Archives of General Psychiatry
Volume 64
Archives of General Psychiatry
Volume 65
Archives of General Psychiatry
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JAMA Psychiatry
JAMA Psychiatry
PSYCHIATRY IN THE ELDERLY
Mental Disorders
DEMENTIA
AUTISM SPECTRUM DISORDERS
PSYCHIATRIC EPIDEMIOLOGY
Psychotropic Drug
PSYCHIATRIC
MINIMAL BRAIN DYSFUNCTION IN CHILDREN
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William McAlpin Award Research Achievement Award, National Mental Health Association (1992)
Elected Fellow, American Academy of Arts and Sciences (1994)
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The Rhoda and Bernard Sarnat International Prize in Mental Health, National Academy of Medicine (2017)

Joseph Coyle began his career in research as a medical student in the laboratory of Solomon Snyder, MD, at Johns Hopkins School of Medicine in 1968. His early research with Snyder and as a postdoctoral fellow with Julius Axelrod at the National Institute of Mental Health focused on the synthesis and inactivation of catecholamines and the development of neurotransmitter systems in the rat brain. While completing his residency in psychiatry at Hopkins, he established his laboratory and began studies on excitotoxic lesions, developing animal models of Huntington's disease and Alzheimer's disease. He identified the degeneration of the basal forebrain cholinergic neurons as an early lesion in Alzheimer's disease and a contributor to the memory deficits in the disorder. For nearly a decade, he served as the director of the Division of Child Psychiatry at Hopkins.
In 1991, he moved to Harvard Medical School where he served as the chairman of the Consolidated Department of Psychiatry for a decade. His most recent research has focused on the role of glutamatergic N-methyl-D-aspartate receptors in the pathophysiology of schizophrenia and related severe mental disorders.

Joseph T. Coyle, MD

Introduction

I want to thank Larry Squire and Tom Albright for inviting me to contribute to this volume of *The History of Neuroscience in Autobiography*. It is a great honor to be included in this series of autobiographies by esteemed neuroscientists. Also, the invitation is particularly timely as it was precisely 50 years ago when as a third-year medical student at Johns Hopkins I began an elective research quarter in the psychopharmacologic laboratory of Solomon H. Snyder. The intervening years seem to have gone by too quickly. But, the opportunity to write these reflections made clear to me that my life in neuroscience research and psychiatry can be best summarized by chance events of unappreciated significance at the time, the generosity of mentors and colleagues, excellent trainees who taught me so much, and a wonderful family that kept me grounded.

The Early Years

I came from a family of physicians. My mother's father was a small-town doctor in Iowa, who attended Rush Medical School in Chicago after emigrating from Luxembourg. My father, the son of a train conductor, worked his way through college and medical school at the threshold of the Great Depression and became an orthopedic surgeon (Coyle 1951; Voris and Coyle 1952[1]). Two uncles and two cousins also became physicians. So, I was probably destined to go into medicine. But, I never gave it much thought as I was growing up on the south side of Chicago.

In retrospect, I was probably a bit odd as a child. I was not particularly interested in playing sports in spite of the fact that during my adolescence my father was the team physician for the Chicago White Sox, a major league baseball team. I was more interested in how things worked: taking a clock apart at age six, playing with my Gilbert Chemistry set at 10, raising a Polyphemous moth from a caterpillar at 12, and struggling with my assignments for the J. W. Ellwood's School of Taxidermy's correspondence course at 14. Adolescence found me in a Jesuit high school hewing to their centuries-honed approach to education—*ratio studiorum*—that extended over the next eight years through college at Holy Cross in Worcester, Massachusetts. This curriculum entailed five years of classical Greek, six years of Latin, and eight years of French. In addition, my college

[1] This article appeared in the journal of which I was appointed editor-in-chief 60 years later.

years were laden with philosophy and theology courses, so that technically I was a French and philosophy major. The science courses required for medical school were painfully dull, mainly concerned with the memorization of mind-numbing facts or conducting canned experiments. The transformative college experience for me was the year that I spent as a student at the Sorbonne, going from a very parochial life at Holy Cross to a very cosmopolitan one in Paris. The return for my last year of college in Worcester was quite painful.

At my interview with the admissions committee at Johns Hopkins Medical School, I was asked whether I had any experience with research. To some puzzled looks, I confidently responded in the affirmative: I did my senior thesis on Samuel Beckett, the Irish playwright, who wrote exclusively in French (later a Nobel Laureate in Literature). Stepping back now, in spite of this naivety about what they really meant by "research," I am convinced that this eight-year immersion in literature, languages, and philosophy was extraordinarily helpful in developing the ability to think critically and to communicate effectively during my scientific career. Of course, the experience also greatly transformed my view of the world and solidified my interests in art, music, and literature.

During the summer between college and medical school, I took a position as a psychiatric orderly at the local community hospital because I was vaguely interested in psychiatry as a result of my readings of Freud, Lacan, and Sartre. After a few weeks, the older brother of my closest childhood friend was admitted to the ward with his first episode of schizophrenic psychosis. Soon, I became enmeshed in his paranoid delusions. I then saw psychosis as the ultimate epistemological conundrum: "what one knows and how does one know it." His thinking had been hijacked by solipsistic (mis)perceptions: paranoid delusions and auditory hallucinations. This experience cemented my decision to focus on psychiatry in medical school as it seemed to blend epistemology, humanism, and medicine.

