control the turnover. Further examinations with different timing of the suppression of human P301L tau are ongoing to validate the amelioration of brain atrophy and tau pathology.

Disclosures: **N. Sahara:** None. **H. Takuwa:** None. **A. Ishikawa:** None. **T. Urushihata:** None. **T. Minamihisamatsu:** None. **M. Tokunaga:** None. **M. Shimojo:** None. **S. Uchida:** None. **I. Matsumoto:** None. **M. Zhang:** None. **T. Suhara:** None. **M. Higuchi:** None.

Nanosymposium

767. Alzheimer's Disease: Animal Models

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Title: Manganese-enhanced magnetic resonance imaging (MEMRI) identification of pre-pathological neuronal dysfunction precedes significant tau pathology in rTg4510 mice.

Authors: *S. N. FONTAINE[1], D. LYONS[2], S. MEIER[1], A. INGHAM[1], M. BELL[1], E. M. MILLER[1], M. VANDSBURGER[3], J. F. ABISAMBRA[1];
[1]Sanders-Brown Ctr. on Aging, [3]Physiol., [2]Univ. of Kentucky, Lexington, KY

Abstract: *Background*: Tauopathies, including Alzheimer's disease (AD), present as significant cognitive decline accompanied by irreversible and severe brain atrophy. The underlying neuronal dysfunction causing these symptoms often occur years before clinical AD develops. Currently, diagnostic tools are ineffective at detecting robust pre-pathological changes in the brain. We hypothesized that manganese-enhanced magnetic resonance imaging (MEMRI) coupled with high-resolution T1-mapping could be used to measure calcium-based neuronal dysfunction in a mouse model of tauopathy. *Methods*: R1-relaxation rates (1/T1) using MEMRI were used to measure the integrity of calcium-dependent broad neuronal function in rTg4510 tau transgenic and wild type mice at 2, 3 and 10 months of age. Additionally, T2-weighted images were used to measure the volume of the hippocampus and cortex in rTg4510 compared to non-transgenic mice. Finally, MEMRI changes were correlated with several standard immunohistochemical analyses of tau pathology including MC1-positive tangle load, and phospho-tau staining. *Results*: MEMRI was highly sensitive and revealed significant alterations in enhancement patterns in the hippocampus and cortex of rTg4510 mice compared to non-transgenic mice at 10 months of age. Differences were detectable in specific sub-regions of the hippocampus: R1 relaxation rates in the CA1 and CA3 regions of the hippocampus were detectable at 3 months of age. Importantly, MEMRI-detected alterations in neuronal function occurred prior to significant hippocampal volume loss and pathological tau progression in rTg4510 mice. *Conclusions*: MEMRI imaging combined with high-resolution T1 mapping is a non-invasive and useful technique for measuring

calcium-based neuronal function. MEMRI detected early alterations in calcium-based neuronal dysfunction in rTg4510 mouse of model of tauopathy, which preceded pathological tau accumulation and hippocampal volume loss. These data show that MEMRI can detect pre-clinical alterations in the tauopathic brain and represent an important technique as a diagnostic tool for determining pre-clinical neuronal dysfunction in patients.

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Nanosymposium

767. Alzheimer's Disease: Animal Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Wincon Research

Title: Tau pathology in aged cynomolgus monkeys with long term type 2 diabetes mellitus

Authors: ***Z. ZHANG**[1,2], F. YUE[3,4], C. LU[4], Y. AI[1], R. C. GRONDIN[1], R. C. GRONDIN[2], G. A. GERHARDT[1], G. A. GERHARDT[2], D. M. GASH[1], D. M. GASH[2];
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Abstract: Epidemiological and clinical studies have shown that type 2 diabetes mellitus (T2DM) increases the risk for Alzheimer's disease (AD). Abnormally hyperphosphorylated tau in paired helical filaments is one of the principal pathological features of AD. To study the link between T2DM and tau, we have conducted a preclinical study to assess tau pathology in aged (>22 years old) cynomolgus monkeys with long-term T2DM. Criteria for inclusion in the study were: fasting plasma glucose (FPG) >6.9 mmol/L, and glycated hemoglobin (Hb-A1c) >6.5% for aged monkeys with T2DM. In addition, T2DM animals had significantly higher levels of plasma insulin ($P<0.0001$) and abnormal lipid profiles. Five additional aged animals with normal range of FPG, and Hb-A1c were used as normal controls. Antibodies (AT 180, AT8, AT100) were used for detecting tau pathogenesis and $A\beta_{1-16}$ was used for detecting senile plaques in some key brain regions associated with AD including the hippocampus, entorhinal cortex, frontal cortex and temporal cortex. Histological analysis demonstrated that significantly higher numbers of AT180 and AT8 positive neurons were found in the CA1, CA2 and CA3, subiculum and

entorhinal cortex in the brains of T2DM animals compared to normal controls. AT100-positive neurons with collapsed basal dendrites were also more often observed in T2DM hippocampus and its surrounding fields. Additionally to increased NFTs in T2DM animals, Aβ positive senile plaques were also significantly increased in T2DM compared to normal controls. These data strongly support the notion that T2DM can accelerate tau pathology in aged cynomolgus monkeys with long-term T2DM, and aging diabetic nonhuman primates might be a relevant AD-model for testing therapeutic interventions.

Disclosures: Z. Zhang: None. **F. Yue:** None. **C. lu:** None. **Y. Ai:** None. **R.C. Grondin:** None. **R.C. Grondin:** None. **G.A. Gerhardt:** None. **G.A. Gerhardt:** None. **D.M. Gash:** None. **D.M. Gash:** None.

Nanosymposium

767. Alzheimer's Disease: Animal Models

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Presentation Number: 767.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: No overt deficits in tau-deficient C57Bl/6. Mapt$^{tm1(EGFP)Kit}$ GFP knockin mice

Authors: *A. VAN HUMMEL[1], M. BI[1], J. VAN DER HOVEN[1], A. VOLKERLING[1], W. S. LEE[1], D. TAN[1], A. BONGERS[2], S. CHUA[1], A. ITTNER[1], Y. D. KE[1], L. ITTNER[1,3]; [1]Dementia Res. Unit, Sch. of Med. Sci., Univ. of New South Wales, Sydney, Australia; [2]Bioolgical Resources Imaging Laboratory, Mark Wainwright Analytical Centre, UNSW, Sydney, Australia; [3]Neurosci. Res. Australia, Sydney, Australia

Abstract: Alzheimer's Disease (AD) is characterized by two main neuropathological events: tau-containing neurofibrillary tangles and extracellular amyloid-β (Aβ) plaques, and considerable evidence has been reported suggesting that tau is required for Aβ toxicity. Several mouse lines with a knockout of the tau-encoding *MAPT* gene have been reported in the past; however, the effects of long-term depletion of tau *in vivo* remain controversial. Here, we used the tau-deficient GFP knockin line *Mapt$^{tm1(EGFP)kit}$* on a pure C57Bl/6 background and subjected a large cohort of males and females (homozygous *tau$^{GFP/GFP}$* males n=8 and females n=12; heterozygous *tau$^{GFP/+}$* males n=18 and females n=20; *tau$^{+/+}$* littermate control males n=12 and females n=15) to a range of motor (Rota-Rod, Hanging Wire Test, Pole Test), memory (Morris Water Maze) and behavior (Elevated Plus Maze, Open Field) tests at the advanced age of 16 months. Mice were trans-cardially perfused at 19-22 months of age and tissue harvested for protein analysis (western blot), immunohistochecmistry or MRI imaging. Neither heterozygous nor homozygous *Mapt$^{tm1(EGFP)kit}$* mice presented with deficits or abnormalities compared to wild-type littermates in

any of the above analyses. Differences to reports using other tau knockout models may be due to different genetic backgrounds, respective gene targeting strategies or other confounding factors, such as nutrition. In this context, we report no functional or morphological deficits due to tau reduction or depletion in aged mice.

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767. Alzheimer's Disease: Animal Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Merck Postdoctoral Program

Title: A novel animal model for spread of tau pathology in mice.

Authors: *M. K. SCHULTZ, JR;
Early Discovery Pharmacol., Merck & Co., West Point, PA

Abstract: Tau protein can aggregate to form pathological neurofibrillary tangles in neurodegenerative disorders such as Alzheimer’s disease (AD). The pathology in AD reportedly spreads in a spatio-temporal pattern along anatomically connected brain regions. We have designed a model of tauopathy using adeno-associated virus (AAV) to express human tau and excitatory DREADD hM3Dq (Tau+hM3D) intracellularly in mouse ventral hippocampus (VHC) neurons so as to investigate the trans-synaptic spread of tau. AAV Tau+GFP was used to control for the amount of tau that spreads under baseline conditions. Neurons expressing hM3D were activated daily with clozapine N oxide for multiple weeks. At necropsy, expression of transgene products (HA, GFP and mCherry) and tau (hyperphosphorylated tau, AT8 and aggregated tau, MC1) were assessed for validation of successful infusions and for induction of pathology, respectively. HA labeling, indicating expression of viral tau, was present at the infusion site in both Tau+GFP and Tau+hM3D mice. Tau+hM3D mice had increased expression of viral tau at the infusion site compared to to Tau+GFP. In the Tau+GFP condition, viral tau was primarily confined to the area of infusion with minimal presence distal to the infusion site. In the Tau+hM3D condition, viral tau spread through the hippocampus, and into synaptically connected cortical regions. Pathological tau (AT8 and MC1 positive) was found in the VHC of all AAV tau-infused mice. Staining for MC1 was elevated in Tau+hM3D mice compared to Tau+GFP

mice. GFP and mCherry were not present in distal regions, indicating that presence of tau away from the infusion site was attributable spread beyond the initially infected neurons. These results indicate AAV-driven tau expression mouse model will prove useful in studying trans-synaptic tau spread. In addition, this spread may be enhanced by inducing neuronal activity.

Disclosures: M.K. Schultz: None.

Nanosymposium

767. Alzheimer's Disease: Animal Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: USF Health Byrd Alzheimer's Institute

Florida High Tech Corridor Grant

Title: Tg4510 mice provide an effective model for testing neuroprotective therapies in early-stage Alzheimer’s disease.

Authors: *P. R. MOUTON[1], H. A. PHOULADY[3], D. GOLDGOF[3], L. HALL[3], M. GORDON[2], D. MORGAN[2];
[1]Pathology & Cell Biol., [2]Mol. Pharmacol. & Physiol., USF Hlth. Byrd Alzheimer's Inst., Tampa, FL; [3]Computer Sci., Univ. of South Florida, Tampa, FL

Abstract: Alzheimer’s dementia is caused by progressive, age-related neuron loss in cortical brain regions that control cognitive, intellectual and emotional behaviors. We do not know why these neurons die and there are no effective treatments to slow or stop this process. Without a treatment or cure the number of Americans suffering from Alzheimer's will increase from about 5.2 million people (annual cost $230 billion) to about 14 million ($1.1 trillion) by 2050. The key to avoiding this coming health care crisis is finding a therapeutic approach to stop this neuron loss in early stages of the disease. Although several *in-vivo* models have been proposed for assessing the effectiveness of potential treatments, few of these approaches show neuron loss with a similar spatiotemporal pattern as Alzheimer’s disease. One promising line is the Tg4510 model of tauopathy, a bigenic mouse with responder and activator transgenes that drive expression of a P301L tau mutation under the control of a tetracycline operon-responsive element. Previous studies of Tg4510 mice using a wide range of morphometric approaches suggest age-related neuron loss and gliosis in neocortex and hippocampus between 2-9 months of age with progressive cognitive impairment and grossly observable forebrain atrophy by 10

months. To establish baseline stereology data for future neuroprotection studies in early stages of neuron loss, we systematically sampled sections through neocortex and hippocampus (CA1 and dentate gyrus) of 6-8 month-old Tg4510 and nontransgenic littermates. These sections were immunostained for neurons (NeuN) and glia (GFAP, Iba-1) using protocols optimized for high signal: noise ratio. Technicians blind to genotype used manual (non-automatic) stereology and a beta version of the automatic Detect-Segment-Quantify (DSQ) stereology program (*Stereologer*, Stereology Resource Center) to assess neuron loss and gliosis in these sections. Stereology results indicate a significant 20 to 25% loss of neocortical NeuN-immunopositive neurons in Tg4510 mice compared to nontransgenic controls with greater than 95% agreement between manual and automatic approaches. Qualitative examination of immunostained astrocytes (GFAP) and microglial cells (IBA-1) from ongoing stereology studies support the view that this neuron loss is accompanied by clear neocortical gliosis. These preliminary findings support the view that middle-aged Tg4510 mice provide a reliable model of neuron loss and gliosis for testing the potential efficacy of neuroprotective therapies for early-stage Alzheimer's patients.

Disclosures: **P.R. Mouton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stereology Resource Center, IP interest in automatic stereology. **H.A. Phoulady:** A. Employment/Salary (full or part-time): University of South Florida. **D. Goldgof:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IP interest in automatic stereology. **L. Hall:** A. Employment/Salary (full or part-time): University of South Florida. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IP interest in automatic stereology. **M. Gordon:** A. Employment/Salary (full or part-time): USF Professor. **D. Morgan:** A. Employment/Salary (full or part-time): USF Professor. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IP interest in automatic stereology.

Nanosymposium

767. Alzheimer's Disease: Animal Models

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Title: A neuronal cell culture model of Alzheimer's disease with amyloid-β (Aβ) and Tau pathology, in the absence of FAD mutations

Authors: *V. MURESAN, Z. LADESCU MURESAN;
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Abstract: Our goal is to identify mechanisms that cause sporadic Alzheimer's disease (AD), and to develop strategies that interfere with these mechanisms, aiming to prevent neuronal death. The exploration of molecular mechanisms leading to the two major lesions in sporadic AD - the generation and oligomerization of Aβ, and aggregation of hyperphosphorylated Tau (pTau) - would be facilitated by a cell culture model that reproduces these AD-specific alterations, in the absence of genetic mutations that cause familial AD. Here, we propose that the CNS-derived, mouse neuronal cell line CAD constitutes an excellent, yet simple, "sporadic Alzheimer's-in-a-dish" model. CAD neurons in culture spontaneously accumulate oligomeric Aβ and aggregates of detergent-resistant pTau, as do neurons in the brain regions afflicted by AD pathology. We show that the degree of accumulation of Aβ and pTau can be experimentally altered, allowing for the investigation of the molecular mechanisms that lead to neuronal pathology. Using the CAD cell system, we identified and dissected in detail a novel and unexpected molecular mechanism that leads to intraneuronal accumulation of Aβ and pTau, and tested means for its prevention. The pathogenic mechanism relies on a signaling pathway triggered by impaired axonal transport, a condition likely associated with old age. We show that, when cargo accumulates in the soma of neurons with experimentally blocked transport, the endoplasmic reticulum (ER)-localized Amyloid-β Precursor Protein (APP) is phosphorylated via a JNK complex that contains the APP binding protein, Fe65 – a regulator of transcription. This phosphorylation increases the amyloidogenic cleavage of APP, and the generation and oligomerization of Aβ inside the ER. The subsequent disassembly of the phosphorylation complex allows for Fe65-dependent upregulation of GSK3β, which phosphorylates Tau, causing its aggregation. We find that the generated pTau disseminates into the extracellular space, and spreads between cells via tunneling nanotubes, which abound in CAD cells. Importantly, knocking down BACE1 prevents accumulation of Aβ, but does not block Tau phosphorylation, and aggregation of pTau. By contrast, blocking APP phosphorylation eliminates both the Aβ and the pTau neuronal pathology. These results suggest that therapeutic strategies based on inhibition of secretases, while diminishing the Aβ pathology, would not prevent the Tau pathology. Blocking APP phosphorylation would prevent both lesions. The cell culture model for sporadic AD developed by us should be useful for studying mechanisms of neuronal pathology, and identify agents that block the pathogenic pathways.

Disclosures: V. Muresan: None. **Z. Ladescu Muresan:** None.

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Belter Neurodegeneration Consortium

Title: Inhibition of p25/Cdk5 attenuates AD-like pathology in P301S mice and iPSC-derived neural cells from fAD patients

Authors: *J. SEO, Y.-T. LIN, O. KRITSKIY, P.-C. PAO, S. ELMSAOURI, W. RAJA, D. DEY, M. RAM, T. KO, L.-H. TSAI;
Picower Inst. for Learning and Memory, Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Increased p25, a proteolytic fragment of p35, is well known to induce aberrant activity of cyclin-dependent kinase 5 (Cdk5), which mediates various pathologies in neurodegenerative disorders including Alzheimer’s disease (AD). Previously, we have shown that genetic manipulation of p35 gene (Cdk5r1) to replace endogenous p35 with non-cleavable protein renders amyloidosis attenuated in the brain of a familial AD mouse model. To address the functions of p25/Cdk5 in tauopathy, we generated double transgenic mice (P301S;Δp35KI), which express non-cleavable p35 with overexpression of mutant tau (P301S). We observed significant reduction of phosphorylated tau by inhibiting p25 generation in P301S mouse brain. This reduction was more predominant in the levels of insoluble tau. Furthermore, brain lysates from P301S;Δp35KI showed significantly reduced tau seeding activity compared to those from P301S. As a human model system to study the role of p25/Cdk5 in AD, we utilized induced pluripotent stem cells (iPSCs) derived from fAD patients, and CRISPR/Cas9 genome editing tool to generate Δp35KI isogenic iPSCs. We then differentiated AD iPSCs and Δp35KI isogenic cells into neural cells. In Δp35KI cells, we observed reduced levels of secreted Aβ and histone deacetylase 2 (HDAC2) compared to those from parent cells. Also, genotoxic agent-induced DNA damage was significantly attenuated in these cells. Together, these data suggest beneficial effects of p25/Cdk5 inhibition in AD. NIH Grant R01 NS051874. Belfer Neurodegeneration Consortium

Disclosures: **J. Seo:** None. **Y. Lin:** None. **O. Kritskiy:** None. **P. Pao:** None. **S. Elmsaouri:** None. **W. Raja:** None. **D. Dey:** None. **M. Ram:** None. **T. Ko:** None. **L. Tsai:** None.

Nanosymposium

767. Alzheimer's Disease: Animal Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Canadian Consortium on Neurodegeneration and Aging

Title: Cerebral microvascular dysfunction in TgAD F334 rats a model of Alzheimer's disease.

Authors: *J. MCLAURIN[1], L. JOO[2], A. LAI[1], J. SLED[3], B. STEFANOVIC[2];
[1]Biol. Sci., [2]Physical Sci., Sunnybrook Res. Inst., Toronto, ON, Canada; [3]Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Alterations in cerebral microvascular network in Alzheimer's disease (AD) may precede and contribute to the onset of clinical symptoms. Better methods for early detection of AD onset can be advanced through animal models that closely recapitulate the vascular aspects of clinical AD. The current study examined the microvascular structure and function in the context of amyloid accumulation and specifically the response to vasodilatory stimulation in the TgF344 rat model that replicates a wide spectrum of human AD pathologies and progressive cognitive decline. In transgenic rats (Tg), cortical parenchymal amyloid plaques concentrated in the lateral-ventral regions of the brain while being sparsely distributed in the somatosensory and motor regions; perivascular cerebral amyloid angiopathy appeared primarily in the somatosensory cortex in both arterioles. Immunoblotting for protein expression of desmin and PDGFR-beta, markers of mural cell activity, were increased in the Tg rats while markers for blood-brain barrier integrity were not significantly different in comparison to those of non-transgenic littermate rats (nTg). We employed *in vivo* two-photon fluorescence microscopy to image the microvessels of the primary somatosensory cortex in the nTg and Tg rats while eliciting global vasodilation by raising inspired CO_2 concentration to 10%. The blood flow was quantified by tracking the passage of a fluorescent dye bolus, injected peripherally, through the local cortical microvascular network. Vascular reactivity of arterioles and venules was significantly impaired in Tg rats, manifested as an attenuated blood flow response to hypercapnia. Tg rats showed greater dispersion of microvascular transit times during CO_2 inhalation. These data demonstrate that TgF344 AD rats recapitulate several key phenotypes of vascular pathology observed in AD patients including reduced cerebrovascular reactivity, morphological manifestation of Abeta deposits on the cortical microvasculature, and perturbation of vascular mural cells.

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767. Alzheimer's Disease: Animal Models

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Time: Wednesday, November 16, 2016, 1:00 PM - 4:30 PM

Presentation Number: 767.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Visual assessment (Electroretinography) in the TgF344-AD rat model: a potential Alzheimer's disease biomarker

Authors: ***F. G. DE OLIVEIRA-SOUZA**[1], M. L. DERAMUS[2], A. GOODMAN[3], L. SMITH[4], R. FARRUKH[5], L. MCMAHON[6], T. KRAFT[2], C. E. STRANG[3], M. BOLDING[7]; [2]Vision Sci., [3]Psychology, [4]Grad Sch. - Biomed Sci., [5]Neurosci., [6]Grad. Sch. Dean's Office, [7]Radiology, [1]Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Alzheimer's disease (AD), the most common dementia, is characterized by grave cognitive enfeeblement. It is associated with the accumulation of neurofibrillary tangles (NFTs) and amyloid beta (Aβ) plaques, extensive neuronal loss and irregularities in several neurotransmitter systems. Visual disturbances in acuity, color, motion perception and contrast sensitivity are also prevalent in AD. AD-induced visual alterations may precede cognitive decline, thus rendering the possibility of early AD diagnosis through visual testing. To assess this hypothesis, we employed electroretinography (ERG) to measure retinal responses in 9 months old (mo) TgF344-AD rats and age-matched wild type (WT) rats. TgF344-AD rats are generated on a Fischer 344 background by co-injecting rat pronuclei with two human genes driven by the mouse prion promoter: Swedish(KM670/671NL) mutant human amyloid precursor protein (APP) and Δ exon 9 mutant human presenilin-1 (PS1ΔE9) (Cohen et al 2013). Our preliminary data showed no difference in the magnitude of long-term potentiation (LTP) at CA3-CA1 synapses up to 12 mo; impaired learning and memory is reported at 15 mo (Cohen et al 2013). Our ERG data showed that the TgF344-AD rats displayed higher rod photoreceptor (a-wave), inner retinal cells (oscillatory potentials) and off bipolar cell responses as compared to WT ($p<0.05$). These augmented retinal responses may stem from a compensatory mechanism to cope with the detrimental consequences of AD pathology. AD is associated with alterations in neurotransmitter levels, calcium homeostasis dysregulation and cell loss. Alterations in the excitation/inhibition balance due to a decrease in inhibitory neurotransmitters, GABA or glycine, or compensatory increase in excitatory neurotransmitters, acetylcholine or glutamate), could yield larger physiological responses. The verification of this hypothesis would require the

administration of receptor agonist and antagonists. In addition, experiments in older animals will be performed to determine the time course of these early compensatory mechanisms as the disease progresses. Our work is the first to report that in the TgF344-AD rats, visual alterations manifest before any measurable cognitive changes appear. The sooner AD is detected, the more effective the treatment may be in delaying and/or attenuating the cognitive decline. Visual assessment (i.e. ERG) may provide a tremendous avenue to facilitate AD's early detection and its timely treatment.

Disclosures: **F.G. De Oliveira-Souza:** None. **M.L. DeRamus:** None. **A. Goodman:** None. **L. Smith:** None. **R. Farrukh:** None. **L. McMahon:** None. **T. Kraft:** None. **C.E. Strang:** None. **M. Bolding:** None.

Nanosymposium

767. Alzheimer's Disease: Animal Models

Location: SDCC 33C

Time: Wednesday, November 16, 2016, 1:00 PM - 4:30 PM

Presentation Number: 767.13

Topic: C.02. Alzheimer's Disease and Other Dementias

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Kleberg Foundation Award

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JMR Barker Foundation Award

Title: Tor as a key regulator of neural and neurovascular function in mouse models of Alzheimer's disease

Authors: *V. GALVAN[1], S. HUSSONG[2], C. VAN SKIKE[2], J. JAHRLING[2], N. DEROSA[3], C. POMILIO[4], A. OLSON[2];
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Abstract: We recently showed that chronic treatment with the target-of-rapamycin (TOR) inhibitor rapamycin, a drug that extends lifespan and delays aging in mice, halted and even reversed Alzheimer's (AD)-like memory deficits, decreased Aβ, and restored cerebral blood flow (CBF) in brains of hAPP(J20) and Tg2576 mice modeling the disease. Reducing TOR activity also restored cognitive function and CBF in mice modeling atherosclerosis, as well as in 36 month-old rats. Attenuating TOR activity was associated with the recovery of cortical network activation and functional hyperemia evoked by somatosensory stimulation. Our data indicate that the mechanisms by which TOR attenuation restores CBF, neuronal activity, and cognitive function may be common to different models of age-associated neurological disease and to brain aging, and singled out (a) vascular NO release, and (b) synaptic bouton remodeling as key mechanisms by which TOR attenuation blocks AD-like progression in mice. To delineate the mechanisms by which TOR regulates synaptic remodeling during aging and in AD we used rapamycin in very old rats, and advanced tissue-specific genetic tools to reduce TOR complex 1 assembly specifically in neurons of adult mice. Moderate, but not drastic reduction of TORC1 assembly in neurons, to levels similar to those achieved by rapamycin treatment, promoted synaptic remodeling and increased autophagy, potentially increasing synaptic vesicle recycling. This was associated with enhanced memory and increased brain glucose uptake, suggestive of increased brain glucose metabolism. We propose that attenuation of TOR in (a) brain vascular endothelial cells and in (b) Aβ-producing parenchymal neurons by pharmacological or genetic means act synergistically to slow the progression of AD dysfunction through the restoration of (1) NO-dependent vasodilation and CBF, increasing vascular Aβ clearance, and (2) by increasing autophagy, leading to synaptic bouton remodeling, decreased Aβ release, and restored neuronal function in AD. Relieving TOR-dependent inhibition of synaptic remodeling and neurovascular NO release may be critical synergistic mechanisms by which rapamycin or genetic TORC1 knockdown preserve network activation, functional hyperemia and cognitive function during aging and in models of AD and other dementias. TOR inhibition may have promise as therapy for AD and potentially other neurodegenerations.

Disclosures: **V. Galvan:** None. **S. Hussong:** None. **C. Van Skike:** None. **J. Jahrling:** None. **N. DeRosa:** None. **C. Pomilio:** None. **A. Olson:** None.