Medical School

Having only a rudimentary background in science in college, I struggled during the first two years of medical school, barely managing a C+ grade point. Nevertheless, the introductory course to psychiatry in the first year was an extraordinary experience, in spite of the fact that it took place on Saturday mornings. Lecturers included Leon Eisenberg (the founder of social medicine), Jerome Frank (a pioneer in subjecting psychotherapy to objective research), Robert Cooke (the chairman of pediatrics, who had two children with *cri du chat* syndrome), Horsley Gantt (one of the last students of Pavlov), Seymour Kety (who applied rigorous genetic analysis to schizophrenia), and Curt Richter (the discoverer of circadian rhythms). Contrary to my expectation, the course spent little time on psychoanalytic theory,

portraying it as one psychological intervention among many, but rather emphasized scientific and evidence-based approaches to psychiatry.

The second year opened with the pharmacology course. Several lectures were allocated to a new faculty member, Solomon Snyder, who was also a resident in psychiatry, to cover the nascent subspecialty of psychopharmacology. The topics included stimulants, antidepressants, antipsychotics, and hallucinogens. He reviewed the research on how these drugs exert their effects by altering chemical neurotransmission in the brain. The obvious implication of this research was that the etiology of serious mental disorders did not derive from psychological disturbances but rather from biochemical abnormalities. So, I chose to write my pharmacology term paper on the etiology of schizophrenia covering the twin studies showing high heritability to the more ephemeral findings, such as the purple spot in urine and taraxein. The epigram that I chose to set the tone for the paper was from Bob Dylan's album, *Highway 61 Revisited*: "you know something is happening here but you don't know what it is, do you, Mr. Jones." Fortunately, we have made some progress over the past 50 years.

I sought out Dr. Snyder to ask whether I could spend the elective quarter in my third year in his laboratory (my second year elective quarter and summer were already committed to a 20-week externship in pathology). He was pleased to have me, not the least because the extra hands would be quite helpful since he was preoccupied with his psychiatric residency training. The focus of research in the laboratory was characterizing the pharmacology of neurotransmitter transporters in the brain. Julius Axelrod, with whom Snyder had just completed a postdoctoral fellowship at the National Institute of Mental Health (NIMH), had recently demonstrated that antidepressant drugs act by inhibiting the neuronal reuptake of norepinephrine, thereby potentiating its action at the synapse, a finding that resulted in the Nobel Prize in 1970 (Axelrod 1971). The primary assay for neurotransmitter uptake used finely chopped rat brain tissue suspended in buffer, which would then be allocated to small beakers containing the radioactive neurotransmitter and drugs dissolved in artificial cerebrospinal fluid (Snyder et al. 1968).

My initial task in the lab was to screen psychotropic medications for their ability to inhibit the uptake of [^{3}H]norepinephrine. I soon realized that substantial variability was introduced in the assay by the fact that the chopped tissue tended to settle in the pipette, resulting in the early beakers getting more tissue than the later beakers. In a biochemistry laboratory assignment in my first year to study metabolism in dissociated fat cells, I stumbled on some articles discussing how brain tissue could be homogenized in sucrose; and with differential centrifugation, metabolically active "pinched-off nerve endings" or synaptosomes could be isolated (Gray and Whittaker 1962; de Robertis et al. 1962). I asked Sol if I could try this synaptosome preparation for the transport studies. The preparation had a uniform

"milky" appearance, and intra-assay variability declined dramatically, leading to my first peer-reviewed publication (Snyder and Coyle 1969). That this seemingly minor technical advance came from my insight was quite gratifying. Science was no longer boring; it was fun and exciting. Thus, this 10-week experience in Sol's laboratory dramatically altered the trajectory of my life because he allowed me the freedom to experience the joy of discovery.

Over the last two years of medical school, I spent 12 months in Sol's laboratory, part of which was funded by the Denison Research Scholarship, a real boon as my father had recently passed away. The scholarship covered half my tuition. I successfully petitioned the dean to allow me to continue in the laboratory for another quarter instead of taking a second surgical rotation in the fourth year. Sol was on "sabbatical" in the Maudsley laboratory of Professor Henry McIlwain, the inventor of the eponymous tissue chopper that was replaced in the Snyder lab with synaptosomes. So, my supervision was conducted by mail and phone calls in this pre-e-mail era. In studying the characteristics of catecholamine transport in synaptosomes in various brain regions, I noticed some anomalies between the striatum, the brain region that receives a very dense dopaminergic innervation, and the rest of the brain, which receives noradrenergic innervation (Coyle and Snyder 1969). Dopamine transport did not exhibit stereoselectivity in striatal synaptosomes in contrast to [^{3}H]norepinephrine uptake in the cortex. At Sol's urging, I screened a large number of drugs that were used to treat Parkinson's disease, many of which were muscarinic acetylcholine receptor antagonists. Benztropine among others clearly differentiated [^{3}H]dopamine transport by striatal synaptosomes from transport for [^{3}H]norepinephrine in other brain regions receiving noradrenergic but not dopaminergic innervation. Published in *Science*, this was the first description of the dopamine transporter, DAT (Coyle and Snyder 1969).

In the second year of medical school, a fellow student from my Paris days, who lived in Washington, DC, invited me to a party at her house. Attending the party was this beautiful, smart, and poised woman, Genevieve Sansoucy, who was working on a master's degree in clinical social work at Catholic University. I could not forget her. Several months later, my friend invited me to her own wedding; and, thankfully, Genevieve Sansoucy was in attendance. I took her to Baltimore for Chesapeake Bay hard-shell crabs for our first date. We were married in the summer of 1968 and took up residence in a fifth-floor apartment on Mount Vernon Place in Baltimore. Within eight years, we went from a couple to a family with three fine sons: Peter, Andrew, and David.

Having enjoyed my rotation in pediatrics but also discouraged by my experience with internal medicine rotations, which were dominated by patients suffering primarily from the consequences of poor life choices (drugs, alcohol, tobacco), I decided to pursue postgraduate training in pediatrics before doing a residency in psychiatry. I also applied for a postdoctoral

position in the Public Health Service at the National Institutes of Health (NIH). This position would satisfy my military service requirements, and thus I could avoid being drafted into the armed services at the height of the Vietnam War, which I strongly opposed. At NIH, I was invited to interview with Julius Axelrod, Floyd Bloom, Erminio Costa, and the Nobel Prize Laureate Marshal Nirenberg. I accepted Julius Axelrod's offer for a position in his laboratory, to begin after my pediatric internship at Johns Hopkins Hospital.

Postdoctoral Training

Starting in Julie's laboratory after my internship was a challenging experience because he took his vacation during July. This meant that the new postdoctoral fellows had to tag along with established fellows to learn what they were doing and thus get a real sense of the methods and projects of the laboratory. I helped Perry Molinoff (currently professor of pharmacology at the University of Pennsylvania) in his project to purify dopamine-β-hydroxylase (DBH), the final enzyme in the synthesis pathway for norepinephrine, to homogeneity from beef adrenals. This was my introduction to protein purification and characterization, which was a strength of the Axelrod laboratory. While this project ultimately failed after Perry's departure, I successfully revisited it when I started my own laboratory at Hopkins, purifying rat adrenal DBH to homogeneity and raising an incredibly avid and specific antisera (Grzanna and Coyle 1976; Grzanna et al. 1978).

Because of my interest in development, I decided with Julie's approval to study the development of the catecholaminergic systems in the rat brain as there was virtually no published information on the maturation of transmitter-specific neuronal systems in the brain, especially antenatally. The project was consistent with the catecholaminergic orientation of the laboratory and took advantage of Julie's skills in enzymology but brought a new developmental perspective to the laboratory. A major challenge was to increase the sensitivity of existing assays by ten- to a hundredfold to reliably measure noradrenergic markers as early as 14 days gestation in the rat brain. We were able to show that the synaptosomal uptake of [^{3}H]norepinephrine, the activity of tyrosine hydroxylase, the rate-limiting step in the synthesis of norepinephrine, and the activity of DBH all appeared at 15 days gestation, a particularly primitive stage of brain development in the rat when the cerebral cortex is in the earliest stages of formation (Coyle and Axelrod 1971, 1972a, 1972b).

Collaborating with David Henry, a fellow in the late Irwin Kopin's Laboratory, we established the most sensitive assay at the time for measuring norepinephrine by exploiting catechol-O-methyl transferase, an enzyme discovered by Julie, to transfer a [^{3}H]methyl moiety from S-adenosyl methionine ([^{3}H]SAM) to norepinephrine; the product was separated from the

[^{3}H]SAM by differential extraction with organic solvents, another of Julie's strategies (Coyle and Henry 1973). As with the other presynaptic markers, norepinephrine appeared in the rat brain at 15 days gestation, suggesting that it might play a role in modulating forebrain development (Coyle 1977). Based on the simultaneous appearance of all four presynaptic markers, we predicted that the locus coeruleus, the primary noradrenergic nucleus in the brain, was formed by 15 days gestation. This proposal was subsequently validated by [^{3}H]thymidine autoradiography (Lauder and Bloom 1974).

Julie was an extraordinary mentor (Coyle 2005). Julie's government-issued steel desk was strategically placed in the laboratory where it was 4 feet from the reagent scale and 10 feet from the scintillation radioactivity counter so that every fellow would have to chat with him when either starting or finishing an experiment. Julie made sure his fellows were visible in the field. Every fellow presented a slide talk at the annual meeting of the American Society for Pharmacology and Experimental Therapeutics, which was *the* scientific meeting of the year. Julie would turn down seminar invitations and recommend a fellow to speak in his stead. Julie would give fellows journal articles to review. After he felt confident in the quality of these reviews, he would let the fellow sign the review so that soon the journal was soliciting reviews directly from the fellow. Once, I wrote a sarcastic review of what was a weak scientific manuscript. Julie caught me at the reagent scale and said, "Joe, a scientific article is like the scientist's child. You shouldn't attack it. Be constructive with your criticism." I learned humility from a Nobel Prize winner.

Julie taught us that the best science is not simply confirming your hypotheses but watching out for the anomalous results that may point to novel insights and new, productive avenues of inquiry. He suggested that the way to succeed in a research career was to identify an important problem on which few were working. "Life is too short to study uninteresting problems." He encouraged us to be aggressive in our research: "Be the firstest with the mostest." He bridled a bit at the introduction of statistics into data analysis, commenting: "If you have to do a t-test to prove something is different, it probably isn't important." This skepticism about statistics resulted from his uncanny ability to pose experimental questions with such clarity that the results were unequivocal. But, he always repeated the experiment to confirm the reliability of the finding.