Nanosymposium

767. Alzheimer's Disease: Animal Models

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Presentation Number: 767.14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NINDS (NS085770)

NIA (AG013854)

Title: Neurotoxicity related to tetracycline transactivator expression in tdp-43 mouse model of frontotemporal lobar degeneration.

Authors: *L. KUKREJA[1], R. SHAHIDEHPOUR[1], G. RODRIGUEZ[2], G. KIM[1], K. R. SADLEIR[2], J. CSERNANSKY[3], M.-M. MESULAM[1], R. J. VASSAR[2], H. DONG[3], C. GEULA[1];
[1]Cognitive Neurol. and Alzheimer's Dis. Ctr., [2]Dept. of Cell and Mol. Biol., [3]Dept. of Psychiatry and Behavioral Sci., Northwestern Univ., Chicago, IL

Abstract: Frontotemporal lobar degeneration (FTLD) constitutes the third most prevalent dementia after those caused by Alzheimer's disease and Lewy bodies, and is among the most prevalent dementias of early-onset. We report on a mouse model for FTLD that overexpresses wild-type TAR DNA-binding protein 43 (TDP-43) gene using tetracycline transactivator (tTA), a system widely used to create transgenic models of neurological disorders. Transgenic expression of tTA is used to activate a second transgene of interest, e.g. the wild-type TDP-43, which is placed downstream of the tetracycline response element (TRE). Exposure to doxycycline, a more stable analog of tetracycline in mouse diet, can cause a conformational change in tTA that inhibits binding to TRE to stop the expression of the TRE-controlled transgene. This allows us to control when the transgene of interest turns on and off. A recent study found that the genetic strain background on which tTA is expressed dramatically influences neurodegeneration caused by the tTA protein. Granule neurons of the dentate gyrus (DG) appeared most sensitive in this process. Our TDP-43 transgenic mice are bred on FVB and 129/SVE backgrounds, which are among the few mouse strains susceptible to neurodegeneration from tTA. Here, we report on the effects of tTA alone in our 3 month- and 7 month old tTA and TDP-43 biallelic transgenic mice. Approximately 40% of the area in DG was lost when tTA protein was expressed by 7 months of age. This effect was smaller at the earlier age. Thin coronal sections showed that the worst loss of granule cells in DG was in the mid-hippocampal region. Interestingly, the CA fields were spared from neurodegeneration. Other unaffected regions included cingulate, motor and somatosenory cortices. The only region affected outside of the hippocampus was the piriform cortex, with 25% reduction in its area. Neuronal loss by tTA is likely caused by off-target interactions involving extraneous proteins or promoters. Similar to an earlier report, we found that doxycycline treatment prevented tTA neurotoxicity. Behavioral tests in our bigenic mice expressing tTA and TDP-43 revealed no hippocampally mediated memory impairment. However, they did display deficits of working memory, novel object recognition and social interaction that are likely related to TDP-43 transgene expression. Although this study adds a note of caution for using the tTA system to generate transgenic mice, our mice remain useful for providing critical insight into the presence of TDP-43 pathology co-occurring with neuron loss and executive function deficits in FTLD.

Disclosures: L. Kukreja: None. **R. Shahidehpour:** None. **G. Rodriguez:** None. **G. Kim:** None. **K.R. Sadleir:** None. **J. Csernansky:** None. **M. Mesulam:** None. **R.J. Vassar:** None. **H. Dong:** None. **C. Geula:** None.

Nanosymposium

768. Oxidative Stress and Antioxidants in Neurological and Psychiatric Diseases

Location: SDCC 32B

Time: Wednesday, November 16, 2016, 1:00 PM - 3:00 PM

Presentation Number: 768.01

Topic: C.03. Parkinson's Disease

Support: Air Force Office of Scientific Research (EOARD, London, UK),

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Title: Nanodelivery of cerebrolysin reduces alpha-synuclein, neuronal nitric oxide synthase and brain pathology in Parkinson's disease

Authors: *A. OZKIZILCIK[1], A. SHARMA[3], R. PATNAIK[4], D. F. MURESANU[5], J. V. LAFUENTE[6], A. NOZARI[7], H. MOESSLER[8], R. TIAN[2], H. S. SHARMA[3];
[1]Biomed. Engin., [2]Chem. & Biochem., Univ. of Arkansas, Fayetteville, AR; [3]Surgical Sciences, Anesthesiol. & Intensive Care Med., Uppsala Univ. Hosp., Uppsala, Sweden; [4]Biomaterials, Biomed. Engin., Indian Inst. of Technology, Banaras Hindu Univ., Varanasi, India; [5]Clin. Neurosciences, Univ. of Med. & Pharm., Cluj-Napoca, Romania; [6]Neurosciences, Univ. of Basque Country, Bilbao, Spain; [7]Anesthesiol. & Critical Care Ctr., Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA; [8]Drug Discovery & Develop., Ever Neuro Pharma, Oberburgau, Austria

Abstract: Parkinson's disease (PD) affects over 80 thousand Americans every year for which no suitable therapy is available till date. Increased oxidative stress and neurotoxic elements in the CSF and in the brain appears to be responsible for PD pathology and decline in cognitive and motor functions. Recent research suggests that increased alpha-synuclein (a-synuclein, ASNC) in the CSF and in several brain areas together with oxidative stress correlates well with the brain pathology and cognitive decline in human cases of PD. Thus, a possibility exists that drugs that are capable to reduce the levels of oxidants and/or ASNC could be useful for novel therapeutic tools in PD.
Previous reports from our laboratory showed that intraperitoneal injections of 1-metyl-4-fenyl-

1,2,3,6-tetrahydropyridin (MPTP, 20 mg/kg) daily within 2-h intervals for 5 days in mice induce PD like symptoms on the 8th day. This model is well-characterized biochemically, histologically and functionally for PD like symptoms. Thus, marked decrease in the number of tyrosine hydroxylase (TH) positive cells in the Substantia Nigra Pars Compacta (SNpc) and striatum (STr) as well as decrease in dopamine (DA) and its metabolites 3,4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) with marked behavioral dysfunctions e.g., Rota-Rod performances, walking on an inclined mesh grid and gait deficits was seen on the 8th day. In this model timed release of CBL using titanate nanospheres (TiNS) treatment results in significant neuroprotection and behavioral improvements. Thus, it would be interesting to examine whether this model of PD is also associated with increased ASNC and free radical nitric oxide in the CSF and brain areas of mice and cerebrolysin treatment could modulate these elements in our model. ASNC was measures using commercial ELISA kit in the CSF and in brain whereas neuronal nitric oxide synthase (nNOS) was examined using immunohistochemistry on paraffin sections. Our results showed a significant increase in ASNC by 2- to 4-fold in the CSF and in various brain areas from normal control group (control: SNpc 1.78±0.08 ng/µg, STr 6.34±0.21 ng/µg, frontal cortex 8.24±0.32 ng/µg; CSF 1.21±0.07 pg/µl). In these brain areas nNOS expression was also increased by 4- to 8-fold as compared to control group. Nanodelivery of cerebrolysin (3 ml/kg, i.v. 2 days after MPTP for 5 days) significantly reduced ASNC levels in the CSF and in all the brain areas examined. In the treated PD mice downregulation of nNOS was also seen in the above brain regions. These results are the first to show that nanodelivery of cerebrolysin induces neuroprotection in PD by reducing ASNC and nNOS expression, not reported earlier.

Disclosures: **A. Ozkizilcik:** None. **A. Sharma:** None. **R. Patnaik:** None. **D.F. Muresanu:** None. **J.V. Lafuente:** None. **A. Nozari:** None. **H. Moessler:** None. **R. Tian:** None. **H.S. Sharma:** None.

Nanosymposium

768. Oxidative Stress and Antioxidants in Neurological and Psychiatric Diseases

Location: SDCC 32B

Time: Wednesday, November 16, 2016, 1:00 PM - 3:00 PM

Presentation Number: 768.02

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Science Foundation - GRFP

National Science Foundation - GROW

Swiss Gov. Excellence Research and Innovation Fellowship

Swiss National Science Foundation

Title: High dimensional analysis of the myeloid landscape in Multiple Sclerosis

Authors: *B. P. LEUNG[1,3], R. F. CATENA[4], A. BARRANTES[5], B. SCHREINER[3], C. STADELMANN-NESSLER[5], T. TOWN[2], B. BODENMILLER[4], B. BECHER[3]; [1]Neurosci., USC, Rancho Palos Verdes, CA; [2]Neurosci., USC, Los Angeles, CA; [3]Exptl. Immunol., [4]Dept. of Mol. Life Sci., Univ. of Zurich, Zurich, Switzerland; [5]Neuropathology, Univ. of Goettingen, Goettingen, Germany

Abstract: Multiple sclerosis (MS) is a demyelinating inflammatory disorder that leads to chronic, progressive neurological disability. Presently, evidence points to MS as an autoimmune/autoinflammatory disorder of the CNS in which deregulated T cells orchestrates the formation of inflammatory lesions leading to neurodegeneration; however, the mechanism by which T cells mediate the destruction of CNS tissue remain largely elusive. There is increasing evidence, that pathogenic T cells secrete GM-CSF and that GM-CSF directly affects myeloid cell activity in these lesions. To study the role of phagocytes in MS, we used Imaging Mass Cytometry on human MS brain biopsies to identify these T_H stimulated myeloid cells endorsing this cytotoxic and neurodegenerative phenotype. Using this high dimensional approach to analyze the human MS brain lesion, we have identified the myeloid "immunome" within the MS lesion, providing a spatial, functional, and subcellular characterization of pathogenic phagocytes in situ.

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Nanosymposium

768. Oxidative Stress and Antioxidants in Neurological and Psychiatric Diseases

Location: SDCC 32B

Time: Wednesday, November 16, 2016, 1:00 PM - 3:00 PM

Presentation Number: 768.03

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Adelson Foundation

Burke Medical Research Institute

Title: Utilizing a tunable antioxidant enzyme to define specific oxidative stress related biomarkers versus non-selective oxidative tombstones: implications for aging and neurodegeneration.

Authors: *I. ALIM, J. T. CAULFIELD, S. S. KARUPPAGOUNDER, D. E. WILLIS, R. R. RATAN;
Burke Med. Res. Inst., White Plains, NY

Abstract: Oxidative stress reflects a disruption of redox homeostasis and has been associated with a variety of neurological disorders, including stroke. Clinical trials using chemical antioxidants to treat both ischemic and hemorrhagic stroke have been disappointing, suggesting a complex role for ROS in oxidative death. In order to understand the events associated with neuronal death due to oxidative stress, we need methods that allow us to temporally regulate the expression of antioxidant enzymes, which possess selectivity for potentially damaging oxidants. Here, we developed a simple method using a destabilization domain (dd; developed by Thomas Wandless) to finely regulate known antioxidant enzyme expression in cultured neurons. We fused a dd domain to antioxidant enzymes glutathione peroxidases 4 (GPX4) and catalase (CAT). GPX4 is a phospholipid hydroperoxidase that reduces lipid peroxidation and CAT is a hydroperoxidase that reduces H_2O_2. When these constructs are transduced in neurons, antioxidant proteins can be dose dependently and rapidly stabilized by the application of the small molecule trimethoprim (TMP). Stabilization of GPX4 provided significant neuroprotection following glutathione depletion-induced oxidative stress, while, stabilization of CAT failed to provide neuroprotection. The stabilization of GPX4 is protective when stabilized up to 8-9 hours after initiation of glutathione depletion, defining a “commitment” point for oxidative death. Using this commitment point for oxidative death we have evaluated which previously described oxidative markers occur prior to this commitment point, and which occur afterwards. We found that protein carbonylation and activity of oxidative stress dependent transcription factor ATF4 is increased prior to the commitment point and are regulated by GPX4 stabilization. DNA oxidation occurred after the commitment point and was not affected by GPX4 stabilization. These results suggest that protein carbonylation and ATF4 activity are primary oxidative events that lead to neuronal death, while DNA oxidation is a consequence of cells committed to oxidative death. Taken together we have developed a method to distinguish events that are causal to oxidative death from those that are consequential; and also identified early upregulation of GPX4 expression as a therapeutic strategy. The implications for aging and disease in the CNS are discussed.

Disclosures: I. Alim: None. **J.T. Caulfield:** None. **S.S. Karuppagounder:** None. **D.E. Willis:** None. **R.R. Ratan:** None.

Nanosymposium

768. Oxidative Stress and Antioxidants in Neurological and Psychiatric Diseases

Location: SDCC 32B

Time: Wednesday, November 16, 2016, 1:00 PM - 3:00 PM

Presentation Number: 768.04

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CHDI

NIH Grant DA000266

NIH Grant MH18501

Title: Aberrant redox regulation of amino acid homeostasis mediates neurodegeneration in Huntington's disease

Authors: *B. D. PAUL, J. I. SBODIO, S. H. SNYDER;
The Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Huntington's disease (HD) is a neurodegenerative disorder triggered by expansion of polyglutamine repeats in the protein huntingtin. Mutant huntingtin causes widespread damage ranging from transcriptional dysregulation to metabolic deficits. Perturbations in amino acid disposition have frequently been reported in HD, but their origins have remained obscure. We have previously shown that cystathionine gamma lyase (CSE), the biosynthetic enzyme for cysteine is depleted in HD and mediates disease progression by altering redox homeostasis. Cysteine, being a component of the endogenous antioxidant glutathione and the precursor of the gaseous signaling molecule hydrogen sulfide plays a central role in mitigating redox imbalance in cells. Here, we report that activating transcription factor 4 (ATF4), the master regulator of amino acid biosynthetic enzymes, including CSE under nutrient stress, is dysfunctional in Huntington's disease. This abnormality results from chronic oxidative stress caused by "cysteine stress" as result of CSE depletion. Mitigating oxidative imbalance by antioxidants rescues the protective response mechanisms to stress. Our findings reveal the molecular basis for the decline in protective pathways and identify a molecular link between redox imbalance and metabolic dysfunction during neurodegeneration in HD. This signaling cascade may be relevant to other diseases involving redox imbalance and deficits in amino acid metabolism.

References

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2. **Paul BD**, Sbodio JI, Xu R, Vandiver MS, Cha JY, Snowman AM and Snyder SH. Cystathionine γ-lyase deficiency mediates neurodegeneration in Huntington's disease. ***Nature***. 2014; 509(7498):96-100.

Disclosures: B.D. Paul: None. **J.I. Sbodio:** None. **S.H. Snyder:** None.

Nanosymposium

768. Oxidative Stress and Antioxidants in Neurological and Psychiatric Diseases

Location: SDCC 32B

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Presentation Number: 768.05

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Czech Science Foundation Grant 15-08565S

Czech Health Research Council Grant 15-33115A

Title: Status epilepticus in immature rats is associated with oxidative stress and mitochondrial dysfunction

Authors: *J. OTAHAL[1], J. FOLBERGROVÁ[2], P. JEŠINA[2], H. KUBOVÁ[2], R. DRUGA[2]; [1]Inst. of Physiol. CAS, Praha, Czech Republic; [2]Inst. of Physiol. CAS, Prague, Czech Republic

Abstract: Epilepsy is a chronic neurologic disorder, particularly frequent in infants and children where it can lead to serious consequences later in life. In majority of patient a cause of their epilepsy remains hidden. Oxidative stress and mitochondrial dysfunction has been shown an important patophysiological mechanism of many neurological disorders. In epilepsy, the role of oxidative stress and mitochondrial dysfunction has been well accepted in adults, however, their role in immature epileptic brain is unclear since there have been two contrary opinions: oxidative stress is age-dependent and does not occur in immature brain during status epilepticus and, on the other hand, oxidative stress in immature brain does occur and is thus general phenomenon during status epilepticus (SE). We have therefore decided to evaluate oxidative stress and mitochondrial functions in immature 12-day-old rats after the status epilepticus induced by substances with a diverse mechanism of action, namely 4-aminopyridine, LiCl-pilocarpine or kainic acid. Mild brain damage especially in hippocampus and thalamus were observed on FluoroJade B stained brain sections in each of the tested models. Reduction in glucose and glycogen levels with parallel increase in lactate shows high rate of glycolysis during SE. Elevated production of superoxide anion was revealed by intravital hydroethidium method (by ~60 %) in the hippocampus, cerebral cortex and thalamus during SE. Consequently, we have observed a specific pronounced decrease of complex I activity that persisted for a long period of survival. Complex II and IV activities remained in the control range. Antioxidant treatment with SOD mimetic MnTMPYP, peroxynitrite scavenger FeTPPS or resvratrol (a natural antioxidative drug with complex mechanism of action) significantly attenuated oxidative stress and inhibition of complex I activity. These findings bring direct evidence that oxidative stress and mitochondrial dysfunction are present also in immature rat brain during SE, and may thus be considered a general phenomenon.

Disclosures: **J. Otahal:** None. **J. Folbergrová:** None. **P. Ješina:** None. **H. Kubová:** None. **R. Druga:** None.

Nanosymposium

768. Oxidative Stress and Antioxidants in Neurological and Psychiatric Diseases

Location: SDCC 32B

Time: Wednesday, November 16, 2016, 1:00 PM - 3:00 PM

Presentation Number: 768.06

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Green tea decreases dopamine metabolism in the dorsal Striatum of Haloperidol treated rats: What can we expect?

Authors: ***T. MALIK**[1], D. J. HALEEM[2];
[1]Basic Sci. Dept., Natl. Univ. of Hlth. Sciences, Basic Scie, Lombard, IL; [2]Neurosci., Panjwani Ctr. of Mol. Research, ICCBS, Karachi, Pakistan

Abstract: Schizophrenia is a prevalent illness (1.1 million in average age of ≥18) in the US that is associated with the significant disability in both man & women. Antipsychotic drugs (APDs), are the only approved treatment, inducing-extrapyramidal symptoms (EPS), which limit drug compliance. EPS are the most common iatrogenic effects imposes an enormous human and economic toll. The antipsychotic drug "haloperidol" (HAL) has been widely used for the treatment of a range of neuropsychiatric disorders. However treatment also induces extrapyramidal symptoms (EPS) including short term parkinsonism, neuroleptic anxiety syndrome (NAS) and late complication tardive dyskinesia (TD). These idiopathic symptoms are associated with serious limitations in this therapy. Some studies have suggested that oxidative stress induced during the metabolism of HAL is involved in the elicitation of EPS. We speculated if green tea may prevent HAL-induced EPS because of its antioxidant properties. The efficacy of green tea extract (GTE) given as a sole source of water on HAL-induced EPS male albino wistar rats was examined. We found that HAL-induced motor deficits, NAS and elicitation of TD were precipitated in GTE than water dinking animals. HAL-induced dopamine level was increased and its metabolites concentrations were higher in the nucleus accumbens and lower ($p < 0.01$) in the caudate of GTE-drinking than water-drinking animals. Increased ratios of homovanillic acid (HVA) and 3, 4-dihydroxyphenylacetic acid (DOPAC)/dopamine in the caudate may be involved in the precipitation of HAL-elicited EPS while drinking GTE. Conversely GTE increased the level of dopamine moreover raised its metabolites in the nucleus accumbens may relapsed the schizophrenic symptoms while on the treatment HAL. Increases serotonin in the nucleus accumbens presented significant phenotype of NAS, and anorexia- like symptoms in rats.

Disclosures: **T. Malik:** None. **D.J. Haleem:** None.

Nanosymposium

768. Oxidative Stress and Antioxidants in Neurological and Psychiatric Diseases

Location: SDCC 32B

Time: Wednesday, November 16, 2016, 1:00 PM - 3:00 PM

Presentation Number: 768.07

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Chemoproteomic approach to characterizing the role of 4-HNE in accelerated cognitive impairment and traumatic brain injury

Authors: ***S. H. LEE**[1], E. M. PIERCE[2], M. BEN AISSA[2], E. NEPOMUCENO[2], G. R. J. THATCHER[2];
[1]Medicinal Chem. and Pharmacognosy, Univ. of Illinois At Chicago, Naperville, IL; [2]Medicinal Chem. and Pharmacognosy, Univ. of Illinois At Chicago, Chicago, IL

Abstract: Dementia is characterized by the decline of cognitive abilities and encompasses several disease including Alzheimer's disease (AD), mixed pathology dementia, and develop following moderate or severe traumatic brain injury (TBI). Currently no therapies exist to prevent, reverse, or stop the progression of these various disease. AD is the most common type of dementia and is associated with a distinct disease pathology, however recent reports have demonstrated that approximately 25% of patients diagnosed with dementia display no distinct pathology. The diversity in the types of dementia leads to several challenges when it comes to diagnosis and drug development. To date drug discovery methods in this area have been unsuccessful and future effort will require a better understanding of the underlying mechanisms of disease progression, the identification of new drug targets, and the development of new preclinical models that more accurately mimic the human diseases. Currently, the exact cellular mechanisms and pathways that lead to the development of age-related dementia remain unknown. Increased oxidative stress has been hypothesized to initiate disease progression in age-related dementia and contribute to the secondary cascade in TBI. Oxidative stress leads to the increased production of lipid peroxidation products (LPx) such as 4-hydroxynonenal (4-HNE) which accumulate in the brain. 4-HNE is an electrophilic LPx capable forming protein adducts at reactive cysteine residues. These protein adducts can act as toxic secondary messengers resulting in the dysregulation of signaling pathways involved in mitochondrial and neuronal function. An increase of 4-HNE-adducts is seen early in the development of dementia, however, the exact role of these 4-HNE adducts in the development of dementia remains unknown. The proposed research seeks to understand the role of LPx by utilizing a chemoproteomic approach in neuronal

cells and a mouse model of age-dependent dementia in order to elucidate novel signaling pathways linked to cognitive decline without traditional degenerative pathology.

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Nanosymposium

768. Oxidative Stress and Antioxidants in Neurological and Psychiatric Diseases

Location: SDCC 32B

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Presentation Number: 768.08

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS K08

NARSAD

Title: Glutathione as a glutamate reservoir: GLU that binds inflammation and neurotransmission

Authors: ***T. W. SEDLAK**, M. KOGA, S. SNYDER, A. SAWA;
Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Glutamate is the most abundant excitatory neurotransmitter, present at the bulk of cortical synapses, and participating in many physiologic and pathologic processes ranging from learning and memory to stroke. The tripeptide, glutathione, is one third glutamate and present at millimolar brain intracellular concentrations, contributing to anti-oxidant and anti-inflammatory defense. Because of the substantial amounts of brain glutathione and its rapid turnover, we hypothesized that glutathione is a relevant reservoir of glutamate, and could influence synaptic excitability. We find that diminishing the liberation of glutamate by the glutathione cycle produces decreases in cytosolic glutamate, decreased frequency of miniature excitatory post synaptic potentials (mEPSC), and diminished depolarization-associated calcium flux. In contrast, pharmacologically decreasing the biosynthesis of glutathione leads to increases in cytosolic glutamate, increased frequency of mEPSC, and increased depolarization-associated calcium release. The glutathione cycle can compensate for decreased excitatory neurotransmission when the glutamate-glutamine shuttle is inhibited. Glutathione may be a physiologic reservoir of glutamate neurotransmitter that bridges anti-inflammatory pathways and glutamatergic functioning.

Disclosures: **T.W. Sedlak:** None. **M. Koga:** None. **S. Snyder:** None. **A. Sawa:** None.

Nanosymposium

769. Axon Growth and Trafficking in Neurodegenerative Disorders

Location: SDCC 25A

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Presentation Number: 769.01

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant NS065183

NIH grant NS089578

Title: Par3-mediated retrograde trafficking of BACE1 limits its convergence with APP

Authors: *H. ZHANG, M. SUN;
Neurosci. and Cell Biol., Robert Wood Johnson Med. School, Rutgers Univ., Piscataway, NJ

Abstract: The convergence of APP and BACE1 is a rate-limiting step in the generation of Aβ, which is a key process during Alzheimer's disease (AD) pathogenesis. However mechanisms that keep APP and BACE1 segregated under physiological conditions remain unclear. Here we show the polarity protein Par3 regulates BACE1 trafficking and limits the convergence of APP and BACE1. We found that Par3 facilitates BACE1 retrograde trafficking from the endosomes to the TGN. We further show Par3 functions through aPKC-mediated phosphorylation of BACE1 on Ser498. Finally, we found that Ser498 phosphorylation promotes the interaction between BACE1 and PACS1, which is necessary for the retrograde trafficking of BACE1 to the TGN. In human AD brains, there is a significant decrease in Ser498 phosphorylation of BACE1 suggesting that defective phosphorylation-dependent retrograde transport of BACE1 is important in the AD pathogenic process. Together, our studies provide mechanistic insight into a novel role for Par3 in regulating retrograde trafficking of BACE1 and limiting APP/BACE1 convergence, and shed light on the mechanisms of AD pathogenesis.

Disclosures: H. Zhang: None. **M. Sun:** None.

Nanosymposium

769. Axon Growth and Trafficking in Neurodegenerative Disorders

Location: SDCC 25A

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Title: Parkinson's disease-associated mutant VPS35 causes mitochondrial dysfunction by recycling DLP1 complexes

Authors: *W. WANG[1], X. WANG[1], H. FUJIOKA[2], C. HOPPEL[3], A. L. WHOEN[4], M. A. CALDWELL[6], P. J. CULLEN[5], J. LIU[7], X. ZHU[1];
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Abstract: Mitochondrial dysfunction plays a key role during the pathogenesis of Parkinson's disease (PD), and increasing studies suggest impaired mitochondrial dynamics and quality control as an important mechanism underlying mitochondrial dysfunction and neuronal dysfunction in PD. VPS35 is the third dominant genetic cause of PD. However, how VPS35 mutants cause PD remains elusive. VPS35 is a key component of the retromer complex important for the retrieval of membrane proteins. Here we demonstrated that PD-associated VPS35 mutations caused mitochondrial fragmentation and neuronal death in primary culture and in mouse substantia nigra. For PD patient bearing VPS35 D620N mutation, cultured fibroblasts also showed extensive mitochondrial fragmentation and dysfunction. VPS35-induced mitochondrial deficits and neuronal dysfunction could be completely prevented by the inhibition of mitochondrial fission both *in vitro* and *in vivo*. Further study revealed that VPS35 mutation caused its increased interaction with DLP1 which enhanced mitochondrial DLP1 complex turnover essential for the efficient mitochondrial fission via mitochondria-derived vesicles-dependent trafficking to lysosome for degradation. Importantly, oxidative stress increased VPS35/DLP1 interaction and VPS35/DLP1 interaction was increased in the brains from patients with sporadic PD. These results revealed a novel cellular mechanism of the involvement of VPS35 in the regulation of mitochondrial fission, dysregulation of which is likely involved in the pathogenesis of VPS35 mutations associated familial PD and possibly also in the sporadic PD cases.