Getting Started at Hopkins

During the last year at NIMH, I needed to find a residency in psychiatry. Although several residency programs expressed interest, Johns Hopkins offered the best opportunity for jump-starting a research laboratory. Paul Talalay, the chairman of pharmacology, working with Joel Elkes, the chairman of psychiatry (clearly facilitated by Sol Snyder) offered to appoint me

as an assistant professor of pharmacology when I started the second year of psychiatric residency and to provide start-up funds and laboratory space. The first person that I hired for the laboratory in 1975 was Rob Zaczek as a technician. A graduate of Towson State University, he was working in the steel mills in East Baltimore. Rob turned out to be brilliant in the laboratory and got it functioning while I was distracted with my psychiatric residency. After serving several years as the senior technician in my laboratory, he completed a PhD in pharmacology with me (Zaczek et al. 1987b) and went on to become the head of neuroscience discovery at Bristol-Meyer Squibb.

Sol kindly referred to me an Austrian postdoctoral applicant to his laboratory, Robert Schwarcz, PhD. Robbie did not have fellowship support and sold his father's stamp collection to cover his salary initially. I later learned that both of his parents were Holocaust survivors in Austria, who had lost several family members in the concentration camps. My first RO1 ($26,000 direct costs) was funded after a Floyd Bloom site visit to ensure that my laboratory was adequately equipped to carry out the proposed studies. The gist of my grant was to use cultured chick retina to characterize dopaminergic neuronal differentiation as a model for schizophrenia. While in the Axelrod laboratory, I published the first description of culturing dopaminergic neurons (Coyle et al. 1973). Robbie took on this project and demonstrated the presence of a dopamine-sensitive adenylyl cyclase along with dopamine and tyrosine hydroxylase but not dopamine-beta-hydroxylase in the retina, confirming the presence of retinal dopaminergic neurons (Schwarcz and Coyle 1976).

The retinal project was soon eclipsed by the discovery of in situ excitotoxicity, which arose out of the confluence of two events. While I was in Julie's laboratory, one of his former postdoctoral fellows, Les Iversen, visited from Cambridge and gave a seminar on his postmortem neurochemical study of Huntington's disease (HD). He found a selective degeneration of striatal intrinsic GABAergic neurons with sparing of the dopaminergic afferents and axons passing through the striatum (Bird and Iversen 1974). In early 1976, Frode Fonnum gave a seminar to the Hopkins Pharmacology Department on the neurotoxic effects to the neonatal rat retina of systemic treatment with glutamate (Karlsen and Fonnum 1976), presumably mediated by the excitatory effects of the glutamate that accumulated in the eye (Olney et al. 1969). The two seminars prompted an epiphany for me: Could direct injection of a potent glutamate receptor agonist into the rat striatum replicate the pathology of HD?

Mike Kuhar, PhD (currently the Candler Professor of Neuropharmacology at Emory University School of Medicine), another one of the Sol-trained junior faculty members in the department, was developing a ligand binding assay for excitatory glutamate receptors using the very potent agonist, [^{3}H]kainic acid, as the ligand. Mike was able to provide us with kainic acid for our studies. Two μg of kainic acid injected into the striatum caused the

rat to rotate away from the side of the injection for 24 hours. Measurement of presynaptic markers for the striatal GABAergic neurons, glutamic acid decarboxylase (GAD), and for cholinergic neurons, choline acetyl transferase (ChaT), revealed marked reductions at 48 hours after injection, whereas the marker for the dopaminergic terminals, tyrosine hydroxylase, was actually increased by 100 percent. These preliminary findings supported our hypothesis of neuronal cell body selective and axon sparing effects of in situ injection of a potent glutamate receptor agonist. In short order, Robbie and I published a detailed letter in *Nature* describing the dose response, time course, cellular specificity, and histolopathology of the striatal kainite lesion (Coyle and Schwarcz 1976). We proposed that dysfunction of glutamatergic neurotransmission might account for the neurodegeneration in HD and related neurodegenerative disorders.

Development of Neurotransmitter Systems

Because little was known about the development of specific neurotransmitter systems in the brain, I continued to pursue this line of investigation in my laboratory at Hopkins. With Sam Enna, a senior postdoc in Sol's laboratory with expertise in GABAergic neurochemistry, we characterized the developmental expression of γ-aminobutyric acid (GABA), its synthetic enzyme GAD, the GABA-A receptor and [3H]GABA transport. Similar to the noradrenergic system, GAD, GABA, and the GABA-A receptor were detectable in the last third of gestation in the rat brain but showed major increases in close synchrony after birth, plateauing at four weeks postpartum. Collaborating with Hank Yamamura in Sol's laboratory, we found that accrual of presynaptic markers for cholinergic neurons and their postsynaptic muscarinic receptors exhibited the most delayed pattern of development.

Although histofluorescent microscopy suggested low innervation of the rat neonatal cortex by aminergic neurons, this seemed inconsistent with my findings of the very early formation of the aminergic nuclei and the presence of presynaptic markers in the fetal neonatal cortex. To address this question, I established a collaboration with the late Mark Molliver, MD, a faculty member in the Department of Anatomy. Mark was clinically trained in neurology and was a superb classical neuroanatomist. We hypothesized that this discrepancy was due to the fact that aminergic terminals were deficient in neurotransmitter. To reveal their presence, we treated neonatal rats with 5-hydroxydopamine (5HODA), which is selectively taken up by and concentrated in the vesicles of aminergic terminals and converted to an electron dense precipitate with fixation for electron microscopy (Coyle and Molliver 1977). Although in the early postnatal period the total density of synapses was quite low, a striking 30 percent exhibited aminergic characteristics after treatment with 5HODA. This synthesis-storage capacity was confirmed by

treatment with L-3,4-dihydroxyphenylalanine, which produced a twenty-fold increase in cortical catecholamines in a reserpine-sensitive compartment (reserpine blocks vesicular storage of amines).

With the incredibly avid and specific antiserum against homogeneously purified DBH developed by Reinhard Grzanna in my laboratory, Mark, Reinhard, and I embarked on another very productive collaboration around characterizing the anatomy of the noradrenergic innervation of the cortex. By using the peroxidase-anti-peroxidase (PAP) immunocytochemical method, the noradrenergic neurons were revealed in their Golgi-like entirety so that cell bodies, dendrites, and axons could be traced in thick sections (Grzanna et al. 1978). John Morrison with his blond Afro was conducting his thesis research in Mark's laboratory, which focused on the intracortical trajectory and organization of the noradrenergic projections. He showed that the dorsolateral cortex is innervated by fibers in the medial forebrain bundle that wrap around the frontal pole, running occipitally in the deep layers. This pattern indicates that noradrenergic fibers have the capacity to modulate a vast expanse of cortex in a tangential manner (Morrison et al. 1978, 1979, 1981). John went on to become the dean for research at Mount Sinai School of Medicine. The PAP technique was extended to electron microscopy and revealed that, contrary to expectations, nearly 60 percent of the noradrenergic axonal varicosities formed axodendritic synapses with specialized appositions, indicating restricted synaptic contacts (Olschowka et al. 1981).

Mike Johnston joined the laboratory after completing his military service and his residency in pediatrics and pediatric neurology, an unusually late start for a research career for someone who ultimately became an outstanding translational neuroscientist on developmental disabilities, a professor of neurology at Hopkins, and the chief medical officer at the Kennedy Krieger Institute. The difference in the times of neurogenesis for different populates of neurons suggested a novel way to selectively lesion neuronal populations based on the time of neurogenesis. Methylazoxy methanol (MAM) is a sort-lived DNA alkylating agent that kills mitotic cells. We timed treatment with MAM to late in gestation when brainstem and pyramidal neurons had already completed mitosis, whereas the GABAergic neurons in the ventricular germinal zone were actively dividing.

Treatment of pregnant rats at 15 days gestation with a single dose of MAM results in severe cortical hypoplasia in the offspring. The cortical volume is reduced by nearly 70 percent, primarily because of the loss of cortical GABAergic neurons as evidenced by the reduction in total GAD activity and GABA levels (Johnston et al. 1979a). We were surprised to find that the total innervation of the cortex by noradrenergic terminals was virtually unchanged. DBH immunocytochemistry revealed that the noradrenergic axons were "compressed" to a much greater density within the atrophic cortex and their pattern of innervation was disrupted. More extensive studies suggested that the cholinergic innervation of the cortex was also of extrinsic

origin, as the concentrations of presynaptic markers were increased in the hypoplastic cortex (Johnston and Coyle 1979). We found that the severity of the lesion and the site of GABAergic deficits could be determined by the date of administration of MAM. Notably, with fetal administration, the cerebellum appeared to develop normally, whereas treatment postnatally with MAM when granule cells were in mitoses produced severe hypoplasia of the cerebellum resulting from loss of granule cells (Slevin et al. 1982).

The parallels between the fetal MAM lesion in the rat and schizophrenia did not go unnoticed by me (Coyle and Johnston 1980). Early imaging studies were revealing ventricular enlargement and cortical atrophy in schizophrenia (Johnstone et al. 1976) similar to what is seen in the MAM lesion. Also, early postmortem studies revealed significant reductions in cortical GABAergic markers (Spokes, 1979) similar to the MAM lesion. Furthermore, the striatum was also reduced in volume, thereby increasing the density of dopaminergic terminals, consistent with the hypothesized hyperfunction of forebrain dopamine in causing psychosis in schizophrenia (Beaulieu and Coyle 1981). However, I could not get much traction with this model of schizophrenia with NIMH review committees as schizophrenia was considered a "functional" disease. As a consequence, I discontinued the project after nearly 20 publications. Forty years later, the MAM lesion is considered to be one of the more robust models of schizophrenia pathology (Grace 2016).

Excitotoxicity

After Robbie and I discovered the selective neurotoxic effect of in situ injection of kainic acid, I knew that we needed to move rapidly to characterize its mechanism of action, limitations, and possibly better agonists. Following Julie's dictum ("be the firstest with the mostest"), I wrote up the project as an RO1 using the prior research on the catecholaminergic neurotoxin, 6-hydroxydopamine, as a template. This was one of the best grant applications that I have written, and it was funded on the first round by the National Institute of Neurological Disorders and Stroke with a very high priority score.