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Nanosymposium

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EMBO Young Investigators Program

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Title: Controlled intracellular delivery of AMPA receptors regulates the postsynapse

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Abstract: Fast excitatory signaling in the brain is greatly mediated by AMPA-type glutamate receptors and information storage in the brain relies on the number of these receptors at synapses. As so, AMPA receptors (AMPARs) need to be inserted in the postsynaptic membrane with precise timing and location for the correct integration of the incoming signal from a partner axon. AMPARs might exchange between synaptic and extrasynaptic sites by lateral diffusion in the plasma membrane, or intracellularly through endosomal recycling and trafficking followed by exocytosis back to the plasma membrane. The spatial coordination between these two transport mechanisms is currently unclear, as little is known about the dynamics of AMPAR-containing endosomes. Moreover, how synapse organization and functioning is determined by the positioning of AMPAR-containing endosomes has never been directly explored.
Using live-cell imaging on hippocampal neuron cultures we were able to show that intracellular AMPARs are transported in Rab11-positive recycling endosomes, which frequently enter dendritic spines and depend on the microtubule and actin cytoskeleton. Moreover, intracellular

transported AMPARs are able to be inserted in the postsynaptic membrane. By selectively coupling Rab11-positive recycling endosomes to kinesin (KIF1C) or myosin (MyosinV/VI) motors using a chemically-induced dimerization system, we are able to control their trafficking and positioning relative to the synapse, which allows us to understand how such trafficking route might be involved in postsynapse dynamics. We found that induced removal of recycling endosomes from spines decreases surface AMPAR expression and PSD-95 clusters at synapses, suggesting a mechanistic link between endosome positioning and the postsynaptic structure and composition.

Disclosures: **M. Esteves Da Silva:** None. **M. Adrian:** None. **P. Schätzle:** None. **J. Lipka:** None. **T. Watanabe:** None. **S. Cho:** None. **K. Futai:** None. **C.J. Wierenga:** None. **L.C. Kapitein:** None. **C.C. Hoogenraad:** None.

Nanosymposium

769. Axon Growth and Trafficking in Neurodegenerative Disorders

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Transport deficits of axonal endo-lysosomes augment mitochondria pathology in spinal motor neurons of asymptomatic fALS mice

Authors: *Y. XIE, B. ZHOU, M.-Y. LIN, Z.-H. SHENG;
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Abstract: A pathological hallmark in ALS-linked motor neurons (MNs) is axonal accumulation of damaged mitochondria, which produce energy and buffer Ca^{2+} less efficiently, and initiate apoptotic cascades. It was thought that the accumulation of those mitochondria in axons is due to impaired mitochondrial transport. We previously tested this by genetically crossing fALS-linked $hSOD1^{G93A}$ mice and *syntaphilin* (*snph*) knockout mice. SNPH is an axonal mitochondria docking receptor; deleting *snph* robustly increases axonal mitochondrial motility (Kang et al., ***Cell*** 2008). However, the two-fold increase in axonal mitochondrial motility does not slow ALS-like disease progression (Zhu and Sheng, ***JBC*** 2011), thus challenging the hypothesis that defective mitochondrial transport contributes to rapid-onset MN degeneration. These observations raise a fundamental question: does impaired degradation of damaged mitochondria by the autophagy-lysosome system play a more pathological role during the early stage of fALS-linked mice?

We recently reveal for the first time spinal MN-targeted progressive lysosomal deficits starting at asymptomatic stages in fALS-linked hSOD1^{G93A} mice (Xie et al. ***Neuron*** 2015, Xie et al. ***Autophagy*** 2015). These deficits impair autophagic degradation, resulting in aberrant accumulation of autophagic vacuoles engulfing damaged mitochondria along MN axons. These phenotypes are captured in cultured adult (P40) spinal MNs from the hSOD1^{G93A} mice. Such early deficits are due to reduced retrograde transport of late endosome (LE) via competitively binding of mutant hSOD1^{G93A} to dynein, and can be reversed by introducing dynein adaptor snapin transgene. Snapin competes with hSOD1^{G93A} for binding to dynein, thereby recruiting more dynein to LEs for transport. Thus, snapin and hSOD1^{G93A} play opposite roles in LE retrograde transport. Expressing snapin efficiently reverses lysosome deficits and facilitates removal of damaged mitochondria. Injecting AAV9-snapin into the diseased mice rescues lysosome deficits *in vivo* and slows MN degeneration and disease progression. Thus, our study advances our knowledge of early pathological mechanisms underlying MN degeneration. Elucidating this early pathological mechanism is broadly relevant, because defective transport, lysosomal deficits, and mitochondrial pathology are all associated with ALS, Huntington's, Parkinson's and Alzheimer's diseases. Therefore, enhancing clearance of damaged mitochondria by regulating endolysosomal trafficking may be a potential therapeutic strategy for ALS and perhaps other neurodegenerative diseases. (Supported by the Intramural Research Program of NINDS, NIH)

Disclosures: **Y. Xie:** None. **B. Zhou:** None. **M. Lin:** None. **Z. Sheng:** None.

Nanosymposium

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Charles & Johanna Busch Biomedical Award

Title: Impaired retrograde transport of axonal autophagosomes contributes to autophagic stress in Alzheimer's disease neurons

Authors: *Q. CAI, P. TAMMINENI, X. YE;
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Abstract: Neurons face the unique challenge of transporting nascent autophagic vacuoles (AVs) from distal axons toward the soma, where mature lysosomes are mainly located. Defective autophagy has been linked to Alzheimer's disease (AD). However, the mechanisms underlying altered autophagy are largely unknown. Here, we demonstrate that defects in retrograde transport contribute to autophagic stress in AD axons. Amphisomes predominantly accumulate at synaptic terminals of mutant hAPP mice and AD patient brains. Soluble amyloid-β (Aβ) is enriched in AD axons and interacts with dynein motors. This interaction impairs dynein recruitment to amphisomes through interruption of dynein-Snapin motor-adaptor complex coupling, thus immobilizing them in distal axons. Consistently, deleting *snapin* in mice causes AD-like axonal autophagic stress, whereas overexpressing Snapin in hAPP neurons reduces autophagic accumulation at axonal terminals by enhancing AV retrograde transport. Altogether, our study provides new mechanistic insight into AD-associated autophagic stress, establishing a foundation for ameliorating axonal pathology in AD.

Disclosures: Q. Cai: None. **P. Tammineni:** None. **X. Ye:** None.

Nanosymposium

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Facilitation of axon regeneration by enhancing mitochondrial transport and rescuing energy deficits

Authors: *B. ZHOU[1], P. YU[2], M.-Y. LIN[1], T. SUN[1], Z.-H. SHENG[1];
[1]NINDS, Bethesda, MD; [2]NHLBI, Bethesda, MD

Abstract: While neurons in early developmental stages possess robust ability for axon growth, mature neurons in the central nervous system (CNS) fail to regrow after axonal injury. Regeneration is a highly energy-demanding process, raising a fundamental question as to whether mitochondrial transport is necessary for mature neurons to meet enhanced metabolic requirements during regeneration. Our previous study demonstrated that syntaphilin (SNPH) acts as a "static anchor" specific for axonal mitochondria (Kang et al., ***Cell*** 2008; Chen and Sheng,

JCB 2013). Deleting murine *snph* results in a substantially increased percentage (~70%) of motile axonal mitochondria. Conversely, over-expressing SNPH abolishes axonal mitochondrial transport. Most interestingly, SNPH is strictly developmentally regulated in the brain: its expression is hardly detectable in embryonic stages, very low before postnatal day 7 (P7), and peaks at adult stages. The unique pattern of SNPH expression in the brain and its specific role in anchoring axonal mitochondria allow us to propose an attractive hypothesis: mature neuron-associated decline of mitochondrial transport is an intrinsic mechanism controlling axon regrowth capacity. Thus, *snph* knockout (KO) mice provide an ideal model to investigate how mitochondrial trafficking and anchoring influences axonal regenerative capacity in mature neurons.
Here, we reveal that reduced mitochondrial motility and energy deficits in injured axons are intrinsic mechanisms controlling regrowth in mature neurons. Axonal mitochondrial transport progressively declines with maturation. Applying microfluidic culture devices, we found that axotomy induces acute mitochondrial depolarization and ATP depletion in injured axons. Thus, mature neuron-associated increases in SNPH expression and decline of mitochondrial transport cause local energy deficits. Strikingly, enhancing mitochondrial transport via genetic manipulation facilitates regenerative capacity in mature neurons by replenishing healthy mitochondria in injured axons, thereby rescuing energy deficits. An *in vivo* sciatic nerve crush study further shows that enhanced axonal mitochondrial transport in *snph* KO mice accelerates axon regeneration. Understanding deficits in mitochondrial trafficking and energy supply in injured axons of mature neurons benefits development of new strategies to stimulate axon regeneration. (Supported by the Intramural Research Program of NINDS, NIH)

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Title: Compartment-specific regulation of neuronal autophagy during homeostasis and starvation

Authors: *S. MADAY;
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Abstract: Autophagy is an essential degradative pathway that maintains neuronal homeostasis and prevents axon degeneration. Initial observations suggest that autophagy is spatially regulated in neurons, but how autophagy is regulated in distinct neuronal compartments is unclear. Here, we use live-cell imaging in mouse hippocampal neurons to establish the compartment-specific mechanisms of autophagy under basal conditions, and during stress induced by nutrient deprivation. We find that at steady state, the soma contains multiple populations of autophagosomes that are derived from distinct neuronal compartments, and are defined by maturation state and dynamics. Axonal autophagosomes enter the soma largely LAMP1-positive and remain confined within the somatodendritic domain. This compartmentalization likely facilitates cargo degradation by promoting fusion with proteolytically-active lysosomes that are enriched in this region. The soma also contains LAMP-negative autophagosomes, likely formed locally, that are less mobile and tend to cluster. Thus, while axonal autophagy is a vectorial process that delivers cargo from the distal axon to the soma, the soma contains autophagosomes at different maturation states, receiving input from the axon combined with locally generated autophagosomes. Surprisingly, starvation conditions that effectively activate autophagy in other cell types (e.g. hepatocytes and HeLa cells) did not induce autophagy in either the axonal or somatodendritic compartment. While starvation robustly decreased mTORC1 signaling in neurons, this decrease was not sufficient to activate autophagy. Furthermore, pharmacological inhibition of mTOR with Torin1 also was not sufficient to markedly upregulate neuronal autophagy. These observations suggest that the primary physiological function of autophagy in neurons, unlike in other cell types, may not be to mobilize amino acids and other biosynthetic building blocks in response to nutrient deprivation. Rather, constitutive autophagy in neurons may function to maintain cellular homeostasis and regulate the quality of the neuronal proteome by balancing synthesis and degradation, especially within distal axonal processes far removed from the soma. Supported by NIH grants K99NS082619 and R00NS082619 to S.M.

Disclosures: S. Maday: None.

Nanosymposium

769. Axon Growth and Trafficking in Neurodegenerative Disorders

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Motility and degradation in the endolysosomal system in dendrites

Authors: *C. YAP, L. DIGILIO, L. MCMAHON, B. WINCKLER;
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Abstract: Neurons are among the largest cells in the body and need to regulate membrane traffic precisely in time and space. In addition, neurons are long-lived cells and need to tightly regulate proteastasis and keep a balance between new protein synthesis and degradation. Membrane proteins are trafficked throughout axons and dendrites over long distances and can recycle or ultimately degrade in lysosomes. The balance between recycling and lysosomal degradation also impacts the signal duration emanating from many receptors important during development and throughout the neuron's life. In fact, dysregulation of this balance can lead to accumulation of proteins in lysosomes and autophagosomes and impact viability. Not surprisingly, neurological disorders have been strongly linked to membrane traffic. We have studied the trafficking of NEEP21 and P19 (aka Nsg-1 and Nsg-2), small membrane proteins found in somatodendritic endosomes and important for the correct trafficking of several receptors, including L1, GluA2, and β-APP. We find that NEEP21 and P19 internalize from the plasma membrane and have an unusual short half-life of about 90 minutes. Using live imaging and interference approaches we address the dynamics of degradative endolysosomes in dendrites and characterize where and when NEEP21 and P19 encounter degradative endosomes and in what ways their trafficking differ from long-lived membrane proteins.

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Nanosymposium

770. Inflammatory and Non-Inflammatory Mechanisms of NeuroHIV/AIDS

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Title: Pathway dysregulations associated with neuroAIDS in humans without HIVE

Authors: *P. P. SANNA[1], V. REPUNTE-CANONIGO[1], E. MASLIAH[2], C. LEFEBVRE[3];
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Abstract: To better understand the events leading to cognitive impairments in HIV, we focused on patients without HIV encephalitis (HIVE) in the National NeuroAIDS Tissue Consortium

(NNTC) gene expression profile dataset using the Gene Set Enrichment Analysis (GSEA) algorithm. Gene expression evidence indicates that induction of interferon response genes, cytokine signaling complement, and antigen presentation are early events in neuroAIDS (e.g., up-regulation of pathways such as REACTOME INTERFERON SIGNALING; KEGG CYTOKINE CYTOKINE RECEPTOR INTERACTION; REACTOME CHEMOKINE RECEPTORS BIND CHEMOKINES; REACTOME ANTIGEN PROCESSING CROSS PRESENTATION; KEGG COMPLEMENT AND COAGULATION CASCADES; REACTOME IMMUNOREGULATORY INTERACTIONS BETWEEN A LYMPHOID AND A NON LYMPHOID CELL; BIOCARTA LYM PATHWAY). The analysis also indicated reduced neuronal trophism (e.g., pathways such as REACTOME L1CAM INTERACTIONS; KEGG NEUROTROPHIN SIGNALING PATHWAY) impacting synaptic communication and capacity for neuronal plasticity as an early event in both neocortex and basal ganglia (KEGG PARKINSONS DISEASE, KEGG HUNTINGTONS DISEASE, REACTOME NEURONAL SYSTEM; REACTOME TRANSMISSION ACROSS CHEMICAL SYNAPSES; REACTOME POTASSIUM CHANNELS), and involving both excitatory and inhibitory neurotransmission (REACTOME GABA RECEPTOR ACTIVATION; REACTOME GABA SYNTHESIS RELEASE REUPTAKE AND DEGRADATION; REACTOME GLUTAMATE NEUROTRANSMITTER RELEASE CYCLE; REACTOME TRAFFICKING OF AMPA RECEPTORS; REACTOME ACTIVATION OF NMDA RECEPTOR UPON GLUTAMATE BINDING AND POSTSYNAPTIC EVENTS). Additionally, we observed a significant dysregulation of mitochondrial genes in the early stages of neuroAIDS, pointing to oxidative metabolism dysfunction (KEGG OXIDATIVE PHOSPHORYLATION; REACTOME REACTOME RESPIRATORY ELECTRON TRANSPORT; REACTOME MITOCHONDRIAL PROTEIN IMPORT). Lastly, gene expression evidence indicated dysregulations of RNA processing (REACTOME MRNA CAPPING; REACTOME MRNA SPLICING; REACTOME RNA POL II PRE TRANSCRIPTION EVENTS; REACTOME TRNA AMINOACYLATION) and protein degradation (BIOCARTA PROTEASOME PATHWAY; REACTOME METABOLISM OF PROTEINS). Altogether, the present GSEA-based analysis points to immune activation and early trophic and metabolic insults in the early stages of neuroAIDS well before the onset of HIVE.

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Title: Allopregnanolone regulation of mitochondrial function may attenuate HIV-1 Tat and morphine-mediated neurotoxicity

Authors: *J. J. PARIS[1], S. KIM[2], J. BALINANG[2], S. ZHOU[2], P. E. KNAPP[2], K. F. HAUSER[3];
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Abstract: Human immunodeficiency virus (HIV) infection is associated with a constellation of neurological disorders that may be exacerbated by opioid drug use. One virotoxin mediating these effects is the HIV-1 regulatory protein, trans-activator of transcription (Tat). Opioids, such as morphine, can exert additive or synergistic effects on HIV-1 Tat promoting neuroinflammation and neurotoxicity *in vitro*. We have observed the progesterone metabolite and neurosteroid, 5α-pregnan-3α-ol-20-one (i.e., allopregnanolone), to ameliorate Tat-mediated dysregulation of intracellular calcium homeostasis in microglia and neurons, as well as microgliosis and neuronal cell death *in vitro*. However, the allopregnanolone mechanisms of Tat-mediated protection and their capacity to be sustained when catalyzed by an opioid challenge were not known. Primary striatal neurons or SH-SY5Y neuronal cultures were incubated with media that did, or did not, contain progesterone or allopregnanolone (0.1, 1, 10, or 100 nM) in the absence or presence of morphine (500 nM) and/or HIV-1 Tat_{1-86} (100 nM). Our data demonstrate that the application of Tat was neurotoxic. While, morphine was not toxic on its own, the combination of morphine and Tat displayed additive neurotoxicity. Steroids conferred concentration-dependent protection against Tat-toxicity. In particular, allopregnanolone significantly attenuated Tat-mediated mitochondrial membrane depolarization in a concentration-dependent manner. Allopregnanolone restored mitochondrial membrane homeostasis in response to challenge with morphine and/or HIV-1 Tat. These data support the notion that neurosteroid-based therapeutics may confer prophylactic advantages for HIV-positive opioid users.

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Title: Methamphetamine and HIV-1/gp120 protein induce lasting changes in gene expression affecting dopaminergic and serotonergic neurotransmission

Authors: *A. B. SANCHEZ[1], R. MAUNG[1], M. KAUL[1,2], T. TMARC GROUP[2];
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Abstract: Individuals infected with human immunodeficiency virus type-1 (HIV-1) frequently use methamphetamine (METH). Combination of viral infection and METH in the central nervous system (CNS) is suspected to exacerbate HIV-associated neurocognitive disorders (HAND). METH abuse is a serious public health concern because it can lead to irreversible damage in the brain, compromising several neurotransmitter systems, including the dopaminergic and serotonergic network. However, the combined effects of HIV-1 and METH on the brain are incompletely understood at the molecular level. We recently treated 3-4 months old HIV-1/gp120 transgenic (gp120tg) and wild type (wt) mice with an escalating METH binge regimen for 25 days. At 10-12 months of age, HIV-1/gp120tg and METH-exposed animals showed significant impairment in spatial learning and memory and neuropathology. METH-treated HIV-1gp120tg mice were the most severely affected. In order to investigate underlying mechanisms in the brain, we used quantitative RT-PCR arrays to assess expression of genes related to the dopaminergic and serotonergic neurotransmission systems. Six comparisons between the four experimental groups revealed significant gene regulation due to METH exposure and chronic HIV-1/gp120 expression: 1) WT Saline (SAL) versus (vs.) WT METH - 10 genes, 6 up (e.g. *Htr1d/f, Akt1*), 4 down (e.g. *Vmat2, Sert1, Bdnf*); 2) WT SAL vs. gp120 SAL - 10 genes, 9 up (e.g. *Htr6, Pde4a/d, Synphilin, Gfap*), 1 down (*Cdk5*); 3) WT SAL vs. gp120 METH - 13 genes, 1 up (*Gfap*), 12 down (e.g. *Bdnf, Casp3, Htr1d/7, Drd5*); 4) WT METH vs. gp120 SAL- 4 genes, 3 up (e.g. *Gfap, Htr6*), 1 down (*Cdk5*); 5) WT METH vs. gp120 METH - 21 genes, 2 up (*Gfap, B2m*), 19 down (e.g. *Htr1a/1d/1f/2c/4/7, Vmat1*); 6) gp120 SAL vs. gp120

METH - 23 genes, 0 up, 23 down (e.g. *Gfap, Drd5, Htr1a/1d/2c/4/7*). In summary, histopathology and impaired spatial learning and memory due to METH exposure and HIV-1/gp120 expression are associated with significant alterations of the dopaminergic and serotonergic neurotransmission systems.

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Title: HIV-1 Tat induced loss of dendritic spines: Evidence from longitudinal *In vivo* multiphoton imaging.

Authors: *J. D. RAYBUCK[1], S. A. THAYER[2];
[1]Dept. of Pharmacol., UMN, Minneapolis, MN; [2]Pharmacol., Univ. of Minnesota, Minneapolis, MN

Abstract: HIV-associated neurocognitive disorder (HAND) affects approximately half of HIV-infected patients. Disruption of synaptic connections is a hallmark of many neurocognitive disorders, including HAND. Because the virus does not infect neurons directly HIV neurotoxicity is indirect, mediated by released neurotoxins, including the HIV protein transactivator of transcription (Tat). Tat can disrupt synaptic connections both *in vitro* and *in vivo* and is linked to impaired neurocognitive functions in humans. To examine the time course of Tat's effects, we developed a longitudinal *in vivo* imaging assay. This approach allows us to repeatedly image dendritic spines in Layer 1 cortex of Thy1-YFP transgenic mice, which express fluorescent protein in a subset of pyramidal neurons. Mice were fitted with a cranial window over retrosplenial cortex and a cannula into the lateral ventricle. Following recovery from surgery mice were imaged for a series of spaced sessions to generate baseline measures, subsequently mice received ICV infusion of HIV-1 Tat and were imaged at intervals to capture the time course of spine changes. A single ICV infusion of HIV-1 Tat induced a 17% (±1.4) loss

of dendritic spines, which lasted for 2-3 weeks. Additionally, to determine if this spine loss was accompanied by disrupted cognitive function, we infused mice with Tat (ICV) prior to fear conditioning. In preliminary studies, Tat infusion produced deficits in learning and memory. To determine if *in vivo* spine loss could be rescued, we administered a GluN2B antagonist during the peak of spine loss. Similar to previous *in vitro* reports, administration of a GluN2B antagonist increased spine density to levels comparable to baseline. These findings suggest that NMDA receptor signaling may be a critical mediator of inflamatory driven changes in spine density, and they demonstrate the utility of *in vivo* imaging to model the time course of spine loss and recovery in neuroinflamatory disease states.

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Title: Effects of flaxseed lignin, secoisolariciresinol diglucose, on endogenous antioxidant response pathway in HIV-infected macrophages

Authors: *K. S. WILLIAMS, K. JORDAN-SCIUTTO;
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Abstract: Macrophages and microglia play pivotal roles in pathogenesis HIV associated neurocognitive disorders. During acute infection, activated and/or infected monocytes infiltrate the central nervous system (CNS) and differentiate into perivascular macrophages. HIV can then spread to microglia, creating an active, protected reservoir in the CNS. The ensuing inflammatory macrophage/microglia activation and secretion of a myriad of toxic factors cause damage to neurons, which are not infected. Thus, current work strives to develop strategies to suppress M/M-driven inflammation and oxidative stress. Numerous studies utilizing exogenous anti-inflammatory and antioxidants to mitigate disease progression have been unsuccessful; however, targeting endogenous antioxidant pathways may be more advantageous. A cellular mechanism that mitigates oxidative stress and inflammation is the endogenous antioxidant response (EAR) pathway, which upregulates key antioxidant enzymes, including heme

oxygenase 1 (HO-1), to reduce free radicals and suppress tissue damage. In this study, we investigated the role of a flaxseed lignin, secoisolariciresinol diglucose (SDG), on viral replication and oxidative stress in HIV-infected macrophages. To evaluate EAR, human monocyte derived macrophages were infected with HIV for 1 day. By immunoblot, HIV suppressed HO-1 levels, in macrophages, while concurrent treatment with SDG increased HO-1 levels. This was paralleled by increased translocation of nrf2 protein from the cytoplasm to the nucleus. Prolonged HO-1 deficiency in macrophages has been negatively correlated with increased HIV replication in human macrophages. HO-1 levels were decreased in macrophages 12-day post infection. To determine if SDG can reverse prolonged HO-1 deficits, macrophages were pretreated with SDG for 1 hour prior to infection and replenished every three days. SDG treatment increased HO-1 protein levels after 12 days post infection. Also, pretreatment of SDG treatment partially suppressed viral replication 12 days post infection in human macrophages, assessed by a reverse transcriptase assay. Given these data, SDG increases endogenous antioxidants pathways and may be a possible adjunctive therapeutic for HIV associated neurocognitive disorders.

Disclosures: K.S. Williams: None. **K. Jordan-Sciutto:** None.

Nanosymposium

770. Inflammatory and Non-Inflammatory Mechanisms of NeuroHIV/AIDS

Location: SDCC 4

Time: Wednesday, November 16, 2016, 1:00 PM - 4:15 PM

Presentation Number: 770.06

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant DA15014

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Title: Alterations in dendritic spine density and morphology in neuroAIDS: variable susceptibility amongst brain regions in two rodent models of HAND

Authors: *L. FESTA[1], C. LIN[1], B. PLATT[1], S. FLORESCO[3], B. WATERHOUSE[2], O. MEUCCI[1];
[1]Pharmacol. & Physiol., [2]Neurobio. & Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [3]The Univ. of British Columbia, Vancouver, BC, Canada

Abstract: HIV-associated neurocognitive disorders (HAND) continue to affect a large portion of HIV+ individuals (~10-50%). Focus has shifted from neuronal loss and apoptosis, which

predominantly occurred prior to the cART era, to sub-lethal synaptodendritic alterations since it is well correlated with neurocognitive impairment and may be reversible. Loss of dendritic spines on excitatory neurons in the medial prefrontal cortex (mPFC) has been previously documented by our group in two noninfectious rodent models of HAND: gp120-treated rats and HIV-Tg rats. Both of these animal models display deficits in cognitive flexibility, which is significantly correlated with loss of dendritic spines. While our studies have focused primarily on the mPFC due to its role in executive function, it is presently unclear whether other brain regions are equally susceptible to the effects of HIV proteins and inflammation. Additionally, the morphology of dendritic spines is crucial for their function; mushroom and thin spines, which are associated with LTP and learning, are considered mature, while stubby and filopodia are thought to be immature, since they lack a well-defined neck and AMPA receptors. It remains to be seen if, in addition to changes in dendritic spine density, alterations in spine morphology occur in HAND. The objective of this study was to evaluate dendritic spine number and morphology in three brain regions (mPFC, motor cortex, and somatosensory cortex) of gp120-treated and HIV-Tg rats. Dendritic spines were evaluated using either Golgi stain or biolistic labeling with DiI, followed by neuronal reconstruction with Neurolucida 360. Dendritic spine density of layer II/III pyramidal neurons in the mPFC and motor cortex of both gp120-treated and HIV-Tg rats was significantly reduced compared to controls. The somatosensory cortex, on the other hand, did not exhibit any significant alterations in dendritic spine numbers. Changes in dendritic spine morphology were observed in all three regions analyzed with a shift towards an immature spine type found in both HIV animal models, though slight differences were detected between brain areas and rodent models. These studies highlight the variable regional susceptibility of neurons to the damage induced by HIV proteins and inflammation, which may underlie the cognitive and behavioral symptoms observed in patients. Furthermore, the shift towards an immature spine morphology in our two animal models of HAND suggests that these neurons may not be able to sequester incoming excitatory neurotransmission in a regulated fashion, leading to neuronal injury and dysfunction.