We obtained some structural modifications of kainic acid and showed that there was a close correspondence between reported excitatory potency of the analogues and excitotoxicity with striatal injection. (Schwarcz et al. 1978). Strikingly, reduction of the side chain resulted in complete loss of activity, whereas extension of the side chain as in domoic acid, a deadly toxin released from algae, increased toxicity by threefold (Schwarcz et al. 1978). One of the effects of intracranial injection of kainic acid was that the rats developed limbic seizures with forepaw stereotypies and retropulsion. Rob Zaczek developed a method for quantifying seizures (Zaczek et al. 1986). Using the electroencephalography, he showed that the subtle head shakes

and streotypies exhibited by the rats after kainite injection masked severe limbic seizures, which were associated with widespread neuronal death in the hippocampus and pyriform cortex. Although the convulsant effects of kainite analogues correlated with neurodegeneration in the limbic system, such proconvulsant effects were not observed with quisquate, N-methyl-D-aspartate (NMDA), and ibotenate, which produced small uniform lesions. These disparities were consistent with the emerging evidence of three families of glutamate-gated cation channels: NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainite receptors (Zaczek and Coyle 1982).

We were able to obtain [3H]kainic acid of high specific radioactivity to carry out ligand binding. Edye London, PhD, on whose PhD thesis committee at the University of Maryland I had sat, joined my laboratory as a postdoctoral fellow and accepted this project. She demonstrated that [3H]kainic acid bound to a high- and a low-affinity site. In retrospect, the structure-activity relationships of the former are consistent with the kainite receptor, whereas the latter was likely the glutamate transporter, GLT1. The highest concentration of the high-affinity sites was in the striatum and the lowest concentration was in the medulla and cerebellum. After a striatal kainite lesion, receptor binding was virtually unaffected a week postlesion when intrinsic neurons were cleared; but by four weeks, the high-affinity site was reduced by 64 percent. This delayed clearance probably reflects the predominant presynaptic localization of the kainite receptor in the striatum and delayed retraction of afferents (Jin and Smith 2011).

Because kainite is secreted as a toxin from the seaweed *digenea simplex* and is ascaricidal, Edye examined the phylogenetic distribution of the kainite receptor. High-affinity [3H]kainite binding sites had a broad phylogenetic distribution in the nervous system, including in planaria, earthworm, dogfish, chicken, and human but were undetectable in hydra (London et al. 1980). Edye went on become an accomplished human brain imager and an expert in the neurobiology of substance abuse. She currently is the Thomas and Katherine Pike Professor of Addiction Studies in the Department of Pharmacology at the University of California, Los Angeles.

A French neurologist from Tours, France, Kathleen Biziere, MD, joined my laboratory and continued the elucidation of the mechanism of kainite toxicity. She found that removing the cortex overlying the striatum protected against kainite toxicity within hours of the lesion. The cortical lesion caused a substantial reduction in glutamate in the synaptosomal fraction as well as in [3H]-glutamate uptake in the striatum, consistent with a massive loss of a glutamatergic projection to the striatum. In support of this hypothesis, injection of 1 μmole glutamate with 9 nmole of kainite into the decorticate striatum restored the toxicity of kainite (Biziere and Coyle 1979). These finding were consistent with the presynaptic localization of kainite receptors, which caused endogenous glutamate release.

The interaction between kainite and glutamate in neurotoxicity was supported in acute striatal slices incubated in vitro. Adenosine triphosphate (ATP) levels were used as an index of neurotoxicity. Although 10 mM glutamic acid modestly depressed ATP levels in the striatal slices, the addition of low concentrations of kainite, which itself does not reduce ATP, markedly potentiates the effect of glutamate (Biziere and Coyle 1978). We directly addressed the presynaptic effects of kainate in stimulating endogenous glutamate and aspartate release in perfused slices of cerebellum, striatum, and hippocampus (Ferkany et al. 1982). Kainate (0.5 mM) was as effective as 40 mM K+ in releasing endogenous glutamate and aspartate. Notably, glutamate release was unaffected by tetrodotoxin, unlike 40 mM K+, but was Ca++ dependent, pointing to a presynaptic site of action of kainate, as action potentials were not involved.

Peter Campochiaro was a Hopkins medical student, who took a research rotation in my laboratory. He undertook a project to characterize the development of striatal neuronal vulnerability to kainate. The seven-day-old rat showed minimal vulnerability, whereas three-week-old rats exhibited near adult vulnerability. Striatal vulnerability paralleled the postnatal increase in [3H]kainate high-affinity binding, synaptosomal uptake of [3H] glutamate, and endogenous glutamate levels (Campochiarro and Coyle 1978). Peter took a residency in ophthalmology and a neuroopthalmology fellowship, returning to my laboratory for his research requirement, characterizing neurotransmitter disposition in the retina. He is currently the Eccles Professor of Ophthalmology and Neuroscience at Hopkins and a world-recognized expert on macular degeneration (Campochiaro 2015).