Disclosures: **L. Festa:** None. **C. Lin:** None. **B. Platt:** None. **S. Floresco:** None. **B. Waterhouse:** None. **O. Meucci:** None.

Nanosymposium

770. Inflammatory and Non-Inflammatory Mechanisms of NeuroHIV/AIDS

Location: SDCC 4

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Presentation Number: 770.07

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Role of atf6b in HIV-associated neurocognitive disorders

Authors: *C. AKAY ESPINOZA, P. LIN;
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Abstract: The underlying mechanism of cognitive impairment and brain injury in patients with HIV-associated neurocognitive disorder (HAND) on suppressive antiretroviral therapy are not completely understood. However, synaptic injury, neuronal dysfunction and damage in these patients are partially driven by immune activation and chronic inflammation in response to soluble factors released by HIV-infected and/or activated macrophages, as well as a low level of HIV replication in CNS reservoirs. A majority of these mediators as well as many of the comorbid conditions were shown to induce a ubiquitous cellular response, unfolded protein response (UPR) *in vitro* and *in vivo*. We have previously shown UPR activation in post-mortem tissue from HAND patients *in vivo.* One of the three master UPR initiator proteins, ATF6b, upon cleavage by site-1 and site-2 proteases (S1P and S2P), translocates to the nucleus for transcriptional induction of ER resident chaperones, apoptotic genes, and secretory pathway regulatory genes. We hypothesized that activation of the ATF6b pathway of the UPR contributed to neuronal damage and death observed in HAND. We found that infection of primary human monocyte-derived macrophages (MDMs) with HIV led to the nuclear translocation of the cleaved, thus active, ATF6b (N-ATF6b), which could be blocked by S1P inhibition. We also observed that blocking nuclear accumulation of N-ATF6b in macrophages led to attenuation of death of primary rat cortical neuroglial cultures exposed to supernatants from HIV-infected MDMs. Finally, we found nuclear N-ATF6b accumulation in neurons exposed to HIV-infected MDM supernatants or excitotoxic stimulus. These findings suggest that altered function of ATF6b in several cell types within the brain might be contributing to neuronal dysfunction and damage in patients with HAND.

Disclosures: C. Akay Espinoza: None. **P. Lin:** None.

Nanosymposium

770. Inflammatory and Non-Inflammatory Mechanisms of NeuroHIV/AIDS

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Title: Intraneuronal β-amyloid accumulation in HIV-1 transgenic rats

Authors: *H. LI, C. MACTUTUS, R. BOOZE;
Psychology, Univ. of South Carolina, Columbia, SC

Abstract: Many HIV positive patients are reaching older age. According to the Centers for Disease Control's assessment, half of all people living with HIV infection in the United States will be older than 50 years of age by 2015. However, an increase in mild cognitive impairment has become prevalent in these patients. It is critical to elucidate the cause of the worsening cognitive impairment. We used HIV-1 transgenic rats (Fisher 344) as a model to study the amount of abnormal protein aggregates (β-Amyloid) in the brain, which may play a role in the neurodegenerative process of HIV. First, we used immunohistochemistry staining to detect the intraneuronal β-Amyloid expression in the hippocampus and the cortex. Second, we observed the expression of amyloid precursor protein (APP) in the hippocampus and the cortex both in HIV-1 transgenic rats and F344 control rats. The results indicated that an abnormal intraneuronal β-Amyloid accumulation was found in hippocampal CA3 region (1.34 fold increase) and cortex (4.06 fold increase) in HIV-1 transgenic rats compared with the F344 control rats. Interestingly, a higher amount of amyloid precursor protein was detected in CA3 region of hippocampus in F344 control rats (3.81 fold increase) relative to the HIV-1 transgenic rats. However, there was no significant difference of amyloid precursor protein expression in cortex between the F344 control and HIV-1 transgenic rats. Further experiments will elucidate the potential effects of intraneuronal β-Amyloid accumulation on the HIV-induced neuronal dysfunction. Collectively, in HIV patients, an accumulation of β-Amyloid suggests that long-term survival with HIV might interfere with the elimination of harmful proteins like β-Amyloid that might worsen the neurodegenerative process and cognitive impairment.

Disclosures: **H. Li:** None. **C. Mactutus:** None. **R. Booze:** None.

Nanosymposium

770. Inflammatory and Non-Inflammatory Mechanisms of NeuroHIV/AIDS

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Title: Examining the effect of HIV on measures of medial prefrontal cortex functioning

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Abstract: Introduction: Executive functioning deficits are common in adults with HIV and cause significant functional impairment. Compared to the executive functions carried out by the dorsolateral prefrontal cortex (e.g., working memory), executive abilities associated with medial prefrontal regions (anterior cingulate and superior) have received considerably less attention in the context of HIV. The medial prefrontal cortex contributes to the initiation and maintenance of mental processes and behavior, and the inhibition of prepotent responses. With little research in this area, it is currently unclear whether these executive abilities are impaired in HIV-positive (HIV+) adults on antiretroviral therapy (ART). The current study assessed HIV+ and seronegative healthy control (HC) adults on several measures of medial prefrontal function.
Method: We compared HIV+ participants (n=32) with minimal comorbidity to a demographically similar HC group (n=26) on composite measures of cognitive initiation and maintenance (Letter Fluency, first 15 seconds; Continuous Performance Test, reaction time variability), response inhibition (Antisaccade task; Flanker task), and an apathy self-report measure (Apathy Evaluation Scale). Composite scores were calculated by averaging standardized z-scores within each executive domain. Associations with demographic and HIV disease variables were assessed.
Results: HIV+ and HC groups did not differ significantly in age, male:female ratio, ethnicity, substance use history, or rate of hepatitis C infection; however, significant differences were observed on a measure of literacy ($t=2.40$, $p<.05$). Controlling for literacy, the HIV+ group performed worse than the HC group on measures of cognitive initiation/maintenance ($F[2, 51]=6.77$, $p=.01$, $d=.72$) and response inhibition ($F[2, 51]=4.01$, $p=.05$, $d=.56$). The groups did not differ significantly on self-reported apathy levels ($F[1,55]=.05$, $p>.05$, $d=.06$). In the HIV+ group, cognitive initiation/maintenance, response inhibition, and apathy were not significantly associated with HIV disease variables (current viral load, current CD4, nadir CD4, disease duration), substance use history, or age. However, hepatitis C co-infection was associated with poorer response inhibition ($r_{pb}=.30$, $p<.05$).
Conclusion: Our results indicate that HIV+ individuals on ART with minimal comorbidity display deficits on measures of cognitive initiation/maintenance and response inhibition. These findings suggest that medial prefrontal brain regions are vulnerable to the pathophysiology of HIV, and that these effects may be compounded by hepatitis C co-infection.

Disclosures: K. Walker: None. **R. Hegde:** None. **D. Byrd:** None. **S. Morgello:** None. **U. Clark:** None.

Nanosymposium

770. Inflammatory and Non-Inflammatory Mechanisms of NeuroHIV/AIDS

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Title: Impact of age on Ca^{2+} influx through voltage-gated Ca^{2+} channels in the context of HIV infection.

Authors: *C. KHODR[1], L. CHEN[1], L. AL-HARTHI[2], X.-T. HU[1];
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Abstract: HIV-associated neurocognitive disorders (HAND) is a co-morbid condition of HIV, even in the era of combined antiretroviral therapy. HAND is characterized by deficits in executive function, attention, memory and/or motor function. Such deficits are associated with dysregulation of the medial prefrontal cortex (mPFC), a brain region altered in HIV^+ patients. HIV-related neurotoxicity can result from effects of HIV proteins and HIV-induced inflammatory responses on Ca^{2+} homeostasis. We have been examining the effects of HIV on the activity of voltage-gated Ca^{2+} channels (VGCCs) in pyramidal neurons in the mPFC, using the HIV-1 transgenic (Tg) rat that express 7 of the 9 HIV-1 genes. We have shown that HIV abnormally enhances Ca^{2+} influx through VGCCs after 6-7 weeks of exposure, which is associated with mPFC neuronal hyper-excitation. We have also reported that, after 12 months (m) of HIV exposure, pyramidal neurons from HIV-1 Tg rats remain hyper-excitable compared to those in age-matched non-Tg rats. In the current study, we evaluated VGCC activity in mPFC pyramidal neurons in 12m-old HIV-1 Tg rats. Whole-cell current-clamp recordings were performed in brain slices from 12m-old HIV-1 Tg and non-Tg rats. Na^+ and K^+ channels, as well as excitatory and inhibitory inputs, were blocked to assess Ca^{2+} influx through VGCCs (represented by Ca^{2+} plateau potentials) in mPFC pyramidal neurons. We found that neurons in 12m-old HIV-1 Tg rats required a stronger excitatory current stimulation (i.e., rheobase) to elicit a Ca^{2+} potential than those in 12m-old non-Tg rats (Tg: 256.0±6.9 pA *vs*. non-Tg: 195.0±10.8 pA, $p \leq 0.001$). But there was no significant difference in the Ca^{2+} potential duration or area (reflecting Ca^{2+} influx) between 12m-old HIV-1 Tg and non-Tg rats. These results suggest that the function of VGCCs is differentially affected by age in the context of HIV infection, and that the increased intrinsic excitability of mPFC pyramidal neurons among older HIV-Tg rats is likely due to the involvement of other non-VGCC ion channels. In fact, a parallel study by our

research team (Chen et al., 2016, presented in this meeting) reveals a reduction of K^+ channel activity that is associated with this increase of mPFC neuronal excitability. Together, our current and previous studies demonstrate that aging affects the nature of ion channel dysregulation that leads to neuronal hyper-excitation in the mPFC, in the context of HIV infection.

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Nanosymposium

770. Inflammatory and Non-Inflammatory Mechanisms of NeuroHIV/AIDS

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: HIV-1 Tat causes structural abnormalities in CA1 regional microcircuitry, and disturbances in CA1 function and memory formation

Authors: W. D. MARKS[1], A. J. BARBOUR[1], J. J. PARIS[1], M. D. DENTON[1], A. R. MCQUISTON[1], P. E. KNAPP[1], *K. F. HAUSER[2];
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Abstract: The hippocampus, which plays a significant role in memory, is known to be susceptible to HIV-1 Tat. We previously found that HIV-1 Tat causes pronounced loss of long-term potentiation (LTP) and deficits in spatial memory-dependent tasks. Despite these deficits, CA1 pyramidal cells, which are the only neurons that project axons from this region, showed only modest decreases in the density of apical dendritic spines, without losses in dendrite length or synaptic integrity at the ultrastructural level. By contrast, there were selective alterations in proteins involved in inhibitory GABAergic synaptic function, including a significant decline in synaptotagmin 2 (Syt2) expression only within stratum radiatum and marked increases in the expression of gephyrin throughout CA1 (Fitting et al. 2013, *Biol Psychiatry*). With each layer comprised of multiple inhibitory interneuron subtypes with distinct functions and morphology, we hypothesized that one or more subsets of CA1 hippocampal interneurons might be selectively vulnerable to HIV-1 Tat. To address this question, we used inducible transgenic Tat-expressing mice to examine structural and functional changes in CA1. Immunohistochemical and morphological analyses revealed several vulnerable types of interneurons within CA1;

specifically nNOS+/NPY− interneurons of the stratum pyramidale and stratum radiatum (interneuron specific interneuron type 3 and neurogliaform cells respectively), parvalbumin+ cells of the stratum pyramidale (putatively bistratified cells), and somatostatin+ cells of the stratum oriens (putatively oriens-lacunosum moleculare projection cells). Interestingly, these interneurons are a significant part of a local feedback and input gating circuit known to be involved in spatial memory formation. Electrophysiological analysis of CA1 pyramidal cells in slices shows that Tat shifts the resting membrane potential to a slightly more depolarized state, and increases the amount of current needed to depolarize cells. Ongoing electrophysiological studies will look at pyramidal cell activity in the absence of feedback circuit activity, spontaneous inhibitory postsynaptic currents, and probe the network properties of the identified microcircuit domain within CA1. Tat induction resulted in deficits in spatial memory as assessed using the Barnes maze and novel object recognition tasks, similar to those observed in other HIV models, and recapitulating spatial memory deficits observed in human patients. Our observations of morphological and functional deficits provide new insight into the origins of the cellular and molecular pathology underlying neuroAIDS.

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Nanosymposium

770. Inflammatory and Non-Inflammatory Mechanisms of NeuroHIV/AIDS

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Type 1 interferon signaling in morphine-potentiated LP-BM5 murine AIDS

Authors: *V. D. MCLANE, L. CAO;
Col. of Osteo. Med., Univ. of New England, Biddeford, ME

Abstract: Opioid abuse increases the severity of HIV-associated neurocognitive deficits (HAND). Morphine attenuates the inflammatory response of glia, which are critical in central nervous system (CNS) immune defense. Using the LP-BM5 murine AIDS model, we investigated the effect of morphine on the CNS immune response in the presence of a live viral infection. In our model, morphine treatment increased viral RNA expression (‘viral load’) in hippocampus while decreasing viral load in striatum. Using quantitative RT-PCR, we found that morphine increased striatal expression of interferon (IFN)-α and IFN-β, correlating to a decline

in viral load. To further explore this, we performed an 84-gene PCR array to assess type 1 IFN signaling in hippocampus, striatum, and frontal lobe. Morphine and LP-BM5 had significant effects on 35 of the 84 genes in our three regions of interest. In the frontal lobe, morphine and LP-BM5 led to significant upregulation of IFN regulatory factor (IRF)-7 and IRF-8; however, we did not observe a change in frontal lobe viral load with morphine treatment, potentially due to morphine-induced suppression of the proinflammatory response. In the hippocampus, we found that multiple genes in the IFN signaling pathway were significantly downregulated by both LP-BM5 infection and morphine treatment, correlating to a morphine-induced increase in hippocampal viral load. Overall, we observed region-specific changes in viral load that correlated to regional differences in expression of type 1 IFNs, IFN responsive genes, and IFN regulatory factors. With this work, we aim to provide fresh insight into the influence of morphine on CNS type 1 IFN activation during the development of HAND.

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Nanosymposium

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Title: Creation of a humanized mice brain for studies of hiv-1 neuropathogenesis

Authors: W. LI, S. GORANTLA, H. E. GENDELMAN, *L. Y. POLUEKTOVA;
Pharmacol. and Exptl. Neurosci., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Rodent models of human immunodeficiency virus type one (HIV-1) brain infections poorly reflect human glial-virus interactions during progressive viral infection and consequent innate and adaptive immune responses. NSG mice reconstituted with human hematolymphoid and glial cells were infected with HIV-1, sacrificed then subjected to histopathologic and molecular tests. Glial precursors (GP) generated from human brain tissue neurospheres were injected into lateral ventricle of neonates. The mice were simultaneously intrahepatically injected with human CD34+ hematopoietic stem cells. At 4 months of age animals were infected for 7 weeks with HIV-1ADA. Human astrocytes were the major type of cells populated white matter

tracts and distributed in periventricular areas. Distribution of human GFAP+ cells in brain hemispheres was anatomically symmetric. Map2+ and NG2+ cells with neuronal and oligodendrocyte morphology were also present in limited number. No evidence of mouse microglial activation was found. Human immune cells (macrophages and lymphocytes) were present in meninges, perivascular spaces. Hippocampus and corpus callosum brain regions from control unmanipulated, humanized and HIV-1 infected mice were isolated for deep sequencing with an Illumina HiSeq 2500 analyzer to find species-specific changes in transcriptomes by alignment to the mouse database and re-alignment of the unmapped reads to the human data base. In comparison with uninfected mice, virus-infected human glia-containing brain samples exhibited unique expressions of 45 and 56 genes in the hippocampus and corpus callosum, respectively. These included up-regulated genes known to be highly associated with interferon signaling pathway, type 1 and 2, defense response to virus, cytokine-mediated signaling, negative regulation of viral genome replication and transcription (PLSCR1, EIF2AK2, ISG15, IFIT1, IFI16, ADAR, OAS3, MX1, BST2, RSAD2), ISG15-protein conjugation and regulation of type 1 interferon production (HERC5, ISG15, UBE2L6, DDX58). The down-regulated genes are critical in cell growth, cytoskeleton reorganization and glial structural constituent (Sept4, CLDN11, MOBP, MAG, MOG, PLP1, STMN2). These data overlap, in part, with disease profile found in human HIV-1 encephalitic brains. Dual reconstituted murine models of human disease provide molecular cues that glial-immunocytes crosstalk are affected by virus. This potentially led to compromise in the integrity of glial system and consequent neural dysfunction.

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Nanosymposium

771. Global Organization of Extrastriate Cortex

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Topic: D.06. Vision

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Title: Adult-like organization of high-level visual cortex in human infants

Authors: *B. M. DEEN[1,2], H. RICHARDSON[2], D. D. DILKS[3], A. TAKAHASHI[2], B. KEIL[4], L. L. WALD[4], N. KANWISHER[2], R. SAXE[2];
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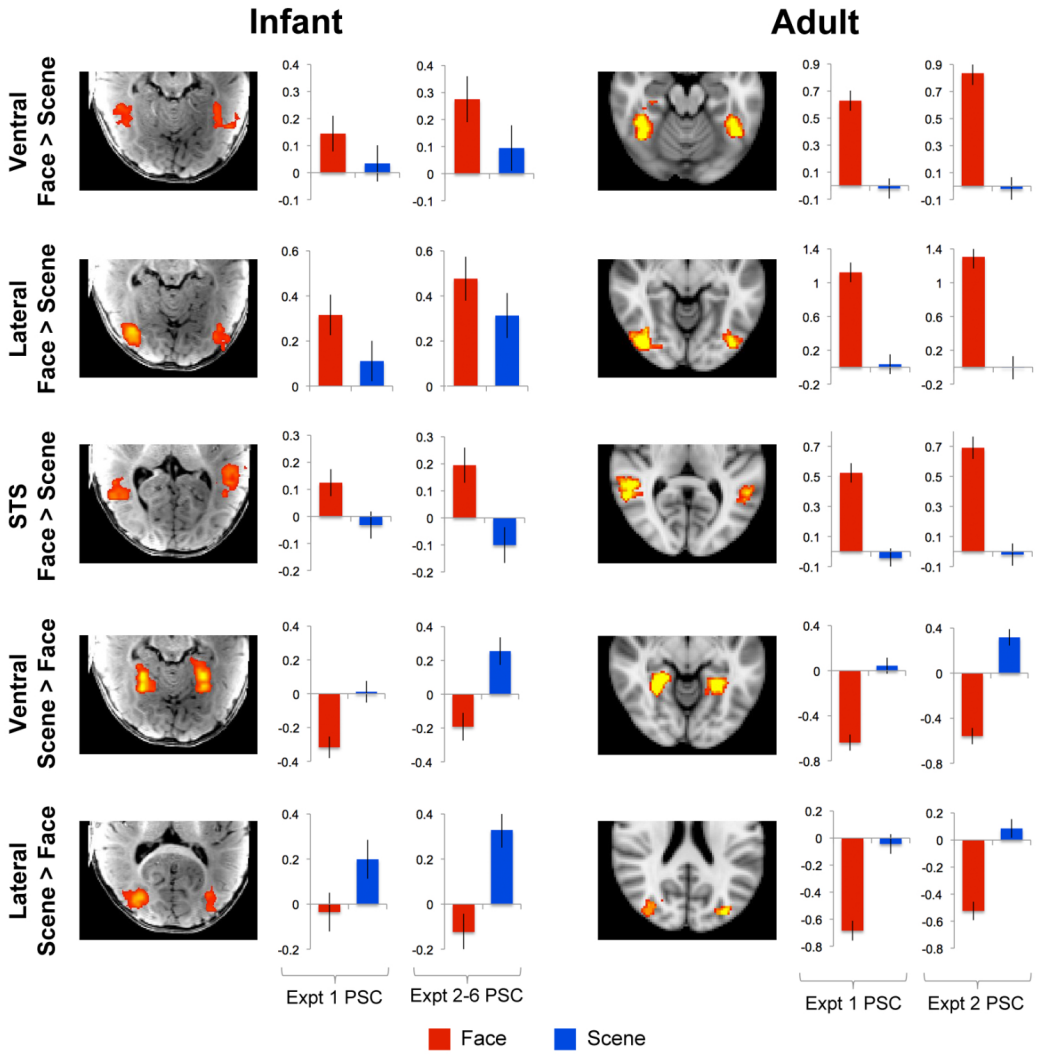
Abstract: The extrastriate visual cortex in humans has a stereotyped functional organization, in which regions selective for specific visual categories are found in approximately the same location in virtually every normal adult. How does this systematic structure arise in development? Are category-sensitive regions largely innately specified and present from early in development, or do they develop gradually, after extensive visual experience with specific categories? Here we develop and apply methods for fMRI in awake infants to address this question. We scanned 2-8-month-old infants (N = 17, 63 total scan sessions) while viewing infant-friendly videos depicting faces, bodies, inanimate objects, natural scenes and scrambled scenes. Several novel methodological approaches were employed, including infant-sized head coils, quiet pulse sequences, and a combination of novel and extant data analysis techniques for reducing the impact of head motion. In infants with enough low-motion data (n=9 infants), we observed face- and scene-preferring regions in the same locations as the corresponding category-sensitive extrastriate regions in adults. These response profiles were replicated in independent data, and in two sets of movie stimuli. At least in some cases, responses in these regions were better explained by high-level category preferences than by low-level stimulus features such as spatial frequency and rectilinearity. However, strongly selective regions, preferring one visual category over all others, were not observed in infants, in contrast to adults. These results demonstrate that the location of multiple category-sensitive regions is already staked out in cortex within a few months after birth, but strong adult-like category selectivity does not emerge until later in development.



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Nanosymposium

771. Global Organization of Extrastriate Cortex

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Title: Connectivity precedes function in the development of the visual word form area

Authors: *Z. M. SAYGIN[1], D. E. OSHER[2], E. NORTON[3], D. YOUSSOUFIAN[4], S. BEACH[1], J. FEATHER[1], N. GAAB[5], J. GABRIELI[1], N. KANWISHER[1];
[1]MIT, Cambridge, MA; [2]Boston Univ., Boston, MA; [3]Northwestern Univ., Evanston, IL; [4]Columbia Univ., New York, NY; [5]Boston Children's Hosp., Boston, MA

Abstract: What determines the cortical location where a given functionally specific region will arise in development? Here we test the hypothesis that functionally specific regions develop in their characteristic locations because of pre-existing differences in the extrinsic connectivity of that region to the rest of the brain. We exploit the Visual Word Form Area (VWFA) as a test case, scanning children with diffusion and functional imaging at age five, before they learned to read, and at age 8, after they learned to read. We find the VWFA develops functionally in this interval and that its location in a particular child at age 8 can be predicted from that child's connectivity fingerprints (but not functional responses) at age 5. These results suggest that early connectivity instructs the functional development of the VWFA, possibly reflecting a general mechanism of cortical development.

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Nanosymposium

771. Global Organization of Extrastriate Cortex

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German Research Foundation DFG HO2002/10-2

Title: Altered retino-cortical connections and visual cortex reorganization in the recently discovered fhonda syndrome

Authors: *K. AHMADI[1], A. FRACASSO[2,3,4], J. VAN DIJK[3,4], M. VAN GENDEREN[5], S. O. DUMOULIN[3,4], M. B. HOFFMANN[1,6];
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Abstract: Purpose: In congential visual pathway disorders, such as albinism and achiasma, the visual cortex receives input from both visual hemifields, due to a misrouting of the retino-cortical connections at the optic chiasm. In these cases the visual cortex is organised as a retinotopic overlay of both ipsilateral and contralateral visual hemifields with bilateral population receptive fields [1]. Here we studied a recently discovered novel congenital visual pathway disorder: the FHONDA syndrome (Foveal Hypoplasia, Optic Nerve Decussation defects and Anterior segment dysgenesis) [2]. FHONDA displays an abnormal crossing of the temporal retina in the absence of albinism, which indicates an alternative mechanism for enhanced crossing of the optic nerves. We determined the cortical organization in FHONDA and its relationship to other visual pathway disorders.
Methods: For a participant with FHONDA and two control subjects, we modeled the population receptive field properties (pRF) [3] using ultra high-field MRI (7 Tesla, Philips). The right eye was stimulated with a horizontally or vertically moving bar exposing a 100%-contrast checkerboard pattern. pRF-mapping data were acquired for 3 stimulation conditions: (1) left, (2) right hemifield and (3) full field (11 deg diameter) mapping.
Results: We report three main findings for FHONDA: (1) The activation in V1 was confined to the left occipital lobe, i.e. contralateral to the stimulated right eye. (2) pRF-hemifield mapping demonstrated retinotopic mappings for both the representation of the contralateral and ipsilateral hemifield. (3) The retinotopic representations of both hemifields in V1 are overlays of mirror-symmetrical visual field locations along the vertical meridian. These findings underline bilateral population receptive fields in the primary visual cortex contralateral to the stimulated eye as also described in other congenital visual pathway disorders.
Conclusion: The similarity of the cortical mapping observed for FHONDA with other visual pathway disorders, such as albinism, achiasma and hemihydranencephaly, confirms that the developmental mechanisms acting in the visual system are independent of the cause of the misrouting of the optic nerves. We conclude that the conservative geniculo-striate projections that explain the observed mapping in congenital chiasmatic malformations reflect a general principle of human visual system organization [1].
[1] Hoffmann MB & Dumoulin SO (2015) Trends in Neurosciences 38: 55-65
[2] Poulter JA et al. (2013) Am J Hum Genet 93:1143-50
[3] Dumoulin SO & Wandell BA (2008) Neuroimage 39: 646-660

Disclosures: K. Ahmadi: None. **A. Fracasso:** None. **J. van Dijk:** None. **M. van Genderen:** None. **S.O. Dumoulin:** None. **M.B. Hoffmann:** None.