The term "excitotoxin" posits that kainite and related ionotropic glutamate receptor agonists kill neurons by overwhelming depolarization, thereby depleting their energy supplies through persistent activation of Na+ K+ ATPase. To test this hypothesis, we had to establish a full battery of microassays for high-energy phosphates and adapted a microwave oven so that the microwaves were focused on the rat brain so that it reached more than 100°C in less than a second to instantaneously stop metabolism (Retz and Coyle 1982). Thirty minutes after the striatal injection of kainite, there were significant reductions in phosphocreatine, ATP, and glucose and elevations in adenosine diphosphate (ADP) and adenosine monophosphate (AMP). By 2 hours, ATP had fallen by 32 percent, adenosine phosphates by 20 percent, and glucose by 56 percent, whereas AMP increased by 60 percent and lactate by 44 percent. These results were consistent with the hypothesis that excitotoxins killed neurons by profound energy depletion.

By the early 1980s, the "generic" glutamate receptor had been pharmacologically dissected into three distinct subtypes: kainite, AMPA, and NMDA receptors. The proconvulsant effects accounted for the very uneven neuropathology of kainic acid. Robbie Schwarcz pioneered the use of NMDA receptor agonists for making more uniform lesions. He showed the striking differences

between the effects of kainic acid and ibotenic acid injected into the rat hippocampus in cases in which kainate exhibited a very uneven vulnerability of neurons in contrast to the uniform lesion caused by ibotenate (Kohler et al. 1979). Ibotenate did not cause seizures and other morbidity associated with kainate, perhaps because of its metabolism to the GABA-A receptor agonist, muscimol, thereby limiting excitatory diffusion (Curtis et al. 1979). In situ injection of an excitotoxin, increasingly ibotenic acid, has replaced electrolytic lesions to identify the role of specific nuclei, because of the axon-sparing effect of the excitotoxin lesion, thereby eliminating the confound of damage to axons of passage or termination (Coyle 1985). After thirty years, the excitotoxin method for selective pathway lesioning is now used without citation to its origins.

Alzheimer's Disease

In the 1970s, evidence was accumulating that senility, long accepted as a normal consequence of aging, in fact, was a disease (Roth 1980). The brains of the elderly with senility exhibited the same neuritic plaque and neurofibrillary tangle pathology as presenile dementia or Alzheimer's disease (AD). The notion of selective neuronal vulnerability in neurodegenerative disorders was solidified with postmortem neurochemical studies on Parkinson's disease and HD (Bird and Iversen 1974). In 1977, a few brief reports appeared describing selective reduction in the presynaptic marker for cholinergic neurons, ChAT, in the cerebral cortex but no change in the GABAergic presynaptic marker GAD in AD (Davies and Maloney 1976; Perry et al. 1977). The inference of cholinergic involvement in AD was supported by the finding that the muscarinic acetylcholine receptor antagonist, scopolamine, replicated the memory deficits of AD in healthy volunteers (Drachman and Leavitt 1974). Given that AD was considered to be a prototypic cortical dementia, it was unclear whether these cholinergic deficits reflected cholinergic neuronal death in the cortex or elsewhere.

The basal forebrain nucleus stains intensely for acetyl cholinesterase (AChEs) and sends fibers to the cortex, prompting Lewis and Shute (1967) to propose it as the major source of cholinergic innervation. However, many noncholinergic neurons stain intensely for AChEs, and McGeer et al. (1974), using antiserum purported to be selective for ChAT, reported that ChAT was confined to cortical neurons, supporting the notion that the cortex was the primary site of pathology in AD. Given our interest in neurodegenerative disorders, we felt that the excitotoxin lesion method could resolve these uncertainties (Johnston et al. 1979b). We first made exploratory electrolytic lesions in various potential sources of cholinergic innervation of the cortex, including the thalamus, and found that only the lesion in ventral pallidum of the rat cause a 45 percent reduction of ChAT activity in the ipsilateral cortex. We then made a kainite lesion in the basal forebrain in the ventral

pallidum that caused a striking loss of the AChEs-stained neurons in the basal forebrain and selective 50 percent loss of ChAT activity, acetylcholine, and [3H]-choline uptake and loss of AChEs staining in the ipsilateral cortex. We concluded: "studies directed at understanding the pathogenesis of (AD) . . . might profitably be directed towards the cholinergic neurons in the basal forebrain." (Johnston et al., 1979, p. 5396).

Don Price, MD, the head of neuropathology, was just across the street. With Peter Whitehouse, a young neurologist, we decided to look at the nucleus basalis of Meynert in a subject who died with a clinical diagnosis of AD. We found a profound loss of the large multipolar neurons with the remaining ones laden with neurofibrillary tangles (Whitehouse et al. 1981). We published in *Science* a follow-up postmortem study involving five controls and five subjects with a clinical diagnosis of AD. Consistent with the preliminary report, we reported an 80 percent loss of the large neurons in the nucleus basalis. This was "the first example of loss of a transmitter-specific cell population in a major disorder of higher cortical function" (Whitehouse et al. 1982). The findings were quite surprising because AD was the prototypical "cortical dementia"; yet, the results indicated that a critical lesion was located in the midbrain. We found that loss of the nucleus basalis cholinergic neurons also occurred in dementia pugilistica, which is now known as chronic traumatic encephalopathy or CTE (Uhl et al. 1982). With Don Price, MD, and Mahlon DeLong, MD (who later won the Lasker Award for electrophysiologically characterizing the circuitry exploited for deep brain stimulation in Parkinson's disease), we then published a highly cited review (more than 2,000 citations) on AD as a disorder of cortical cholinergic innervation, linking together the cortical pathology with senile plaques, the selective loss of cortical cholinergic markers, degeneration of cholinergic nucleus basalis, and the memory-disrupting effects of cholinergic antagonists (Coyle et al. 1983).