Nanosymposium

771. Global Organization of Extrastriate Cortex

Location: SDCC 7B

Time: Wednesday, November 16, 2016, 1:00 PM - 3:15 PM

Presentation Number: 771.04

Topic: D.06. Vision

Support: NSF Grant #BCS 1063774

Title: Population receptive field estimation applied to high-resolution measurements of visual eccentricity representations in human superior colliculus

Authors: *E. HALFEN[1], S. KATYAL[2], I. AKBAR[3], D. RESS[1];
[1]Neurosci., Baylor Col. of Med., Houston, TX; [2]Univ. of Minnesota, Minneapolis, MN; [3]Rice Univ., Houston, TX

Abstract: Superior colliculus (SC) is a small midbrain structure critical for eye movements and attention. Visually responsive neurons form a retinotopic map in its superficial layers. Functional magnetic resonance imaging (fMRI) of SC in humans is hindered by its small size, deep location, and nearby vasculature, which make data weak and noisy. Standard population receptive field (pRF) analysis can be confounded by the low data quality. Here, we describe a modified form of pRF analysis that measures pRF properties across the entirety of SC. **Methods:** 5 subjects (10 SC) fixated on a point at the edge of the screen and performed a speed discrimination task on radially moving (4°/s) black and white dots within a unilateral 50° polar angle segment of visual space presented at 6 eccentricities (5–30°). 8 quasi-axial slices that covered both SC were acquired using a spiral acquisition (1.2 mm). Functional data were transformed into a high-resolution (0.7 mm) anatomy and depth-averaged onto the surface of SC (0–1.6 mm). For each voxel, correlation coefficients (R^2) between models and measurements were evaluated across a 2D parameter space (size and eccentricity). Size and eccentricity were then calculated as the central moment of these R^2 values with their corresponding parameter. 3D surfaces were created from each subject's segmented anatomy, and manifold distance from each surface node to a foveal coordinate was calculated to quantify how pRF parameters vary with collicular distance. **Results:** Good pRF model fits ($R > 0.2$) were found across the majority of each SC. Eccentricity versus distance is described by a logarithmic function with $R^2 > 0.5$. There is a clear linear relationship between size and eccentricity. Size data obtained by the moment method are much more reliable than those obtained using the usual minimum-error approach. **Conclusions:** The visual response of superficial human SC can be reliably mapped using a wide-field stimulus, high-resolution fMRI, and the moment method of pRF analysis. Results quantify the logarithmic character of collicular magnification.

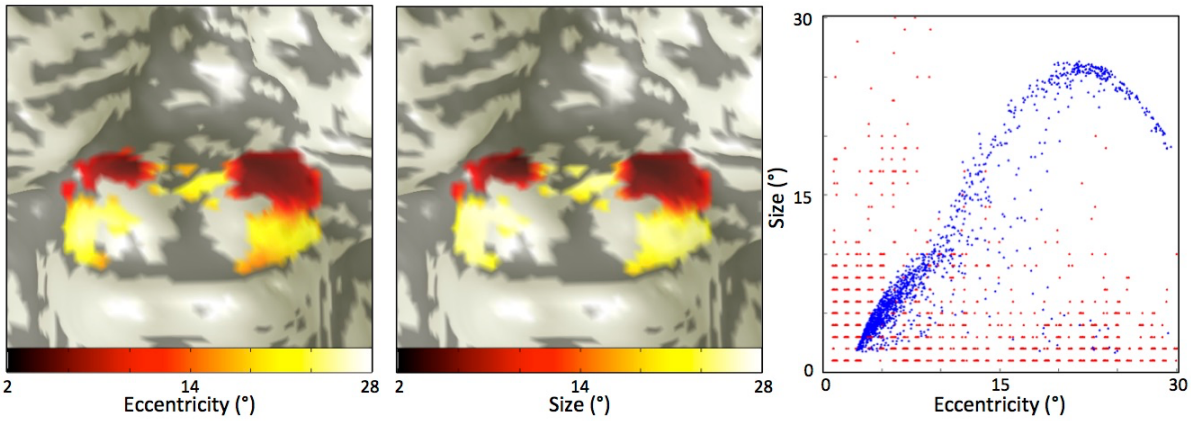

Fig. 1 In this session, stimulus was presented to left visual hemifield. Parameter maps of population receptive field (pRF) eccentricity and size from the moment method are on the left. The scatter plot shows the relationship between pRF size and eccentricity across all sessions. Results from the standard method are shown in red. Results from the moment method are shown in blue. Only the top 50% of data, based on correlation coefficient, are displayed.

Disclosures: **E. Halfen:** None. **S. Katyal:** None. **I. Akbar:** None. **D. Ress:** None.

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Topic: D.06. Vision

Support: NWO Grant 433.09.223 (S.O.D.)

Title: Population Receptive Fields reorganization in a subject born with one hemisphere

Authors: ***A. FRACASSO**[1], Y. KOENRAADS[2], G. L. PORRO[2], S. O. DUMOULIN[1]; [1]Spinoza Ctr. for Neuroimaging, Amsterdam Zuidoost, Netherlands; [2]Univ. Med. Ctr., Utrecht, Netherlands

Abstract: Introduction Congenital hemihydranencephaly (HH) represents an extreme case in human development, where the subject is born with only a single hemisphere. The hemispheric loss occurs during prenatal development, likely due to a vascular insult at the level of one of the carotid arteries, which prevents the development of one hemisphere. Astonishingly, the destruction of one hemisphere is not always associated with severe neurologic impairments. Here we describe a HH subject. Surprisingly, the subject's visual field is largely normal and extends into both visual hemifields including visual locations normally processed by the missing hemisphere. This suggests a considerable degree of reorganization of the visual pathway in HH.

This subject is different from previous reports of HH because both eyes and hemifields converge towards a single hemisphere.
This makes patients affected by HH an extremely interesting model to assess the principles of plasticity and stability of visual field map representations in humans. We measured population receptive field (pRF) properties using fMRI.
Methods Data were acquired by means of a 7T MRI (Phillips) while the patient affected by HH viewed conventional pRF stimuli in two separate sessions. Two possible pRF reorganization schemes were tested: where every cortical location processed information from (i) a single region of the visual field or (ii) from two bilateral regions of the visual field. We compared different models using signal detection theory. Two healthy control subjects without any known neurological condition also participated in this study.
Results The patient moved severely in the scanner due to tremor, thus we limit the analysis to the first functional run of each session. PRF mapping results shows an interleaved representation of both the right and left visual hemifields in the occipital lobe. For each cortical location two separate visual field locations are represented, one on each single hemifield. This result is consistent with a reorganization at the thalamus, with preserved thalamo-cortical projections.
Conclusions We found that in a subject born with one hemisphere the pRFs consist of an overlaid representation of both right and left visual hemifields located in the occipital lobe of the contralesional hemisphere. This study lends further evidence to the hypothesis that in humans congenital visual pathway malformations, such as hemihydranencephaly, achiasma and albinism, developmental mechanisms of local wiring within cortical maps compensate for the improper gross wiring to preserve visual function.

Disclosures: **A. Fracasso:** None. **Y. Koenraads:** None. **G.L. Porro:** None. **S.O. Dumoulin:** None.

Nanosymposium

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Topic: D.06. Vision

Support: ECOR 2015A051305

NIH 5P41-EB-015896-17

Title: Column-level segregation of magno- and parvo-cellular streams in human extrastriate cortex

Authors: *R. B. TOOTELL[1,2], S. NASR[1,2];
[1]MGH-NMR Ctr., Charlestown, MA; [2]Harvard Med. Sch., Boston, MA

Abstract: Among known segregated parallel information processing 'streams' in macaque monkeys, the 'magno- and parvo-cellular' (M-P) streams are primary. M-P streams are segregated from retina through LGN, into V1. The M stream extends further into cortical area V5/MT, and likely into thick V2 stripes. Why would M-P information be segregated at low levels of visual processing, but not at higher levels? Perhaps M-P information is encoded differently at higher levels. Alternatively, perhaps M and P streams do remain segregated at higher (but currently unknown) levels. Moreover, it is unknown whether M-P streams exist in humans. In macaques, these streams are often segregated within specific layers or columns, but such layers/columns are difficult to spatially resolve in humans using conventional fMRI techniques. We conducted high resolution (0.8 - 1 mm, isotropic) fMRI at high field (7T), in 8 human subjects, measuring resting state functional connectivity (FC) with eyes closed, and responses to multiple stimuli, like those used earlier to distinguish electrophysiological responses in M vs. P streams in non-human primates. Specifically, we measured the: 1) response to color vs. luminance across different spatial patterns, 2) luminance contrast gain, and 3) spatial frequency (SF) tuning curve. If M-P streams selectively activate thick and thin V2 stripes (respectively), this predicts higher color-selective activity in thin stripes, and higher contrast sensitivity (plus possible selectivity for lower SFs) in thick stripes. BOLD responses were sampled in lower cortical layers to minimize pial BOLD artifacts, tested for consistency, then signal-averaged across sessions (Nasr et al. 2016). All M-P predictions were confirmed in V2 thin vs. thick stripes, and also found in V3 columns. In both areas, we found more emphasis on the central (compared to peripheral) retinotopic representation in thin (compared to thick) stripes/columns, supporting some reports in macaques. Finally, FCs were selectively stronger between like (compared to unlike) functionally driven stripes/columns, within and across hemispheres. In contrast, all results in V3A suggested a strong M stream influence, without significant influence from P stream: 1) no color-selective columns, 2) M-like contrast gain, 3) low SF tuning, 4) stronger FCs with peripheral visual field in V2/V3, and 5) stronger FCs between V3A and thick (compared to thin) columns in V2/V3. Compared to lower areas, V4 showed a weaker segregation between M-P streams. Thus, a column-level segregation of M and P streams also exists in humans. These streams extend to higher areas, compared to that in current diagrams of M-P streams in macaques.

Disclosures: R.B. Tootell: None. **S. Nasr:** None.

Nanosymposium

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Title: Network-level interactions drive response properties in word- and face-selective cortex

Authors: *J. D. YEATMAN[1], K. N. KAY[2];
[1]Speech and Hearing Sci., Univ. of Washington, Seattle, WA; [2]Univ. of Minnesota, Minneapolis, MN

Abstract: High-level perceptual functions depend on complex interactions within networks of brain regions. However, the typical approach in high-level vision is to localize brain regions that respond selectively to particular categories of images and to probe response properties focusing on these specific regions. Here we show that to understand the computations performed by high-level visual regions, it is essential to analyze the connectivity of the region and model responses in the context of the larger network in which the region resides. We used fMRI to localize word-selective (VWFA-1, VWFA-2) and face-selective (FFA-1, FFA-2) regions in ventral temporal cortex (VTC). We then measured responses in these regions to a wide range of carefully controlled grayscale images while subjects performed different tasks that isolate bottom-up, stimulus-driven responses from top-down, task-dependent modulations. Based on diffusion MRI (dMRI) and fiber tracking we identified the major white matter fascicles that terminate in each VTC region. Our measurements demonstrate that VTC is not only sensitive to stimulus properties (e.g. image category) but is also substantially affected by the task performed on the image. Importantly, we find that the observed task sensitivity is predictable based on the pattern of white-matter connections between each region and the rest of the brain. VWFA-1 and FFA-1 are directly connected with the intraparietal sulcus (IPS) through the vertical occipital fasciculus (VOF), and we find that task-related response modulation in VWFA-1 and FFA-1 can be predicted with a model that incorporates the evoked response in IPS to each stimulus. In contrast, VWFA-2 is directly connected with Broca's area through the arcuate fasciculus, and we find that this connection imparts substantial word selectivity when subjects perform tasks involving linguistic analysis of visually presented words. These results show that anatomical connections between VTC and parietal and frontal cortex support functional interactions that fundamentally shape response properties in VTC.

Disclosures: J.D. Yeatman: None. **K.N. Kay:** None.

Nanosymposium

771. Global Organization of Extrastriate Cortex

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Topic: D.06. Vision

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Title: Visual field map clusters in high-order visual processing: An analysis of a new cluster in the posterior superior temporal sulcus

Authors: *B. BARTON[1], A. A. BREWER[2];
[1]Univ. of California Irvine, Irvine, CA; [2]Cognitive Sci., Univ. of California, Irvine, Irvine, CA

Abstract: The cortical hierarchy in the human visual system has been shown to be organized around retinal spatial coordinates throughout much of lower- and mid-level visual processing. These regions (e.g., V1, V2, V3, hV4, V3A, V3B, etc.) are comprised of visual field maps (VFMs) that each follow the organization of the retina, with neighboring aspects of the visual field processed in neighboring cortical locations. On a larger, macrostructural scale, groups of such sensory cortical field maps (CFMs) in the visual (Brewer et al., 2005; Wandell et al., 2005; Kolster et al., 2010) and auditory systems (Barton et al., 2012) have additionally been shown to be organized into roughly circular cloverleaf clusters. CFMs within clusters tend to share properties such as receptive field distribution, cortical magnification, and processing specialization. Here we investigate the extent of VFM and cluster organization with an examination of visual processing in temporal cortex. The posterior superior temporal sulcus (pSTS) of human cortex has been implicated in various functional magnetic resonance imaging (fMRI) studies as subserving higher-order visual processing, including faces (e.g., Hoffman and Haxby, 2000), biological motion (e.g., Grossman and Blake, 2002), and audiovisual integration (e.g., Beauchamp et al., 2004). We present fMRI data from population receptive field (pRF) modeling using flickering checkerboard bar visual stimuli (Dumoulin and Wandell, 2008) that show that pSTS contains four VFMs (pSTS-1, pSTS-2, pSTS-3, and pSTS-4) bilaterally and that these are organized in each hemisphere into one cloverleaf cluster: the pSTS cluster. Compared to low-level VFMs, we characterize these VFMs in the pSTS cluster as being relatively small at ~125 mm^2 and as containing relatively large pRFs with sizes ranging from 3-6 degrees of visual angle throughout the visual field. In addition, cortical magnification measurements show that a larger extent of the pSTS VFM surface areas are devoted to the peripheral visual field than those in occipital VFMs like V1, V2, V3, or hV4. Our findings add to the growing number of measurements of widespread sensory CFMs organized into cloverleaf clusters, indicating that CFMs and cloverleaf clusters may both be fundamental organizing principles in cortical sensory processing.

Disclosures: **B. Barton:** None. **A.A. Brewer:** None.

Nanosymposium

771. Global Organization of Extrastriate Cortex

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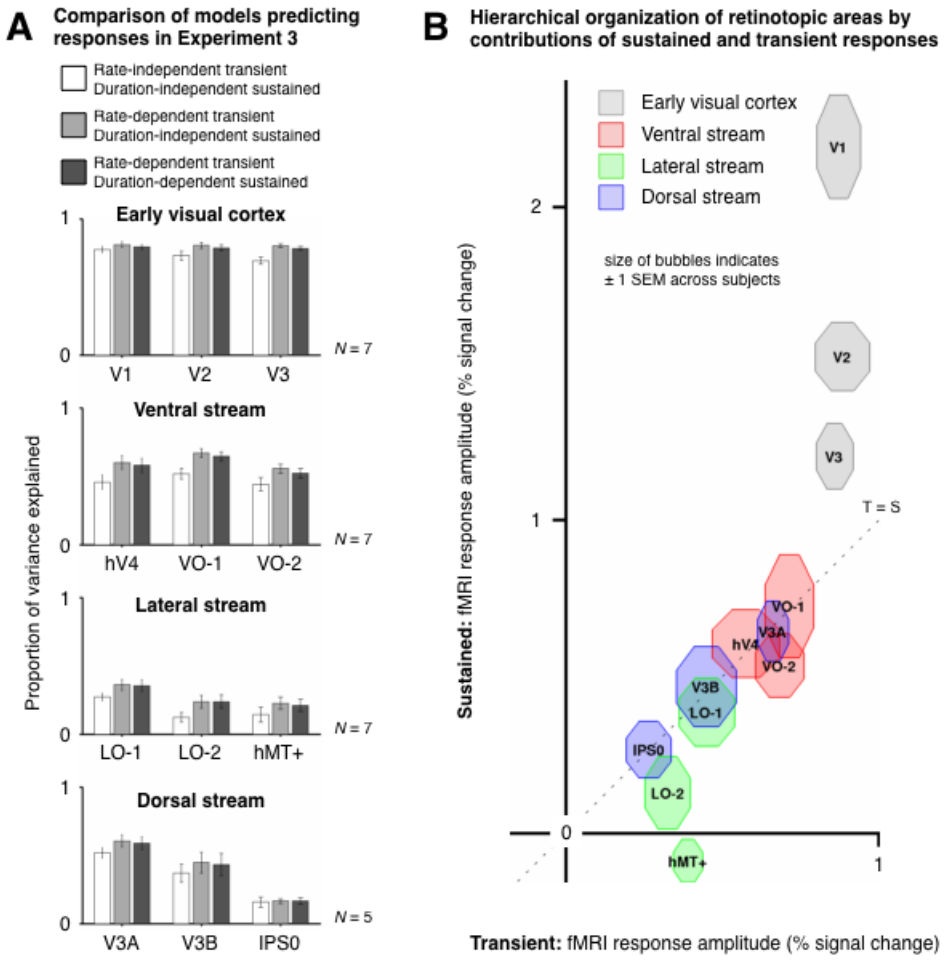
Topic: D.06. Vision

Support: NIH Grant R01 EY02391501A1

Title: Independent responses to transient and sustained stimulation across the cortical visual processing hierarchy

Authors: ***A. STIGLIANI**[1], B. JESKA[1], K. GRILL-SPECTOR[1,2];
[1]Dept. of Psychology, [2]Neurosciences Inst., Stanford Univ., Stanford, CA

Abstract: Early stages of the human visual system contain separate channels for parvocellular (P) and magnocellular (M) processing that differ in their temporal characteristics. M neurons code rapid visual transients, while P neurons code static stimuli and have larger sustained responses. However, it is unknown if P and M channels, and consequently sustained and transient processing, remain segregated beyond V1. To address this gap in knowledge we used fMRI to measure sustained and transient visual responses across a hierarchy of retinotopic regions. In Experiment 1, we measured sustained responses to single static gray-level phase-scrambled images presented continuously for durations of 1-30 s. In Experiment 2, we measured transient responses to images presented for 33ms, followed by a blank screen. In each trial, 30 images were shown at rates of 1-30 Hz. In Experiment 3, we varied both the duration and rate of stimulation. Our data reveal three main findings: (1) Responses to sustained and transient stimulation are separable in retinotopic areas: responses to sustained stimuli are duration independent and responses to transient stimuli are rate dependent. Importantly, separable contributions of sustained and transient responses measured in Experiments 1 and 2, respectively, predict responses in Experiment 3. (2) Ascending the hierarchy from V1, the magnitude of sustained responses decreases more than transient responses. (3) Transient responses gradually decline with rate in all regions, but remain above noise floor at 30 Hz in V1-V3 and drop below noise floor at rates > 8 Hz in later regions. These findings reveal independent transient and sustained channels in extrastriate cortex, and a hierarchical clustering of visual areas according to contributions from these channels.



Disclosures: **A. Stigliani:** None. **B. Jeska:** None. **K. Grill-Spector:** None.

Nanosymposium

772. Enduring Consequences of Early Stress II

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Time: Wednesday, November 16, 2016, 1:00 PM - 3:45 PM

Presentation Number: 772.01

Topic: F.04. Stress and the Brain

Support: NIH RO1 grant: 4R01MH101183-04

Duke Dean's Graduate Fellowship

Title: Prenatal air pollution and maternal stress alters early communication and social behavior in developing offspring

Authors: *C. L. BLOCK[1], R. HANAMSAGAR[1], J. M. TANDLER[2], C. EROGLU[3], S. D. BILBO[1];
[1]Pscyhology and Neurosci., [2]Cognitive Neurosci. Admitting Program, [3]Cell Biol., Duke Univ., Durham, NC

Abstract: Prenatal air pollution (diesel exhaust particles; DEP) combined with maternal stress (MS) during the last trimester of gestation act synergistically on offspring to promote long lasting changes in immune function and deficits in cognition and anxiety later in adulthood (Bolton et al. 2013). . Both changes in immune function and behavioral deficits are consistently more severe in males, in agreement with literature suggesting males are more vulnerable to immune activation early in life, resulting in increased vulnerability to neurodevelopmental diseases such as autism (Bolton et al. 2013; Pardo et al. 2005). We aimed to determine whether DEP and MS (DEP/MS) during pregnancy induces a neuroinflammatory response and subsequent social communication and anxiety deficits in developing offspring. Mouse dams were intermittently exposed via oropharyngeal aspiration to DEP (50 μg × 6 doses) or vehicle (VEH) throughout gestation. This exposure was combined with standard housing for dams or nest material restriction (a model of maternal stress) during the last third of gestation. Male offspring exposed to DEP/MS emitted more calls with a longer call duration and at a decreased frequency range at P5 in ultrasonic vocalization (USV) recordings. At P15, prenatal DEP/MS resulted in a stereotyped preference for familiar environments, reduced exploration of novel environments and increased anxiety in both male and female offspring during a social exploration task. Taken together, these results suggest that environmental risk factors can have synergistic effects in utero, resulting in changes in brain development and social and communication dysfunction that are more severe in male offspring. This model thus affords a unique opportunity to explore environmental factors that may contribute to the early-life neurodevelopmental male bias reported in humans.

Disclosures: C.L. Block: None. **R. Hanamsagar:** None. **J.M. Tandler:** None. **C. Eroglu:** None. **S.D. Bilbo:** None.

Nanosymposium

772. Enduring Consequences of Early Stress II

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Time: Wednesday, November 16, 2016, 1:00 PM - 3:45 PM

Presentation Number: 772.02

Topic: F.04. Stress and the Brain

Title: Mechanisms supporting accelerated hippocampus maturation following early life stress

Authors: *K. G. BATH[1], G. MANZANO-NIEVES[2], K. HUNTZICKER[2], T. MOSS[2], H. GOODWILL[2];
[1]CLPS, [2]Neurosci., Brown Univ., Providence, RI

Abstract: Early life stress (ELS) increases the risk for later cognitive and emotional dysfunction. ELS is known to truncate neural development through effects on suppressing cell birth, increasing cell death, and altering neuronal morphology, effects that have been associated with behavioral profiles indicative of precocious maturation. However, how earlier silencing of growth drives accelerated behavioral maturation has remained puzzling. Here, we will discuss recent results in which we tested the novel hypothesis that, ELS drives a switch from growth to maturation to accelerate neural and behavioral development. To test this, we used a mouse model of ELS, fragmented maternal care, and a cross-sectional dense sampling approach focusing on hippocampus and measured effects of ELS on the ontogeny of behavioral development and biomarkers of neural maturation. Consistent with previous results, ELS was associated with an earlier developmental decline in expression of markers of cell proliferation (Ki-67) and differentiation (doublecortin). However, ELS also led to a precocious arrival of Parvalbumin-positive cells, led to an earlier switch in NMDA receptor subunit expression (marker of synaptic maturity), and was associated with an earlier rise in myelin basic protein expression (key component of the myelin sheath). Behaviorally, ELS accelerated the timed developmental suppression of contextual freezing, in a contextual-conditioning task. We will also provide, preliminary data, identifying potential mechanisms underlying the acceleration in maturation. Together, these data provide support for the hypothesis that ELS serves to switch neurodevelopment from processes of growth to maturation and promotes accelerated development of some forms of emotional learning.

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University of Wisconsin Institute for Clinical and Translational Research Translational Pilot Grant Award NIH/NCATS UL1TR000427

Title: Differential DNA methylation of neuro-immune regulatory genes associated with symptom severity and amygdala-prefrontal abnormalities in pediatric post-traumatic stress disorder

Authors: *T. J. KEDING, L. A. PAPALE, R. S. ALISCH, R. J. HERRINGA; Psychiatry, Univ. of Wisconsin-Madison Sch. of Med., Madison, WI

Abstract: Studies of adult post-traumatic stress disorder (PTSD) suggest a role for DNA methylation on genes related to hypothalamic-pituitary-adrenal (HPA) axis regulation, including the glucocorticoid receptor gene NR3C1 and its regulator FKBP5, as well as genes involved in the regulation of the immune system. Furthermore, DNA methylation levels on such genes are dynamically related to symptom severity and symptom improvement. However, studies have yet to investigate whether these relationships exist in pediatric PTSD, especially with regards to symptom severity, trauma exposure, and abnormalities in brain circuitry. Saliva from youth with PTSD (n = 22, 8-18 years) and age- and sex-matched healthy comparison youth (n = 22) was collected. DNA was extracted and genome-wide 5-methylcytosine (5mC) was profiled using the HumanMethylation450 BeadChip. Differentially methylated regions (DMRs) between groups were examined using a mixed effects model, controlling for age, sex, and cell type composition. False discovery rate (FDR)-corrected multivariate and partial correlation analyses were conducted to relate methylation at DMRs to symptom severity, trauma-related measures, gray matter volume, and task-based brain activation and amygdala functional connectivity within the PTSD group. Eighty-one group-associated DMRs were identified. Enrichment analyses revealed that 14 of these genes were associated with signal transduction regulation and 11 were associated with immune response regulation. Additionally, a subset of these genes (N = 28) showed significant associations with symptom severity, trauma exposure, and amygdala-prefrontal structure and function within the PTSD group (FDR-corrected $p < 0.05$). Eleven of these genes were related to cellular and organismal development processes, including GABRA5 and SYNGAP1, which showed unique specificity to the nervous system. Cellular and systems pathways analysis further revealed that these two genes are heavily involved in the development and migration of neurons, neuronal apoptosis, as well as learning, memory, and general cognition. Together, these findings link DNA methylation to symptom severity and trauma-related brain measures in pediatric PTSD. The DMRs found here represent potentially modifiable molecular substrates in the brain that ultimately could be targeted to aid children exposed to early life trauma that otherwise may result in PTSD.

Disclosures: **T.J. Keding:** None. **L.A. Papale:** None. **R.S. Alisch:** None. **R.J. Herringa:** None.

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Title: Developmental changes in emotional neurocircuitry and cellular aging in nonhuman primates: effects of early life stress.

Authors: *E. L. MORIN[1,2,3], B. HOWELL[2,3,5], E. FECZKO[3,4], E. EARL[6], K. ESTEVES[7], M. STYNER[9], S. DRURY[8], D. FAIR[6], M. SANCHEZ[2,3,4];
[1]Neurosci., [2]Dept. of Psychiatry & Behavioral Sci., [3]Yerkes Natl. Primate Res. Ctr., [4]Ctr. for Translational Social Neurosci., Emory Univ., Atlanta, GA; [5]Insititute of Child Develop., Univ. of Minnesota, Minneapolis, MN; [6]Dept. Of Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR; [8]Dept. of Psychiatry and Behavioral Sci., [7]Tulane Univ. Sch. of Med., New Orleans, LA; [9]Dept. of Psychiatry, Univ. of North Carolina, Chapel Hill, NC

Abstract: Childhood maltreatment is associated with increased risk for psychopathology, and social and cognitive deficits. This is in part due to alterations in cortico-limbic circuits (e.g. prefrontal cortex (PFC) and amygdala connectivity) critical for emotional and stress regulation, which are sensitive to early experience and stress due to their protracted development. How these effects unfold during early development is not well understood and difficult to study in humans. This study utilized a well-established nonhuman primate model of infant maltreatment (MALT), which consists of comorbid physical abuse and rejection, leading to infant distress. In this macaque model, the highest rates of abuse and rejection take place during the first three months of life, co-occurring with rapid brain development and maturation of PFC-amygdala circuits. MALT leads to long-term effects on emotional reactivity and social behavior, as well as alterations in amygdala and white matter development. To disentangle the effects of experience

from inheritance we used a unique cross-fostering design with random assignment of infants to control or maltreating foster mothers at birth. This study delves into the effects of MALT on the developmental trajectory of PFC-amygdala functional connectivity (FC) throughout the infant and juvenile periods. Structural and resting state (rs) fMRI scans were collected at ages 2 weeks, and 3, 6, 12, 18 months in 13 animals with history of MALT (7 male, 6 female) and 13 controls (6 male, 7 female). RsfMRI findings indicate reduced PFC-Amygdala FC in MALT animals and an accelerated switch to negative coupling than in controls, between amygdala and medial, dorsolateral and orbitofrontal PFC. The reduced PFC-amygdala FC during infancy and the juvenile period in MALT subjects could underlie the emotional and cognitive alterations they exhibit during development. To test the hypothesis that the developmental alterations in FC are linked to accelerated biological aging we explored the association between the trajectory in leukocyte telomere length (TL) measured at birth, 2 weeks and 3 and 6 months and FC fMRI data. TL is an established marker of cellular aging that reflects cellular allostasis and is sensitive to a range of factors including psychosocial stress, oxidative stress and DNA damage. Accelerated decline of TL has been associated with MALT in adults and children, however limited data exists on the neurobiological correlates. Our preliminary findings indicate a complex interaction between MALT, sex and the trajectory of TL shortening, which predicts some of the maturational effects of maltreatment on PFC-amygdala FC, particularly in females.

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Nanosymposium

772. Enduring Consequences of Early Stress II

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Topic: F.04. Stress and the Brain

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Hope for Depression Research Foundation

Title: Changes in the translational profile of CA3 neurons in BDNF-Val66Met mice mirrors changes observed after early life stress

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Abstract: Early life stress (ELS) has been associated with an increased risk for developing a mental health disorder later in life. Rodent models of ELS show impaired learning and memory in adulthood that is coincident with persistent changes in hypothalamic-pituitary adrenal (HPA) axis reactivity and neuronal morphology, such as decreases in spine density in the CA3 neurons of the hippocampus. In this study, the effects of ELS on the translational profile of CA3 pyramidal neurons was examined in a mouse model of genetic susceptibility to mood disorders, the BDNF-Val66Met mice. Isolation of in vivo translating RNA fractions from a genetically homogenous population of CA3 pyramidal neurons was accomplished using transgenic mice expressing an EGFP fused to the L10a ribosomal subunit that is under the control of a cell-type specific promoter (Gprin3). Reporter mice were crossed with BDNF-Val66Met allele carriers to generate double transgenic animals that were subjected to bedding and nesting deprivation from P2-P12 (Rice·· Baram, 2008) and then given standard housing conditions until 4mos of age. Mice were rapidly decapitated and the hippocampus was dissected for RNA isolation by Translating Ribosomal Affinity Purification (TRAP). TRAP and unbound fractions were subjected to RNA-sequencing using an Illumina Hi-Seq 2500 to collect 100bp reads at a sequencing depth of 30M reads/sample. Results were aligned against the mouse genome (mm10) and the numbers of reads for each transcript were normalized to obtain relative expression levels. Strand software was used to perform statistical analysis to identify differentially expressed genes, which were grouped into pathways using the DAVID tool. Comparisons of TRAP fractions from adult mice between ELS and controls revealed over 5,939 genes that are persistently changed after ELS. $BDNF^{Met/+}$ mice had 1,420 genes changed at baseline, and homozygous $BDNF^{Met/Met}$ mice also show 3,736 changes. Over half of the genes changed after ELS in WT mice were identical to those changed in unstressed $BDNF^{Met/Met}$ mice. This substantial overlap identifies possible common mechanisms of stress susceptibility derived from either genetic or environmental factors. Interestingly, fewer genes were changed in response to ELS in $BDNF^{Met/+}$ mice, suggesting an altered reactivity profile. Together, these data not only reveal common mechanisms of susceptibility to mood disorders in CA3 neurons, but also lay the groundwork for developing novel treatments to reverse the changes induced by ELS or resulting from a genetic predisposition to mood disorders.

Disclosures: J.D. Gray: None. **J.F. Kogan:** None. **T.G. Rubin:** None. **E.F. Schmidt:** None. **N. Heintz:** None. **B.S. McEwen:** None.

Nanosymposium

772. Enduring Consequences of Early Stress II

Location: SDCC 5B

Time: Wednesday, November 16, 2016, 1:00 PM - 3:45 PM

Presentation Number: 772.06

Topic: F.04. Stress and the Brain

Support: NHMRC 1091571

Title: Early adversity affects amygdala-cortical functional connectivity: links to adversity and gut microbial community structure

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Abstract: The amygdala-prefrontal cortex circuit is critically involved in emotion regulation, takes many years to develop and is affected by the environment. For example, early adversity alters the development of the amygdala-prefrontal cortex network and is a significant risk factor for the emergence of mental health problems (such as anxiety). Such adversities are also associated with changes in the peripheral nervous system. For example, early adversity alters community composition of bacteria in the rodent gut, with such microbial changes increasing anxiety risk. Interestingly, rodent data suggests that the microbiome may be linked to amygdala development. Hence, rodent studies support the notion that the effects of adversity on the microbiome may critically contribute to stress-associated neurobiological profiles and anxiety. No human studies have examined how adversity affects the gut microbiome and how such changes are linked to amygdala-prefrontal development and anxiety. This is an important gap in the literature, as non-invasive peripheral manipulations hold much promise for mental health treatment, especially in developing populations. To address that gap in the literature, we examined the effect of early adversity on the community composition of the gut microbiome during middle childhood, and then linked those microbial changes to anxiety and amygdala functional connectivity during an emotional faces task (the emotional go-no go). Measures were collected from a group of adversity-exposed youths and a comparison group of youths not exposed to early adversity. We characterized the effect of adversity on the microbiome using 16s rRNA sequencing of stool samples. Across taxonomic levels, bacterial richness estimates were lower in the adversity-exposed youths than in the comparison sample. Controlling for adversity exposure, richness estimates were negatively associated with separation anxiety symptoms such that lower richness estimates predicted worse anxiety symptoms. Richness was also positively related to amygdala-mPFC functional connectivity during the emotional faces task (fear > baseline contrast). These data are the first to investigate how exposure to early adversity affects the microbiome, and how such microbial changes are linked to anxiety and emotion neurobiology in developing humans. These data suggest that adversity-altered microbiota are important for both anxiety and brain development, highlighting the potential of peripheral interventions following adversity exposure.

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Topic: F.04. Stress and the Brain

Support: NIGMS (1P20GM103653)

Title: Inhibition of DNA methylation rescues deficits in maternal behavior induced by early-life stress

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Abstract: Exposure to caregiver maltreatment results in lifelong changes in DNA methylation in the brain and periphery. Maltreatment likewise alters behavioral trajectories. For example, we have shown in a rodent model that adult females which were mistreated in infancy mistreat their own offspring. However, the role aberrant DNA methylation plays in this maternal behavior is unknown. In the current study, adult female rodents exposed to caregiver maltreatment were infused with the DNA methylation inhibitor, zebularine, for one week following parturition. Maternal behavior was recorded in these dams and levels of nurturing and aversive behavior were analyzed. Preliminary data indicate females with a history of maltreatment which were administered zebularine show levels of maternal care comparable to females with a history of normal infancy (i.e. mainly nurturing behavior). Methylation status of the brain derived neurotrophic factor (*bdnf*) gene, which is critical for development and implicated in psychiatric disease states, is modulated by early-life caregiver experience. Thus, methylation status of the *bdnf* gene as well as global and hydroxymethylation are currently being assayed in these dams in several brain regions implicated in maternal behavior, including the nucleus accumbens and medial preoptic area. Motherhood-induced changes in DNA methylation will also be discussed, as data indicate that motherhood induces divergent methylation changes in females with a history of maltreatment compared to females with a history of normal infancy.

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Topic: F.04. Stress and the Brain

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HDRF

Title: Early life stress enhances susceptibility to depression via long-lasting transcriptional and epigenetic alterations in the brain's reward circuitry

Authors: *C. J. PENA, H. KRONMAN, E. LOH, I. PURUSHOTHAMAN, H. M. CATES, O. ISSLER, R. C. BAGOT, D. M. WALKER, L. A. FARRELLY, T. LEONG, B. PATEL, I. MAZE, L. SHEN, E. J. NESTLER;
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Abstract: Early life stress (ELS) including abuse and neglect tragically impacts >3 million US children every year, and increases the lifetime risk of major depression and other psychiatric disorders by 2-4 fold. Reduced or impaired social behavior and anhedonia are hallmark symptoms of depression and other related disorders which implicate involvement of the brain's reward and motivation circuitry including the ventral tegmental area (VTA), nucleus accumbens (NAc), and prefrontal cortex (PFC). The molecular mechanisms mediating how ELS impacts development of the reward circuitry and vulnerability to depression are largely unknown. We now show that male and female mice subjected to ELS in a postnatal sensitive window exhibit enhanced susceptibility to depression-like behaviors after experiencing additional, chronic stress in adulthood. Interestingly, without adult stress, ELS alone did not alter depression-like behaviors, enabling us to study latent environmentally-induced vulnerability to depression-like behaviors prior to their onset. RNA-seq of VTA, NAc, and PFC of male and female mice reveals broad, long-lasting transcriptional changes similar to that of adult stress alone. In male VTA, bioinformatic analysis identified suppression of the transcription factor orthodenticle homeobox 2 (Otx2) as an upstream regulator of these enduring transcriptional changes. Otx2 has been implicated in genomic enhancer-region activation, and ChIP-seq reveals ELS alters patterns of enhancer-associated histone modifications in regions near Otx2 binding motifs. VTA-specific manipulation of Otx2 by viral-mediated over-expression or knock-out rescues or recapitulates, respectively, the effects of ELS on depression-like behavior after adult stress. In NAc, ELS promotes accelerated rates of histone H3.3 variant turnover, which has been shown to be necessary for activity-dependent gene expression, synaptic connectivity, and cognition. ELS is also associated with altered levels of histone-modifying enzymes in male and female NAc and PFC. Together, we have identified putative epigenetic mechanisms for the long-lasting effects of

ELS on transcriptional alterations within the reward circuitry. Moreover, we have identified a novel molecular target in VTA mediating the impact of ELS on long-lasting vulnerability to depression-like behavior.

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Topic: F.04. Stress and the Brain

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Title: The interaction of childhood trauma and recent stress are associated with heightened ventral striatum-medial prefrontal cortex connectivity: Relations with depression and anxiety

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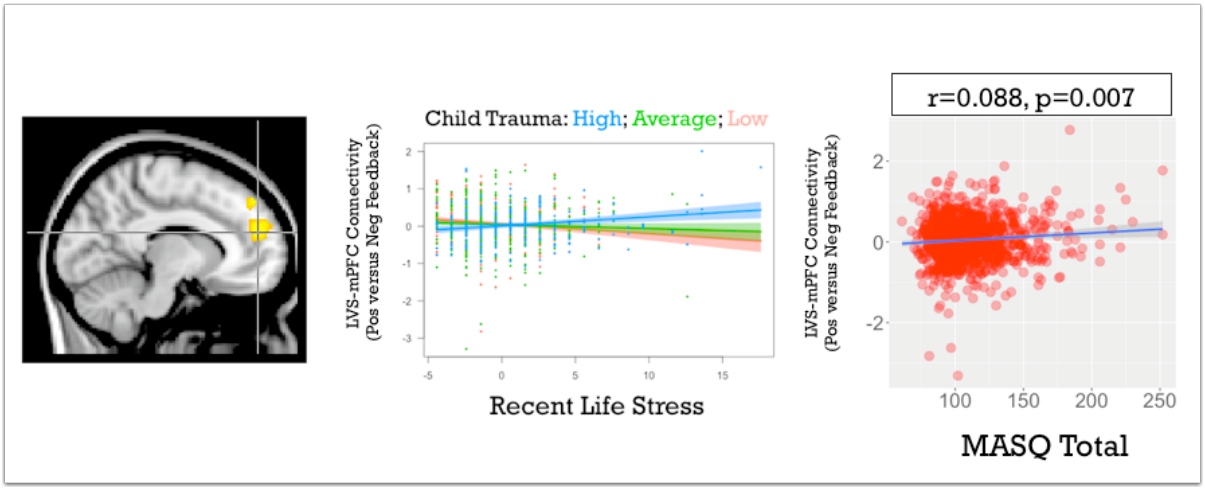
Abstract: The experience of childhood maltreatment is a significant risk factor for the development of depression. This risk is particularly heightened after exposure to additional, more contemporaneous stress. While behavioral evidence exists for such “stress sensitization,” little is known about biological correlates of this putative process. Identifying such correlates may not only substantiate the “stress sensitization” model, but also provide biomarkers of risk for later depression. Suggestive clues have emerged from targeted neurobiological investigations that experiences of early life stress, such as childhood maltreatment, may influence the structure and function of a corticostriatal circuit supporting motivation and action. Moreover, dysfunction of this circuit has been implicated in the pathophysiology of depression. The limited available

research, though informative, has not investigated whether differences in reward-related corticostriatal circuit function may be associated with "stress sensitization," or if any circuit-level effects explain subsequent risk for depression. To begin to fill in these important gaps, we turned to the Duke Neurogenetics Study (DNS), an ongoing project assessing a wide range of behavioral and biological traits in a large cohort of non-patient, 18-22 year-old university students. Investigating reward-related functional connectivity within the corticostriatal circuit of 926 participants, we found evidence for increased connectivity between the ventral striatum and the medial prefrontal cortex (Interaction β=0.199, p<.005) in individuals exposed to greater levels of childhood maltreatment who also experienced greater levels of recent life stress. We also found that this aberrant pattern of connectivity was associated with elevated symptoms of depression, specifically reduced positive affect (β=0.089, p<.005). These findings suggest a novel neurobiological mechanism linking cumulative stress exposure with later depressive symptoms and provide support to the "stress sensitization" model of depression.



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Nanosymposium

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Topic: F.04. Stress and the Brain

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Title: Agmatine attenuates chronic unpredictable mild stress-induced anxiety, depression-like behaviours and cognitive impairment by modulating nitrergic signalling pathway

Authors: *N. B. GAWALI, V. D. BULANI, A. A. CHOWDHURY, M. S. GURSAHANI, A. R. JUVEKAR;
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Abstract: ***Objectives***: Agmatine, a neurotransmitter/neuromodulator, has been shown to exert numerous effects on the central nervous system. Chronic stress is a risk factor for the development of depression, anxiety and deterioration of cognitive performance. Increasing evidence indicates an involvement of nitric oxide (NO) pathway in these disorders. Therefore we decided to investigate the beneficial effects of agmatine on chronic unpredictable mild stress (CUMS) induced depression, anxiety and cognitive performance with the involvement of nitrergic pathway.
Methods: Mice were subjected to a battery of stressors for 28 days. Agmatine (20 and 40 mg/kg, i.p.) alone and in combination with NO modulators like L-NAME (10 mg/kg, i.p.) and L-arginine (100 mg/kg i.p.) were administered daily. The changes in the behavioural, biochemical, corticosterone and BDNF levels were evaluated.
Results: In our study, CUMS produced depression, anxiety-like behaviour and memory impairment as well as alterations in the levels of corticosterone (CORT), acetylcholinesterase, oxidative stress markers and reduced brain-derived neurotrophic factor (BDNF). Results revealed that, agmatine (20 and 40 mg/kg, i.p) significantly produced antidepressant-like behaviour in sucrose preference test (SPT), force swim test (FST), anxiolytic behaviour in elevated plus maze (EPM), open field test (OFT) with improved cognitive function in morris water maze (MWM) test. Treatment with agmatine significantly reduced levels of acetylcholinesterase and attenuated oxidative–nitrergic stress, however, treatment with L-NAME (10 mg/kg) potentiated the effect of agmatine. In addition, agmatine and L-NAME with sub-effective dose of agmatine (20 mg/kg) significantly increased the BDNF level and inhibited CORT level in stressed mice. L-arginine abolished the anxiolytic, antidepressant and neuroprotective effects of agmatine.
Conclusions: Thus, the results of the present study suggests that agmatine has marked effect on depression and anxiety-like behaviour in mice through nitrergic pathway, which may be partly related to modulation of hypothalamic–pituitary–adrenal axis (HPA axis) and BDNF levels.

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Topic: F.04. Stress and the Brain

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CIHR

Title: Effects of maternal stress and fluoxetine on outcomes of offspring as adults

Authors: *V. KIRYANOVA[1,2], S. J. MEUNIER[2], V. M. SMITH[2], M. C. ANTLE[2], R. H. DYCK[3];
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Abstract: Fluoxetine (Flx, Prozac) is the antidepressant most commonly used by pregnant women. We previously demonstrated that perinatal exposure to Flx alters anxiety, aggression, memory, and circadian rhythms of male mouse offspring. However, few animal studies examine effects of antidepressants in combination with maternal stress. The combination of maternal stress and antidepressant exposure likely affects the offspring differently than these variables in isolation.
The present study investigated the effects of maternal stress and perinatal exposure to the SSRI Flx on the behaviour of the offspring as adults. METHODS: Mouse dams were exposed to chronic unpredictable stress (embryonic (E) day 7 to E18), Flx (E15- postnatal day 12), and combination of stress and Flx. A separate control group consisted of animals that were not exposed to stress or Flx. Maternal behaviour was assessed during the early postpartum period. At two months of age, offspring of mothers exposed to prenatal stress, perinatal Flx, prenatal stress and Flx, or neither stress nor Flx, were subject to a behavioural test battery or assessment of circadian behaviour. The behavioural battery included tests of cognitive abilities, memory, aggression, anxiety, sensorimotor information processing, and exploratory and risk assessment behaviours. Circadian organization of wheel running rhythms and phase shifts to photic and non-photic stimuli were assessed in a separate group of mouse offspring. RESULTS: Our results show that perinatal exposure to Flx, maternal stress, and their combination, lead to discernible changes in behavior. Furthermore, some of the long-term, stress-related consequences can be reversed by Flx administration, when administered to the mother during pregnancy and the early postpartum period.

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Nanosymposium

773. Perception and Imagery: Perception and Behavior in Real-World Settings

Location: SDCC 2

Time: Wednesday, November 16, 2016, 1:00 PM - 4:30 PM

Presentation Number: 773.01

Topic: H.02. Human Cognition and Behavior

Title: The high-order brain dynamics (HOBD) model

Authors: *J. R. MANNING[1], H. LI[2], Q. LIU[2];
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Abstract: Our brain's structures do not operate in isolation. Rather, our thoughts and actions presumably arise from dynamic patterns of *interactions between brain structures* that are modulated according to our moment-by-moment experiences and intentions. Gaining insights into these dynamic patterns of interactions has historically been challenging. For example, considering pairwise correlations in brain data (as in functional connectivity analyses) produces a matrix with on the order of n^2 entries (where n is the number of brain regions). When n is large (e.g. the number of voxels in an fMRI volume), the full correlation matrix becomes unwieldy to compute with (e.g. to examine how functional connectivity changes over time, across people, or between different experimental conditions). Further, if one wishes to examine higher-order patterns (e.g. how correlations between correlations change over time), the computational requirements of the resulting patterns increase exponentially.

A second challenge is that correlation matrices are not inherently well suited to studying dynamic activity. Computing correlations requires a time series of measurements. Therefore, studying how correlations change over time requires compute correlations within each of a series of overlapping time windows. However, this "sliding window" approach provides only a poor approximation of the true moment-by-moment patterns of interest. Further, such analyses are often very sensitive to the precise window duration, which must be selected *a priori*.

We propose the *high-order brain dynamics* (HOBD) model for exploring and examining deep dynamic patterns in neural data. HOBD provides an efficient (scalable) means of describing and computing with high-order patterns of brain activity (e.g. correlations, correlations between correlations, and so on). Within the model space, computing with activity-based patterns require (on the order of) the same amount of memory as correlation-based patterns, which require the same amount of memory as higher-order patterns. Second, HOBD provides instantaneous estimates of (low- and high-order) brain patterns at each moment during an experiment. These patterns may then be linked to cognitive states, etc.

Disclosures: J.R. Manning: None. **H. Li:** None. **Q. Liu:** None.

Nanosymposium

773. Perception and Imagery: Perception and Behavior in Real-World Settings

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Topic: H.02. Human Cognition and Behavior

Support: DARPA Grant ARO:W911NF-14-1-0157

Title: Engagement synchronizes brains and warps time

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Abstract: Films, music, and books are often characterized as “engaging”, and arguably, this property can predict their ability to attract an audience. But what does it mean for an audience to be engaged? Here, we propose a precise definition: An audience is engaged to the extent that it is willing to allocate a scarce resource, such as time. To quantify this in the context of digital media, 1000 individuals watched short online videos under a time pressure, thus revealing their relative commitment to the stimulus on a time resolution of 10 s. The neurological validation of this real world audience engagement was obtained by the level of brain synchronization the stimuli induced among a group of 22 individuals. We conclude that when engaged with a story, the subjective experience of time’s distortion can be directly assessed from the behavior of a large audience and has a measurable neural correlate.

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Nanosymposium

773. Perception and Imagery: Perception and Behavior in Real-World Settings

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Presentation Number: 773.03

Topic: H.02. Human Cognition and Behavior

Support: NIMH IRP

Title: Neuronal integration of temporal information compared across three face patches in the macaque temporal lobe

Authors: *B. E. RUSS, N. PERWEZ, K. KOYANO, D. A. LEOPOLD;
Section on Cognitive Neurophysiol. and Imaging, Lab. of Neuropsychology, NIMH/NIH, Bethesda, MD

Abstract: The macaque face processing system includes a set of circumscribed, bilateral patches along the inferior temporal cortex. Previous studies have investigated how neurons in these regions respond to facial attributes such as viewing direction, expression, identity, and other object types. In most of these studies static images, or short dynamic sequences, isolating a feature of interest are presented for a few hundred milliseconds at a subject's center of gaze. Data suggest a progression in the complexity of facial identity information from posterior to anterior, as well as a mediolateral division for the role of motion. Our lab has recently taken a different and complementary approach, mapping neural responses during the free viewing of natural videos. We have shown that neurons in the anterior fundus face patch respond consistently to multiple viewings of a video, but that nearby neurons are nearly uncorrelated in their responses. These results raise challenging questions about how such neurons process visual information under conditions other than brief, flashed presentations. In the present study, we investigate the extent to which neurons in three face patches respond to information integrated over different temporal scales. We address this question by recording neural activity longitudinally over several weeks from the macaque middle lateral (ML), anterior fundus (AF), and anterior medial (AM) face patches. We assessed several features of the neurons, including their selectivity to different categories of static images and their responses during viewings of 5 min naturalistic movies. Critically, we measured the responses of the same cells to short clips from the longer movies, presented in random order. Clip duration was systematically varied to investigate the temporal response integration of cells across the three regions. Based on the responses, we assessed temporal integration of each neuron by comparing responses to the isolated movie clips with those to the same stimulus presented within the original movie. We found the level of temporal integration varied markedly among neighboring neurons. For example, for some neurons in the face patch AF, reshuffling the static frame responses into the original order provided a good approximation of the response to the full movie. For others, such reordering never resembled the continuous response, even when snippets of 800 ms, rather than static frames, were used. Comparing this measure of temporal integration across different face patches provides valuable knowledge about how dynamic biological signals are processed in different regions of the inferior temporal cortex.

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NEI 1R01EY019684-01A1

Title: Using voxel-wise modeling of fMRI responses to natural stories and movies to study semantic representations in human cortex

Authors: *A. G. HUTH, J. L. GALLANT;
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Abstract: For decades the fields of psychology and neuroscience have used neuroimaging to investigate the functional organization of the human brain. Even so, little is known about how specific information is mapped across the cerebral cortex. One factor that has limited progress is over-reliance on the point null hypothesis testing approach that has dominated neuroimaging research. This approach can have high statistical power for testing specific hypotheses, but cannot be used to accurately estimate effect sizes, leaving open the question of whether many "significant" findings are actually meaningful. Furthermore, it is extremely difficult to synthesize results from this approach into a coherent view of cortical organization. We have pursued an alternative approach: voxelwise modeling (VM) using natural stimuli. In VM, subjects are presented with complex natural stimuli while brain responses are recorded using fMRI. Hypotheses about how these stimuli are represented in the brain are instantiated as feature spaces that can be extracted from the stimuli. Potentially high-dimensional encoding models that predict responses based on a linear combination of the features are then estimated separately for each voxel in each subject using regularized regression. To prevent over-fitting, determine effect size, and test generalization, these voxel-wise models are validated by predicting responses in held-out datasets that were not used for model estimation. Valid models can then be examined in order to assess what types of information are represented in each voxel. Here we show that this exploratory approach can be used to construct complex and comprehensive maps of cortical representations for two modalities: vision and language. The results from just two experiments with a small number of subjects can replicate findings from many previous hypothesis-driven studies and help put these findings in context. Our results demonstrate the utility and power of data-driven approaches like voxelwise modeling, as well as the importance of using natural stimuli.

Disclosures: **A.G. Huth:** None. **J.L. Gallant:** None.

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773. Perception and Imagery: Perception and Behavior in Real-World Settings

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Presentation Number: 773.05

Topic: H.02. Human Cognition and Behavior

Support: NIH R01

Princeton Cognitive Science Program

Title: Attention selectively modulates dynamical functional connectivity in processing of simultaneously presented spoken and written narratives

Authors: *M. REGEV[1], E. SIMONY[2], C. BALDASSANO[2], U. HASSON[2];
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Abstract: The topology of brain networks and the anatomical connections across brain areas are relatively stable over time. However, while the anatomical structure may be stable, the dynamics of neural processing within these networks is ever changing, with information routed dynamically across different nodes as a function of task and processing demands. For example, during either reading or listening to a story, linguistic and high-order areas receive semantic information, but in former case from visual cortex and in the latter case from auditory cortex. How can the relatively fixed structural connections in the brain serve as a platform for functional connectivity which dynamically changes to suit the current processing needs of written vs. spoken information? Using fMRI, we recorded neural responses from participants who were simultaneously presented with two 15-minute, unrelated stories, one spoken and one written (presented one word at a time). One group of participants was asked to attend to the written story while ignoring the spoken one, while the other group did the reverse. A detailed post-scan questionnaire revealed good comprehension of the attended story and chance level comprehension of the unattended story in both groups. The neural data was compared to two unimodal control groups, who were exposed to only one of the stories. We correlated the response timecourses obtained from each of the unimodal control groups with responses obtained from the simultaneous presentation groups. In early auditory cortex, we found reliable responses for the spoken story even when attention was directed to the written story. Similarly, in early visual cortex, we found reliable responses for the written story even when attention was directed to the spoken story. Thus, the responses in early sensory cortices were locked to the low-level structure of the spoken/written input irrespective of attention. Nevertheless, response reliability in both early sensory cortices was enhanced when attention was directed to the appropriate sensory modality. In contrast, in language areas and other high order areas, where the stories compete for the same neural resources, there were no reliable responses to the unattended story, but only for the attended modality. Furthermore, using inter-subject functional correlation (ISFC)

analysis we observed that the correlation between high-order areas and sensory areas dynamically shifted as a function of attention. These results reveal how attention can dynamically route information across neural networks as a function of processing demands.

Disclosures: **M. Regev:** None. **E. Simony:** None. **C. Baldassano:** None. **U. Hasson:** None.

Nanosymposium

773. Perception and Imagery: Perception and Behavior in Real-World Settings

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Topic: H.02. Human Cognition and Behavior

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Title: From words to narratives: Amplification of global meaning in the brain

Authors: ***Y. YESHURUN**, U. HASSON;
PNI, Princeton Univ., Princeton, NJ

Abstract: Small local changes of the words used can create a big change in the overall narrative. Thus, as a story unfolds over time, the brain has to simultaneously process each incoming word while embedding it within the overall context. Recently, we suggested a hierarchy of timescales in the brain; from early sensory areas with short processing timescales (10s to 100s of milliseconds) to high-order areas with long processing timescales (many seconds to minutes). In this fMRI study, we hypothesize that short processing timescale regions would be sensitive to local changes in the words used (e.g. ”she” vs “he”), whereas regions with long processing timescales would accumulate and amplify these local changes. In order to test this hypothesis, we generated two stories that differed in 33% of words; keeping the overall structure constant, while creating two coherent yet distinct narratives. Eighteen subjects listened to each story (N = 36). To measure differences in the neural representations of the stories we calculated the Euclidean distance between neural responses to the two stories in each voxel. In line with our hypothesis, we found that the neural distance between the stories was amplified as the information transferred from low-level regions (e.g. early auditory cortex) to high-level regions (e.g precuneus and dorso-medial prefrontal cortex). Our results suggest that small differences in the words used are gradually accumulated and amplified as the information is transmitted from one level of the processing hierarchy to the next, to produce a distinctive neural representation for each narrative.

Disclosures: **Y. Yeshurun:** None. **U. Hasson:** None.

Nanosymposium

773. Perception and Imagery: Perception and Behavior in Real-World Settings

Location: SDCC 2

Time: Wednesday, November 16, 2016, 1:00 PM - 4:30 PM

Presentation Number: 773.07

Topic: H.02. Human Cognition and Behavior

Support: Academy of Finland, grant No. 257811

Title: Increases and decreases in BOLD-responses associated with enhanced comprehension of acoustically degraded sentences

Authors: *M. HAKONEN[1], P. J. C. MAY[2], I. P. JÄÄSKELÄINEN[1], E. JOKINEN[3], M. SAMS[1], H. TIITINEN[1];
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Abstract: The neural mechanisms by which previous experiences and acoustic information are integrated allowing successful comprehension of even severely acoustically distorted speech are largely unknown. One reason for this is that most studies of speech comprehension have compared responses to acoustically different stimuli which makes it difficult to distinguish whether the differences in the responses reflect speech intelligibility or acoustic variability.
In our fMRI study, acoustically distorted sentences were disambiguated by an exposure to their acoustically intact counterparts. In a separate behavioral experiment, intelligibility was significantly enhanced from 41% to 95% by such disambiguation. Contrasting the blood oxygenation level dependent (BOLD) -responses elicited by the acoustically distorted sentences as intelligible vs. unintelligible, i.e., before vs. after the presentation of their intact counterparts, revealed increased activity in higher-order cortical areas including the frontal pole bilaterally, the right inferior frontal gyrus, the left middle frontal cortex, and the anterior cingulate cortex. A reversed pattern was found in the vicinity of the auditory cortex where the BOLD-responses decreased.
Our results suggest that when comprehension of acoustically distorted speech is considerably enhanced after an exposure to the intact version, neurocognitive subsystems (e.g. prediction of incoming content, memory retrieval, integration) become engaged in processing of the distorted speech. Further, neuronal activity in auditory cortex does not simply represent the signaling of acoustic features but is strongly modulated by top-down influences from a widely spread set of brain areas.

Disclosures: **M. Hakonen:** None. **P.J.C. May:** None. **I.P. Jääskeläinen:** None. **E. Jokinen:** None. **M. Sams:** None. **H. Tiitinen:** None.

Nanosymposium

773. Perception and Imagery: Perception and Behavior in Real-World Settings

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Support: DOE Computational Science Graduate Fellowship to AK

NEC award to JHM

McDonnell Scholar Award to JHM

Title: Noise-robustness of cortical responses to natural sounds increases from primary to non-primary auditory cortex

Authors: *A. J. KELL, J. H. MCDERMOTT;
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Abstract: In everyday listening, the sounds from sources we seek to understand are often embedded in background noise. This noise alters the pattern of spikes in the auditory nerve, often to a profound degree. In order to recognize sources of interest, the brain must to some extent become robust to the effects of these background noises. To study the neural basis of listening in real-world background noise, we measured fMRI responses in auditory cortex in eight human listeners to a broad set of thirty natural sounds, presented in quiet as well as embedded in thirty different everyday background noises (e.g., a bustling coffee shop, crickets chirping, heavy rain hitting pavement). We quantified the extent to which neural responses were robust to background noise by correlating each voxel's response to the natural sounds in isolation with its response to those same natural sounds mixed with background noise. We then squared this correlation coefficient to get a measure of variance explained, and normalized it by the reliability of the voxel's response, measured across split halves of the data. This measure quantifies the extent to which a voxel's pattern of response across natural sounds is the same when the natural sound is presented in quiet and when it is embedded in noise. Responses in anatomically-defined primary areas (TE 1.1 and 1.0) were substantially affected by background noise ($r^2 \sim 0.40$). However, voxel noise-robustness increased with distance from these primary areas: nearby non-primary areas were slightly more robust, while more distal areas were hardly affected by the background noises ($r^2 \sim 0.85$). Mean responses in primary and non-primary regions were both only slightly lower in the presence of background noise, indicating that this effect was not due to the background noises differentially suppressing responses in primary areas. Our results illustrate the neural basis of a core aspect of real-world listening, and offer evidence of a potential hierarchy in human auditory cortex.

Disclosures: **A.J. Kell:** None. **J.H. McDermott:** None.

Nanosymposium

773. Perception and Imagery: Perception and Behavior in Real-World Settings

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Topic: H.02. Human Cognition and Behavior

Support: NSERC Grant RGPIN-2014-04465

DFG Grant MU 3875/2-1

Title: Short-term memory of natural speech: processes of semantic regeneration and control

Authors: ***K. MÜSCH**[1], K. HIMBERGER[1], T. A. VALIANTE[2], C. J. HONEY[1];
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Abstract: How do we maintain and recall information that is structured in time such as a sequence of words? On the one hand, maintenance of sequential information may rely on rehearsal of low-level features, supported by fronto-parietal control mechanisms. On the other hand, the temporal structure of the sequence may not be explicitly maintained, but is rather reconstructed as the sequence is regenerated during recall. We hypothesize that the explicit rehearsal is necessary for maintaining the structure of arbitrary (incoherent) information sequences, while the regeneration mechanism contributes especially to the recall of meaningful sequences, such as coherent naturalistic speech. Finally, we hypothesize that these two mechanisms operate in parallel, but must ultimately compete to determine the sequence of information that is recalled. We investigated these hypotheses in the context of speech repetition, predicting that (i) repeating incoherent sentences will place greater demands on fronto-parietal control systems; and (ii) neural systems associated with maintenance of coherent and incoherent information patterns will be anticorrelated in their ongoing activity. We recorded electrocorticographic (ECoG) signals from the lateral surface of the human cerebral cortex in 17 patients with pharmacoresistant epilepsy. In a sentence repetition task, on each trial (i) two sentences were presented sequentially, (ii) participants silently rehearsed the second sentence, and (iii) they repeated the second sentence aloud. Half of the sentences to be repeated were semantically coherent, and half were semantically incoherent. Sentence coherence was associated with different patterns of broadband power (70-200 Hz) in the lateral cerebral cortex during subvocal sentence rehearsal. Incoherent sentences were associated with broadband power increases in the bilateral superior temporal and left inferior frontal gyri, while semantically coherent sentences were associated with greater “lexico-semantic” activations in the angular

gyrus, and the middle and inferior temporal gyri. During rehearsal, the temporal broadband power dynamics of the inferior frontal gyrus and middle temporal gyrus were anticorrelated over time. Furthermore, this anticorrelation was stronger for semantically incoherent relative to coherent sentences. These data suggest that at least two processes operate in parallel when maintaining and recalling sequences of natural language: explicitly controlled rehearsal of perceptual features and regeneration of semantic features. These two processes interact, seemingly competitively, to determine the sequences produced at recall.

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Nanosymposium

773. Perception and Imagery: Perception and Behavior in Real-World Settings

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Topic: H.02. Human Cognition and Behavior

Support: ERC-StG 261177

NWO-Vidi 452-12-009

Title: Updating memory hierarchies in hippocampus and medial prefrontal cortex

Authors: ***S. H. COLLIN**, B. MILIVOJEVIC, C. F. DOELLER;
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Abstract: Episodic memories are not stored in isolation, but rather as networks of related events. These networks are susceptible to change which enables integration of new events. However, the neural mechanisms underlying the updating of memory networks, and integration of new information during memory reactivation remain elusive. Here, we created two virtual families using the life-simulation game TheSims3 and then exposed participants to complex multi-event narratives that each comprised a typical day of one of the virtual families. Unknown to participants, the narratives formed a three-level hierarchy with levels family, day and event. In the MRI scanner 24 hours later, we presented participants with the narrative-events (pre-block), followed by an updating block with new related events (updating events) along with control events, and concluding with a post-block with the original narrative-events. We observed higher neural similarity in the anterior hippocampus between all updating events, compared to control events. Additionally, in the post-block (relative to the pre-block), a day-level and event-level representation of the original narratives was observed in the anterior hippocampus, selectively

for the updated narratives. In contrast, the posterior hippocampus showed higher neural similarity between updating events and their corresponding narratives, relative to controls. In sum, these data suggest that the hippocampus can integrate new information after reactivation of the original memory, by representing the original memory as well as the new information in the anterior hippocampus while the posterior hippocampus is crucial for the flexible integration of this new information into the initial mnemonic representation. An additional behavioral experiment showed that the new events had a neutral relationship to the original narratives. To further examine whether this updating mechanism is different when the new information is consistent with the original memory network, we performed an additional fMRI experiment. We found that updating narratives with consistent information leads to higher neural similarity between updating events and original narrative-events only in the medial prefrontal cortex (mPFC). This suggests that updating with consistent information is performed preferentially by the mPFC, independent of the hippocampus.

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Nanosymposium

773. Perception and Imagery: Perception and Behavior in Real-World Settings

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Topic: H.02. Human Cognition and Behavior

Support: NIH (R01-MH094480)

CIHR (MOP-115148)

Title: Is the hippocampus necessary for long-timescale dynamics in the default network?

Authors: ***J. CHEN**[1], M. D. BARENSE[2], D. CROMBIE[2], L.-K. YEUNG[2], U. HASSON[1], C. J. HONEY[2,3];
[1]Psychology, Princeton Neurosci. Inst., Princeton, NJ; [2]Psychology, Univ. of Toronto, Toronto, ON, Canada; [3]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: The timescales of information processing appear to vary hierarchically in the human cerebral cortex: early sensory areas typically process information over 10s to 100s of milliseconds, while higher-order areas process information over seconds to minutes of time [Honey 2012; Hasson et al, 2008; Lerner et al, 2011; Stephens et al, 2013]. In particular, when engaging with naturalistic narrative stimuli, activity patterns in regions within the default network depend on memory of stimulus input from minutes earlier. Is this memory within

default network regions supported by generic cortical mechanisms (such as recurrent activity [Murray et al, 2014; Chaudhuri et al, 2015]) or does it require a contribution from specialized systems (such as the hippocampus)? In this study, we estimate the timescales of processing in default network regions in an amnesic individual with bilateral hippocampal damage, as he engaged with a naturalistic narrative stimulus. The amnesic participant listened to a 7-minute continuous auditory narrative during brain imaging (2 repetitions). He also listened to a temporally-scrambled version of the same narrative, in which the sentences were presented in random order (2 repetitions). Preliminary results indicate that the scrambled narrative elicited reliable activity (consistent across repetitions) in auditory cortex, but not in higher-level default network areas; in contrast, the intact narrative elicited reliable activity in both auditory and default network areas. This pattern of results matches those observed in healthy control subjects [Lerner 2011]. Moreover, it was not only the overall pattern of reliability across the cortex that matched controls: the moment-by-moment BOLD activity observed in lateral parietal regions of the default network of this amnesic individual was well-matched to control subjects' activity timecourses in the same regions while listening to the same narrative. These data indicate that at least some of the memory-dependent narrative processing is preserved in this individual, without an intact hippocampus. Thus, it appears that stimulus information can persist within default network regions over surprisingly long periods -- on the order of minutes -- independent of interactions with the hippocampus.

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Nanosymposium

773. Perception and Imagery: Perception and Behavior in Real-World Settings

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Presentation Number: 773.12

Topic: H.02. Human Cognition and Behavior

Support: NSERC

CIHR

Title: Neural reactivation of naturalistic memories in developmental amnesia

Authors: *B. R. BUCHSBAUM[1,2], M. ST-LAURENT[1], C. DANG[1], R. OLSEN[1], S. ROSENBAUM[3];
[1]Rotman Res. Institute, Baycrest Ctr., North York, ON, Canada; [2]Psychology, Univ. of Toronto, Toronto, ON, Canada; [3]York Univ., Toronto, ON, Canada

Abstract: Neural reactivation occurs when brain activity patterns elicited during event perception are reinstated at retrieval. Recent evidence has linked the strength of pattern reactivation to successful episodic recall, indicating that neural reinstatement is fundamentally related to the fidelity and vividness of complex memories. Here we examine the extent and spatial topography of memory reactivation in two amnesic patients during a task requiring mental replay of short, audio-visual video clips depicting naturalistic events. We tested two patients: NC, an 19-year old individual with developmental amnesia linked to a diencephalic stroke; HC, a 21-year old developmental amnesic with a compromised hippocampal system; and a set of 19 control participants on a functional magnetic resonance imaging (fMRI) task during which participants viewed and recalled 11 short videos multiple times. We found that activation patterns associated with video perception were at least as reliable for the amnesic patients as for the control participants. During mental replay of the same videos, however, the amnesics showed markedly reduced neural reinstatement as measured by the similarity between perception- and memory-related activation patterns for the corresponding videos. Despite this pronounced reduction in the ability to reinstate distributed activation patterns during memory, both patients showed reliable video-specific activation during mental replay. Thus, the two amnesic patients showed *consistent* memory activation patterns that nevertheless diverged from the activation pattern evoked during direct perception. Follow-up regional analyses using a multivariate searchlight approach showed that both amnesic patients showed relatively increased video-specific pattern information in heteromodal cortices including the angular gyrus, anterior temporal lobe, and anterior ventrolateral prefrontal cortex. These results suggest that although the two patients fail to reinstate patterns of activation evoked during stimulus encoding -- leading to a perceptually impoverished memory experience -- they may draw more heavily on higher-order semantic representations to support memory-guided behavior.

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Nanosymposium

773. Perception and Imagery: Perception and Behavior in Real-World Settings

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant 1RO1NS089729

Title: Overlap between real-world spatial routes triggers divergence of their hippocampal representations

Authors: *A. J. CHANALES[1], S. E. FAVILA[1], B. A. KUHL[2];
[1]New York Univ., Brooklyn, NY; [2]Univ. of Oregon, Eugene, OR

Abstract: The hippocampus is thought to play a critical role in the disambiguation of overlapping experiences so that corresponding memories are free from interference. Despite substantial evidence demonstrating that the hippocampus maintains distinct representations of overlapping events, the mechanisms driving the disambiguation of hippocampal activity patterns remain unclear. A prominent account is that the hippocampus 'pattern separates' overlapping events by forming sparse representations at the time of encoding. This is thought to produce orthogonalized representations of overlapping events. An alternative, though not mutually exclusive, idea is that disambiguation of overlapping events continues over the course of learning, with hippocampal activity patterns becoming further separated through a competitive process that minimizes feature overlap between event representations. If the hippocampus specifically eliminates shared event features from the representations of overlapping events, this makes the paradoxical prediction that overlapping events may actually move farther apart *from each other* than from other, non-overlapping events. To test for evidence of pattern differentiation, we ran three separate studies (1 behavioral, 2 fMRI) in which participants studied naturalistic, real-world spatial routes around the NYU campus. The routes were structured into overlapping pairs that followed the same path before diverging to distinct destinations (similar to rodent T-maze paradigms). Results from an initial behavioral study demonstrated that participants' ability to discriminate between the overlapping routes robustly improved with learning. In two fMRI studies we utilized spatiotemporal pattern analysis to look for parallel learning-related changes in hippocampal representations. Strikingly, hippocampal activity patterns for overlapping routes diverged over learning to the point where they became *less similar* to each other than to patterns for non-overlapping routes. This pattern was completely absent in the 'parahippocampal place area' (PPA). Rather, PPA activity patterns were more similar for overlapping compared to non-overlapping routes at the beginning and end of learning. Lastly, we found evidence that the divergence of overlapping route patterns within hippocampus was driven by targeted decrease in similarity between voxels that were initially 'moderately shared' across the overlapping segments, providing critical mechanistic insight into how pattern differentiation occurs. Taken together, these findings inform theoretical perspectives on how the hippocampus disambiguates overlapping events.

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Nanosymposium

773. Perception and Imagery: Perception and Behavior in Real-World Settings

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Presentation Number: 773.14

Topic: H.02. Human Cognition and Behavior

Support: CIHR: MOP 49566

Title: Using google streetview to examine cognition in virtualized real-world environments

Authors: *J. D. OZUBKO[1], J. ROBIN[2,3], B. BELLANA[2,4], I. BRUNEC[2,4], C. GRADY[2], S. ROSENBAUM[5,2], G. WINOCUR[2,6], M. MOSCOVITCH[2,4];
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Abstract: In recent decades, researchers have begun to use virtual reality environments to examine human spatial memory. This research has been instrumental in identifying the hippocampus as a key structure that supports the learning and representation of spatial environments. Due to technical and practical limitations, however, virtual reality techniques have been restricted to artificial, contrived, and unfamiliar environments. That research, therefore, is limited in the degree to which it can address issues about long-term, remote spatial memory retention and retrieval of real-world environments, and how those representation may change with time and experience. As a first step in addressing these types of questions, we used Google Streetview to investigate naturalistic spatial navigation. Google Streetview offers an effective method for virtualizing large-scale, real world environments, allowing researchers to investigate naturalistic types of navigation and spatial memory. We present the results of one study that examined the role of the hippocampus in supporting spatial memories while participants navigated along routes in virtual representations of real-world environments in their home city (Toronto) that varied in terms of familiarity, or had their first-person views mirror (L-R) transformed. Our results showed that the hippocampus played a role in the navigation of less familiar or spatially translated routes but that other areas, such as the mPFC, may support navigation along highly familiar routes. Building on this first study, We discuss several other ongoing lines of research that expand on this study and use Google Streetview to investigate temporal vs. sequential event representation in the hippocampus, the decoding of hippocampal representations during navigation, and potential clinical applications of our software.

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Nanosymposium

774. New Electrode Technologies

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Presentation Number: 774.01

Topic: I.04. Physiological Methods

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UCSD Center for Brain Activity Mapping

Title: High density individually addressable nanowire arrays record intracellular neuronal potentials

Authors: *R. LIU[1], R. CHEN[1], A. T. E. YOUSSEF[1], S. LEE[1], S. HINCKLEY[4], M. L. KHRAICHE[1], J. SCOTT[2], Y. HWANG[1], A. TANAKA[3], Y. RO[1], A. K. MATSUSHITA[3], X. DAI[5], C. SOCI[5], S. BIESMANS[4], A. JAMES[6], J. NOGAN[6], K. L. JUNGJOHANN[6], D. V. PETE[6], D. B. WEBB[6], Y. ZOU[2], A. BANG[4], S. A. DAYEH[1];
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Abstract: Nanowire geometries are ideal for interfacing with cells and measuring intracellular potentials of neurons with minimal invasiveness. Prior works have demonstrated single nanowire devices or devices encompassing ensembles of several nanowires but individual electrical addressability of a single nanowire in a vertically standing array of nanowires, which is important for localizing the origin of action potentials, has not been accomplished before. Additionally, sensitivity to subthreshold potentials has not been clearly observed with high-density nanowires and interfacing with human neurons, important for drug screening is yet to be demonstrated. We report a new hybrid integration scheme that offers for the first time a nanowire-on-lead approach which enables independent electrical addressability, is scalable, has superior spatial resolution down to submicrometer site-to-site spacing, permits natural internalization into neurons, and can be combined with standard integrated circuit fabrication technologies. Physiological recordings from mouse primary neurons and human induced pluripotent stem cell (hiPSC)-derived neurons revealed high signal to noise ratios with clear subthreshold potential detection for both intracellular and extracellular configurations. We measured electrical activity from neurons penetrated by nanowires for up to 6 weeks, as validated by transmission electron microscopy. This records the first long-term non-destructive measurement of intracellular potentials from a single neuron, as opposed to the standard patch-clamp-which is destructive, unscalable to large neuronal densities and to long recording times or to microelectrode arrays that enable long-term recordings but lack the sensitivity to subthreshold potentials. Overall, our platform paves the way for longitudinal electrophysiological experiments on synaptic activity in human iPSC based disease models of neuronal networks, critical for the development of drugs for neurological diseases.

Disclosures: The Disclosure Block has exceeded its maximum limit. Please call Tech support at (217) 398-1792 for more information.

Nanosymposium

774. New Electrode Technologies

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NIH 1R01EY023173

New York Stem Cell Foundation-Robertson Award

NIH Director's Pioneer Award 1DP1NS087724

Title: A novel implantable electrode for chronic electrical interfacing with the murine cervical vagus nerve

Authors: *A. CARAVACA[1], G. RIGGOT[2], R. DESIMONE[2], K. J. TRACEY[3], H. SOHAL[2], E. S. BOYDEN[2], P. S. OLOFSSON[1];
[1]Karolinska Institutet, Stockholm, Sweden; [2]MIT, Cambridge, MA; [3]Feinstein Inst. for Med. Res., Manhasset, NY

Abstract: Recent advances in neuroscience and immunology have revealed that inflammation is regulated by neural reflexes. Electrical stimulation of the cervical vagus nerve reduces splenic release of tumor necrosis factor alpha (TNF-α) in endotoxemia. This new discovery has spawned human clinical trials with chronically implanted devices for electrical stimulation of the vagus nerve in treatment of inflammatory diseases such as rheumatoid arthritis and Crohn's disease. Current technology for electrical interfacing with the vagus nerve does not permit high channel count chronic stimulation or recording in relevant murine models. To address this, we developed a 16-channel electrode array on a 10 μm layer of highly biocompatible parylene configured with fourteen 30 x 30 microns recording electrodes diagonally arranged between two 80 x 80 stimulation electrodes. The array is designed to record elements of directional and spatial properties of vagus nerve electrophysiology. The probe has an integrated ribbon cable that interfaces with a standard Omnetics connector allowing connectorisation to most commercial electrophysiology systems.
The left cervical vagus nerve was surgically isolated in adult, male BALB/C mice under

anesthesia and the electrode wrapped around a segment of the nerve. The integrated cable was subcutaneously tunneled to the back of the animal and a connector externally fixated in position for recording and stimulation. The wound was closed and mice were allowed to recover until being returned to housing, where they were kept for 3 months before euthanasia. No postoperative complications such as excessive weight loss, hunching, abnormal grooming behavior, and immobility were observed. Another group of mice were subjected to electrode implantation followed by either electrical stimulation of the vagus nerve by delivering a 1 mA current (250 μs biphasic pulse, 50 μs interphase delay, 10 Hz) for 60 seconds or sham stimulation. After 3 hours of recovery, the mice were injected with 5 mg/kg lipopolysaccharide (LPS) intraperitoneally and euthanized 90 minutes post-injection for serum collection. Enzyme-linked immunosorbent assay (ELISA) showed reduced TNF-α in the electrically stimulated group as compared to sham, a key validation of this electrode technology. The development of this novel device is a critical step towards a viable chronic interface for vagus nerve stimulation and for recording in the murine peripheral nervous system.

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Nanosymposium

774. New Electrode Technologies

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Presentation Number: 774.03

Topic: I.04. Physiological Methods

Support: UT Brain award #365459

UT Austin Cockrell School of Engineering and Department of Biomedical Engineering start-up fund

Title: Sub-cellular, ultra-flexible electrodes for reliable gliosis-free neural interface

Authors: ***L. LUAN**[1], C. XIE[2];
[1]Dept. of Physics, The Univ. of Texas At Austin, Austin, TX; [2]Dept. of Biomed. Engin., UT Austin, Austin, TX

Abstract: Despite great success and promise, current implanted neural electrodes are limited by their poor reliability and strong invasiveness to the hosting brain tissues both acutely and chronically. Electrodes fail to track the same neurons even within hours due to their relative movements with respect to brain tissues induced by biological motions. Moreover, over extended

period of time (weeks to months), the implanted probes elicit sustained tissue response, leading to blood-brain-barrier (BBB) leakage, neuronal loss, fibrotic accumulation, and consequential probe performance deterioration. This chronic neural interface degradation is a multifaceted process with leading causes attributed to (*i*) relatively large physical dimension of the neural probe compared with cells and capillaries, whose chronic presence interrupts local biological functions and triggers immune responses, (*ii*) substantial mechanical mismatch between the probes and brain tissues, which induce persistent perturbation to surrounding neural tissues, and (*iii*) relatively large initial surgical tissue damage that leaves unrecoverable fibrotic tissue at the interface. Despite extensive efforts in the past decades addressing the aforementioned problems, reliable long-term neural electrical recording has not been achieved. In this presentation, we demonstrate a viable solution to this long-standing challenge by drastically reducing the electrodes' dimension to subcellular scale and matching their compliance to brain tissues. We show that ultra-flexible, multiplexed electrodes with subcellular dimensions and cellular surgical footprint form reliable, gliosis-free neural interface. We characterize the interface by chronic recording and in-vivo two-photon microscopy for over four months in mice brain. We have monitored 80 implanted electrodes and show that the electrode impedance, the noise level, the single-unit recording yield, and the signal amplitude remain stable for the entire course. We have followed 20 single-unit activities by bi-weekly measurements, which consistently show slow trackable evolution in the waveforms. Using in-vivo two-photon microscopy, we monitor the chronic evolution of neurons, astrocytes and capillaries at the interface and reveal the non-invasive nature of the interface. We observe no neuronal loss, complete absence of gliosis, fully recovered local capillary network and intact BBB. We also track individual neurons' movements with respect to the implanted electrode, and observe < 10 μm/month migration, which is consistent with the single unit waveform evolution we observe in chronic recording.

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774. New Electrode Technologies

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Topic: I.04. Physiological Methods

Support: Nuviant Medical Inc.

Title: Electrical activation of the rat tibial nerve with a chronically-implanted wireless stimulation system

Authors: *J. P. PAQUETTE[1], Z. MOAZZAM[1], N. KHODAPARAST[3], A. R. DUKE[3], P. B. YOO[1,2];
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Abstract: Overactive bladder (OAB) is characterized by symptoms of urinary frequency, urgency and incontinence. Percutaneous tibial nerve stimulation (PTNS) is an effective OAB therapy, where electrical stimulation is applied for 30 minutes in a clinical setting. Therapeutic effects are achieved by 12 weeks of weekly stimulation, and maintained by treatment sessions every 3 weeks thereafter. Although effective, long-term treatment is recognized as a concern. Repeated needle insertions, logistics and recurrent office visits are considered major contributors to low compliance among patients. This motivated the current study where the feasibility of a novel wireless stimulation device was investigated as a means of providing chronic tibial nerve stimulation.

Long-term implants were conducted in six adult Sprague-Dawley rats. Under sterile conditions, an incision in the lower back region, followed by blunt dissection of the underlying fascia, provided a subcutaneous pouch for implanting the main body of the implantable pulse generator (IPG, Gecko 1.0 prototype, Nuviant Medical Inc). Additional incisions were made in the lower abdominal area and the posterior-medial aspect of the lower leg. This allowed subcutaneous tunneling of the lead wire from the lower back to approximately the medial malleolus. The four electrode contacts located at the distal end of the lead wire were positioned in close proximity to the tibial nerve (TN). All incisions were sutured closed and the stimulation threshold for evoking a foot response (T) was tested for each individual electrode contact. The foot twitch threshold was tested repeatedly every two weeks until the end of the study (12 weeks post-implant). Overall, channel 1 (most distal electrode contact) achieved the lowest foot twitch threshold across all 6 animals. Immediately following surgical implant, the average threshold amplitude (T_0) was 0.49 ± 0.07 mA (mean ± SE). At 2 weeks post-implant, the average threshold for channel 1 exhibited a notable increase to 0.75 ± 0.09 mA (78 ± 42% increase from T_0), but remained consistent until the end of the study (0.77 ± 0.15 mA at 12 weeks post-implant). The increase in foot twitch threshold was attributed to electrode encapsulation and possible electrode migration.

The results of this long-term implant study suggest the feasibility of using the Gecko system as part of a chronic tibial nerve stimulation therapy for OAB patients. An implantable system would offer patients an at-home treatment option that would obviate the need for recurrent office visits.

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Topic: I.04. Physiological Methods

Support: NSF ECCS-1351980

UCSD Center for Brain Activity Mapping

Title: Novel 3d minimal tissue-penetrating probes on conformal flexible substrates for in-vivo brain mapping

Authors: *S. LEE[1], F. AZZAZY[1], M. GANJI[1], M. KHRAICHE[2], J. HERMIZ[1], N. ROGERS[1], A. YOUSSEF[1], V. GILJA[1], S. DAYEH[1];
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Abstract: To better understand the local brain activity and isolate neuronal circuits with specific functionality in the brain, it is essential to develop new probes that can (i) enable single unit recordings, (ii) cause minimal tissue scarring and biofouling, and (iii) remain in intimate contacts with the sulci and gyri of the brain. We introduce sharp, needle-like Si-based neuronal probes on ultra-thin, biocompatible flexible substrates for penetrative, high-resolution, 3D mapping of brain activity. The flexible polyimide substrate carrier allows conformal adhesion to the cortex surface and embeds electrical leads for data streaming. This technology has the potential for higher fidelity recordings and localized stimulation to better understand neuronal activities and possibly to assist in the local and efficient treatment of neurological diseases. We utilize a polyimide layer and Si substrates with conventional Si micromachining processes and double-side photolithography alignment in order to fabricate 3D Si neuronal micropillars on a flexible substrate. Our dual-array device consists 2Fins with each comprising 16 high aspect ratio pillars (4×4 array, L=100μm and W=2μm) on a sub-10μm thin flexible substrate. The measured electrochemical impedance in phosphate buffered solution (PBS) for each pillar was in the range of 162kΩ-247kΩ range for the functional pillars that are suitable for single unit recordings. The impedance was reduced from several 10-100MΩs to the 100KΩ range through an engineered Pt, Ti, or Au metal coat at the Si micropillar tips. The Nyquist plot of the characterized probes on flex illustrates a charge-transport dominant impedance and mostly capacitive coupling to the neuronal probes. In-vitro measurements on retinal slices demonstrated single unit activity detection. We will present physiological and behavioral measurements in rodent animal. These preliminary results indicate that our technology is capable of high resolution, penetrating, in-vivo recordings with individual electrical addressability on conformal flexible substrate. Funding Sources: NSF ECCS-1351980 and UCSD Center for Brain Activity Mapping.

Disclosures: **S. Lee:** 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; NSF, UCSD Center for Brain Activity Mapping. **F. Azzazy:** 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; NSF, UCSD Center for Brain Activity Mapping. **M. Ganji:** 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; NSF, UCSD Center for Brain Activity Mapping. **M. Khraiche:** 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; NSF, UCSD Center for Brain Activity Mapping. **J. Hermiz:** 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; NSF, UCSD Center for Brain Activity Mapping. **N. Rogers:** 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; NSF, UCSD Center for Brain Activity Mapping. **A. Youssef:** 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; NSF, UCSD Center for Brain Activity Mapping. **V. Gilja:** 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; NSF, UCSD Center for Brain Activity Mapping. **S. Dayeh:** 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; NSF, UCSD Center for Brain Activity Mapping.

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Topic: I.04. Physiological Methods

Support: BrainSEED grant

Title: Virtual steerable acousto-optic waveguides for non-invasive deep brain imaging and stimulation

Authors: *M. CHAMANZAR[1], N. DO[2], J. BLOCH[2], M. HUH[2], M. ALAM[2], M. MAHARBIZ[2];
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Abstract: Optical methods are now being widely used for recording and stimulation of activity in the brain with high temporal resolution. All existing techniques, however, suffer from an inability to deliver light deep into the tissue with high spatial resolution in a non-invasive way. Additionally, in the context of the central nervous system, which harbors widely distributed neural processes, system-wide interrogation would require either fast optical beam-steering capability or simultaneous multi-site illumination. As light propagates through tissue, it undergoes diffraction, scattering, and absorption; as a result, the beam widens up and the intensity of light rapidly falls below the threshold of excitation of opsins and optical tags. This would limit optical techniques to superficial layers of the tissue. To alleviate these issues, either the intensity of light on the surface of the brain must be increased, which can be detrimental to the tissue, or invasive light guides such as fiber optics or optical waveguides need to be inserted inside the tissue. Steering light to different locations within the tissue using implantable waveguides is difficult and invasive, limiting common optical imaging and stimulation techniques to fixed positions in the tissue. Here, we introduce a radical approach to use ultrasonic waves to confine and steer light deep (a few millimeters) into the tissue without having to insert a physical light guide. Ultrasonic pressure waves launched from outside can propagate in the brain tissue with minimal loss and change the density of medium locally and interact with light to define and steer the trajectory of light. The flexibility of sculpting complex ultrasonic patterns in tissue to confine and guide light would enable creation of *virtual* optical components such as lenses, gratings, and waveguides without having to implant a physical component or displace the tissue. We will show how an array of ultrasonic transducers can be used to generate reconfigurable complex optical patterns entirely within the tissue. We will show that a large intensity enhancement of ~35 folds can be achieved by forming a ~200 μm virtual waveguide in the tissue. We will demonstrate that the required intensity of ultrasound to form and reconfigure virtual optical components is well below the established safe limits. Specifically, we can realize optical waveguides within a few millimeters of the tissue depth, while maintaining a mechanical index of ~1.3 and the de-rated spatial-peak temporal average ultrasonic intensity of I_{SPTA} = 503.7 mW/cm^2 to ensure safe operation. This technology provides unprecedented optical access to deep regions of the brain in a non-invasive way.

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Presentation Number: 774.07

Topic: I.04. Physiological Methods

Title: The neuropixels project: design and performance of 966 selectable site 384 channel Si probes

Authors: *T. HARRIS[1], J. JUN[2], B. KARSH[2], B. BARBARITS[2], W.-L. SUN[2], S. MUSA[3], J. PUTZEYS[3], A. ANDREI[3], C. LOPEZ[3];
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Abstract: [**Introduction**] The ability to record and resolve large numbers of neurons using Si probes has been limited by the fabrication technology needed to produce probes with large numbers of dense recording sites. HHMI Janelia Research Campus, the Allen Institute, the Gatsby Charitable Foundation and the Wellcome Trust have formed a consortium to fund a probe fabrication project that addresses this deficiency. Janelia, Allen, and University College London, the latter though grants from Wellcome and Gatsby, have designed a set of probes engineered and fabricated at imec, Leuven Belgium.
[**Methods**] We have developed four probe types to assess the relative advantages of technology options. These probes are fully integrated recording systems, with sensors, amplifiers, multiplexers and digitizers fabricated into the device. All versions of this first generation of engineering prototypes are single shank devices with two adjacent sites at twenty micron intervals beginning at the shank distal end. Shanks are 50 or 70 um wide and 20 um thick. Two versions have only 384 sites, one with passive sites, and the other with a unity gain CMOS amplifier under each of the sites. The other two versions have 960 and 966 sites respectively. The 960 site version has a switch under each site so that 384 channels of output can be selected from among the 960 sites. The last version has both a unity gain amplifier and a switch. To keep the shank width from exceeding 70 um, output from version four is 276. Performance of each version was tested in vitro, in vivo acute, and in vivo chronic recordings. We report signal to noise for each version, single unit yield, and chronic stability.
[**Results**] The performance of all four types is excellent. Version 1, with only passive sites and no amplifiers has the lowest noise, ~5 uV rms. To accommodate the extra shank circuitry, the other three versions are 70 um wide. As expected, Version 4 with both amplifiers and switches on every site has highest noise, but still less than 10 uV rms. Many hundred well resolved single units are obtained in a typical recording. We find no difference in chronic stability between any of the four versions. The low impedance path from site to base in the amplified versions is thought to reduce transients from impacts or tooth grinding. We report the variety of methods to

cope with these transients.
[**Conclusions**] Even though these probes are first engineering prototypes, performance is outstanding. They illustrate the power of using advanced microelectronic design and fabrication to create a large dense array of recording sites. The objective of the consortium is to make these probes commercially available after another engineering refinement cycle.

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Nanosymposium

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Topic: I.04. Physiological Methods

Support: Cognitive Science and Technologies Council

Title: Electrical imaging from high density neural recordings

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Abstract: Electrical recordings have been central to the study of function of neural systems. With recent advances in the design of high density electrode arrays, this begs the question of whether electrical recordings can be converted into time-varying images of neural activity. In principle, such images may be possible well beyond the density of electrodes as the activity of neurons is extremely sparse in space and time. The possibility for electrical imaging could be extremely useful for quantifying the structure of neural circuits in vivo in a way complementary to optical methods such as calcium imaging.
Here we thus develop methods to estimate spatially distributed neural current sources from high-density measurements of electrical fields. The underlying model are quasi-static maxwell's equations and the goal is to produce a clear image. Our method consists of three steps: 1) Point sources: using regularization method to find a sparse representation of electrical point sources in the volume. 2) Grouping: link these point sources together based on their values and proximity in distance and form the wave of activities. 3) Tracking the activity: match the waves of activity at different times to track the movement of charge distribution. The final image contains the anatomical structure and functional activity of brain tissue. We test this method on data from our own simulations, where we know the ground truth. We also analyze its results on real data.

Disclosures: **R. Farhoodi:** None. **K.P. Kording:** None.

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774. New Electrode Technologies

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Title: Integrated autopatching and close-packed extracellular recording in headfixed mice

Authors: B. ALLEN[1], J. BERNSTEIN[1], C. CHRONOPOULOS[2], J. KINNEY[2], C. LAMANTIA[2], C. MOORE-KOCHLACS[3], *J. SCHOLVIN[1], C. FONSTAD[1], S. KODANDARAMAIAH[4], N. KOPELL[3], E. BOYDEN[1];
[1]MIT, Cambridge, MA; [2]LeafLabs, Cambridge, MA; [3]Boston Univ., Boston, MA; [4]Univ. of Minnesota, Twin Cities, MN

Abstract: Close-packed nanofabricated silicon microelectrodes allow a great number of neurons to be recorded in a spatially oversampled fashion, supporting automated data analysis, but are restricted to the recording of spikes due to the extracellular nature of the recordings. Autopatching, the robotic performance of in vivo whole-cell patch clamp neural recording, enables gold standard subthreshold neural activity monitoring, but cannot scale to as many neurons as extracellular recording. Here we integrate autopatching and close-packed microelectrode recording in order to enable the examination of subthreshold neural activity in conjunction with massively parallel neural recording.
Using close-packed electrodes with 64, 128, 256 and 1020 recording sites, we present dense microelectrode recordings of neurons from the visual cortex of awake headfixed mice, in the presence of varying kinds of visual stimuli. For a subset of probe geometries, we establish a

ground-truth data-set by performing close-packed extracellular recordings while patch clamping simultaneously a neuron within the network. This may not only provide important data-sets for automated spike sorting efforts and for understanding design tradeoffs for close-packed extracellular probes, but also provide a new way to integrate multiple levels of neural dynamics data for more comprehensive understanding of neural network dynamics.

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Topic: I.04. Physiological Methods

Support: DARPA Contract N66001-15-C-4017

NIH Grant 1R43EB018200-01A1

Title: Impedance stability, long-term stimulation stability, and efficacy of IrOx tip metallization for Utah electrode arrays

Authors: ***L. RIETH**[1,3], B. BAKER[1], R. CALDWELL[2], H. MANDAL[3], R. SHARMA[1]; [1]Electrical and Computer Engin., [2]Dept. of Bioengineering, Univ. of Utah, Salt Lake City, UT; [3]Blackrock Microsystems, Salt Lake City, UT

Abstract: Achieving reliable recording and stimulation of neural signals is required for neural interface technologies to be used in long-term therapeutic applications. Tip metal degradation has been observed for Utah Electrode Arrays (UEAs) used for both recording and stimulation of

the central and peripheral nervous system. Our tip metal research has focused on decreasing the impedance magnitude and distribution, increasing the lifetime under aggressive stimulation conditions, understanding the relationship between in-vitro and in-vivo impedances, and exploring the ability to predict stimulation waveforms from wide-band impedance data. The impedance magnitude of arrays using IrOx tip metallization was decrease to maintain median values for individual arrays of < 10 kOhms with standard deviations of less than 4 kOhms for typical electrode deinsulations (50 microns). Controlling the interface between the tip metallization to mitigate degradation mechanisms associated with 1) oxidation, 2) Kirkendall void formation, 3) surface roughness, and 4) uniformity of metallurgical junction formation were address and will be reported. Electrode stimulation stability was characterized under aggressive conditions including voltages above the water window and constant current stimulation of up to 2,500 uA, and some tests extending to billions of cycles. Periodic voltage waveforms were used to monitor electrode impedance and degradation. Electrodes have survived > 0.1 billion cycles at > 400 uA, with only modest changes in the voltage waveform. Comparative SEM findings for electrodes before and after stimulation suggest that no discernable IrOx degradation is occurring for electrodes at currents less than 800 uA, which is well above the 100 uA limit of our in-vivo stimulation paradigms, strongly suggesting electrochemical degradation is not an endemic challenge. Comparison of electrode impedances from PBS to in-vivo found significant impedance increases in-vivo, particularly in the resistive portion of the spectra above 1 kHz. In addition, a method to develop a transfer function from broad-band impedance spectroscopy data to predict stimulation waveforms was developed. This method was found good agreement with actual collected stimulation waveforms, particularly for <1 Volt stimulation.

Disclosures: **L. Rieth:** A. Employment/Salary (full or part-time): Blackrock Microsystem. **B. Baker:** None. **R. Caldwell:** None. **H. Mandal:** A. Employment/Salary (full or part-time): Blackrock Microsystems. **R. Sharma:** None.

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UCSD CTRI support

Nanovision Biosciences

Title: Towards scalable integrated nano-engineered retinal interfaces

Authors: ***A. AKININ**[1,2], S. HA[2], M. KHRAICHE[1], Y. JING[1], W. R. FREEMAN[3], G. A. SILVA[2], G. CAUWENBERGHS[2];
[2]Bioengineering, [3]Ophthalmology, [1]UCSD, La Jolla, CA

Abstract: Common retinal degenerative disorders such as retinitis pigmentosa and age-related macular degeneration cause irreversible loss of vision. Retinal prosthetic devices replace the retinal photoreceptor functions by producing electrical stimulation for inducing visual perception artificially. However, recent retinal prostheses have achieved very limited success in regaining vision-achieving only up to a few dozens of pixelized phosphenes. This project pursues a fully integrated nano-engineered retinal prosthesis that completely departs from the status quo with a path forward to restore natural, high-resolution vision in the affected population. The engineering effort pertains the design and development of high-performance light-sensitive electrode arrays, as well as innovation in the wireless interface, communication, stimulation waveform generation, system control, and biocompatible packaging and surgical assembly. In vitro experiments show restored light responsivity through the nano-engineered implant, and a path forward towards in vivo validation and clinical trials.

Disclosures: **A. Akinin:** 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; UCSD Institute for Engineering in Medicing: Galvanizing Engineering in Medicine, UCSD Clinical Translation Research Institute, Nanovision Biosciences. **S. Ha:** None. **M. Khraiche:** None. **Y. Jing:** None. **W.R. Freeman:** None. **G.A. Silva:** None. **G. Cauwenberghs:** None.

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Cleveland Clinic

Title: Software tools for removing noise from intracortical spiking data and local field potentials

Authors: *D. M. TAYLOR[1,2,3], T. JOHNSON[1], J. JIANG[1,3];
[1]Neurosciences, Cleveland Clin. Lerner Res. Inst., Cleveland, OH; [2]Louis Stokes Cleveland VA Med. Ctr., Cleveland, OH; [3]Biomed. Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: Multichannel intracortical microelectrode arrays detect signals of interest comprised of both spikes and local field potentials. However**,** they also detect a lot of background 'noise' that inhibits our ability to accurately extract the desired information from the neural recordings. For spike detection, the background noise consists of both biological noise made up of small overlapping spikes from many unisolatable neurons as well as electromagnetic noise from the environment and the recording system itself. Using software to subtract out a single 'reference' channel from all remaining channels can reduce common noise but requires finding an ideal reference channel. Subtracting out a common average reference (CAR) is more robust but still has its limitations--especially when there are small numbers of channels or multiple channels that detect the same neuron. Here we show how subtracting out a weighted average reference (WAR) can do a significantly better job than CAR or single-channel referencing at reducing background noise. Using identical objective spike sorting methods showed that WAR out performed CAR in terms of spike sorting accuracy and number of detectable neural units. Similarly, task-specific weighted average referencing can maximize the useful information extracted from local field potentials. We also show how customizing separate weighted average referencing schemes for individual frequency bands of interest can maximize the information extracted from local field potentials. WAR methods can be used offline as well as online to improve real-time information extraction in closed-loop experiments.

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Topic: I.04. Physiological Methods

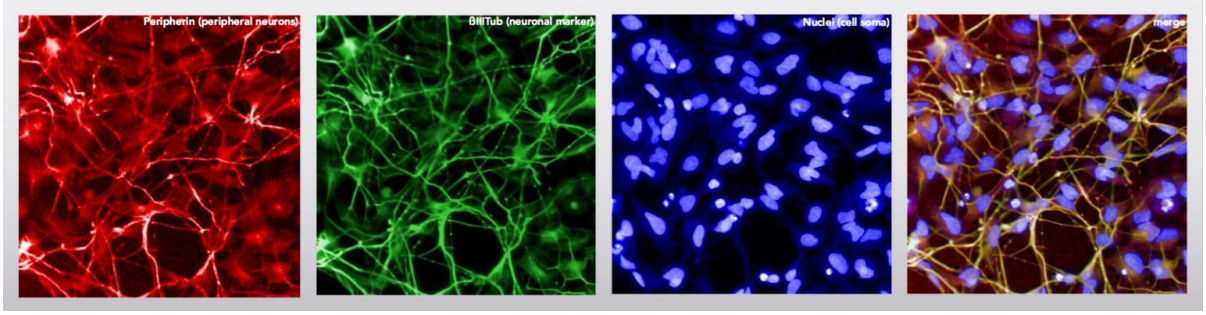
Title: Functional assessment of *In vitro* neurotoxicity and network activity using human iPSC derived peripheral neurons on microelectrode arrays

Authors: *G. C. LUERMAN[1], D. HESS[2], E. GUENTHER[3], H. BOHLEN[2];
[1]Axiogenesis Inc, Plymouth Meeting, PA; [2]Axiogenesis AG, Cologne, Germany; [3]NMI TT GmbH, Reutlingen, Germany

Abstract: In vitro pharmacology profiling of new chemical entities during early phases of drug discovery has recently become an essential tool to predict clinical adverse effects. While for cardiac safety testing high technology platforms are available, specific in vitro neurotoxic panels are not, and in-vivo models are used instead. However, correlations between animal and human data are often weak; in addition, animal studies are expensive, ethically questionable and require large amounts of chemical compounds. In order to directly address human targets and toxicity, we developed a 48-well platform based on the usage of human induced pluripotent stem-cell derived neurons. Compound related effects on the spontaneous activity of the Peri.4U neurons were assessed by performing field potential recordings using multiwell microelectrode array (MEA)- technology (Maestro System, Axion, Atlanta). When cultivated on the MEA chips, spontaneous activity of these neurons was recorded in 100% of the wells after a cultivation period of 3-4 days. Recordings with clear detectable burst-like activity indicating the presence and establishment of a functional neuronal network were chosen for testing neurotoxic compounds. Reference compounds with a known neurotoxic potential, such as neuroleptica, antidepressiva, neurotransmitter blockers, pesticides or plant toxins, were analyzed for their effect on neuronal network behavior, thereby demonstrating the potency of this in vitro system for reliable detection and quantification of neurotoxic compound actions.

EFFECT OF COMPOUND ON MEAN SPIKE FREQUENCY

Neurotoxic Compound	**Peri.4UNeurons**	**Rat CorticalNeurons***
Fipronil	decreased IC50 = 4.4 +/- 1.6 uM	decreased > 10 uM
Loxapine	decreased IC50 = 1.4 +/- 0.3 uM	Not tested
Nomifensine maleate	decreased IC50 = 6.2 +/- 0.8 uM	decreased IC50 > 10 uM
Trimethyltin	decreased IC50 = 50.2 +/- 8.5 uM	decreased IC50 > 10 uM
Eugenol	decreased > 10 uM	decreased IC50 > 1 uM
Nicotine	increased > 100 nM	increased > 10 uM
Diphenhydramine	decreased IC50 = 4.1 +/- 0.7 uM	decreased >10 uM
Mepiquat chloride	no effect up to 100 uM	no effect > 100 uM
* data from Defranchi et al, 2011 4(6):1-10		



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