Collaborating with David Olton, PhD, who unfortunately died young of pancreatic cancer at the age of 51, we characterized the memory impairments resulting from ibotenate lesions in the nucleus basalis magnocellularis (NBM) and the medial septal area (MSA) in the rat (Hepler et al. 1985). The NBM lesion caused a selective reduction in ChAT in the frontal cortex, whereas the MSA lesion caused a selective reduction of ChAT in the hippocampus. The four tasks—T-maze, radial arm maze, active avoidance, and passive avoidance—differed in response-reinforcement contingencies. Nevertheless, regardless of the lesion site, the performance of the lesioned rats was significantly impaired for all four of the memory tasks, indicating the important role of these midbrain cortical cholinergic projections for memory. The impact of the cholinergic denervation of cortex with an NBM ibotenate lesion was confirmed by the 30 percent reduction in glucose consumption (London et al. 1984), similar to what had recently been described in subjects with AD (Friedland et al. 1983).

To better understand the molecular determinants of the risk for AD, I collaborated with John Gearhart, PhD, and Mary Lou Oster-Granite, PhD, in the Department of Physiology, who were studying a mouse model of Down syndrome (DS). DS, which results from trisomy of chromosome 21, is associated with cognitive limitations, but of particular relevance is the fact that all DS individuals exhibit the neuropathology of AD, including the cortical cholinergic deficits by the age of 40 (Yates et al. 1980; Price et al. 1982). The gene encoding the precursor protein for amyloid A-beta, *APP*, is located on human chromosome (HSA) 21 in the so-called Down's region; it maps to mouse chromosome 16 in a region that has synteny with the human DS region on HSA 21 (Reeves et al. 1987). As the TS16 mice die by birth, we focused on prenatal development of the NBM and found that neurogenesis for the TS16 fetal NBM AChEs-positive neurons peaked two days earlier than in littermate wild-type (WT) mice. Furthermore, at the end of neurogenesis of the NBM, the TS16 mice had many fewer AChEs-positive cells than did littermate controls (Sweeney et al. 1989a).

We also used tissue culture to study postnatal maturation of the NBM in TS16 mice (Corsi and Coyle 1991). We dissociated the NBM of TS16 and WT fetuses at 15 days gestation and grew the cells in completely defined medium. In the TS16 NBM cultures, there were fewer ChAT-positive neurons, and these neurons were smaller, and emitted fewer processes, which were simpler and smoother. The addition of nerve growth factor to the cultures resulted in more and bigger ChAT-positive TS16 neurons with processes that formed varicosities. These results were replicated in the DN mouse, which has a much more restricted triplication of the DN region of HSA 21 (Kleschevnikov et al. 2012). These studies on the TS16 mouse were my first foray into genetic approaches to neuroscience and opened my eyes to new, more powerful methods for understanding brain disorders (Bendotti et al. 1988).

Forty years ago, I met Ken Davis, MD, who is now the president of Mount Sinai Health System in New York City, when he was an assistant professor of psychiatry at Stanford. At the time, he was doing pioneering studies on the cognitive enhancing effects of the AChEs inhibitor, physostigmine, in normal adults and in AD patients (Davis and Mohs, 1982; Davis et al. 1983). His wife, Bonnie, an endocrinologist, scoured the scientific literature to find the best AChEs inhibitor for treating AD while she stayed at home raising their two children. She identified galantamine, a natural product isolated from snowdrop bulbs, which was used in various "tonics" in Eastern European medicine. Bonnie asked whether I could determine its ability to reverse the cognitive deficits in the NBM lesion model of AD.

I had a smart, enthusiastic student, who was going to do her PhD thesis in my laboratory and had already worked on the NBM during an elective rotation. Joanne Sweeney, who is now the president of Trinity College in Hartford, showed that galantamine treatment reversed the memory deficits

in the Morris water maze caused by a prior ibotenate lesion of the NBM (Sweeney et al. 1988). She found that galantamine had a relatively long effect on memory in the NBM-lesioned mice of more than six hours after a single dose, whereas it showed a mirror effect on control mice, inhibiting their performance (Sweeney et al. 1989b). The results of these animal studies served as the basis for carrying out successful clinical trials by Janssen that resulted in galantamine (Reminyl) being approved as a treatment for AD. However, galantamine differs from other U.S. Food and Drug Administration–approved AChEs inhibitors because it also acts as a positive allosteric modulator of α-7 nicotinic acetylcholine receptors, which may contribute to its efficacy (Coyle et al. 2007).

Oxidative Stress

A popular assay for a glutamate receptor was the quisqualate-sensitive, chloride-dependent "binding" of [3H]glutamate to brain homogenates at 37°C (Foster and Fagg 1984). We and other laboratories used this "quisqualate receptor" assay in many published studies without question. However, Rob Zaczek was troubled by the peculiar features of this purported receptor-binding assay and took on the task of deconstructing the "receptor" as his thesis project. He showed that it was in fact a Cl^- dependent antiporter, exchanging glutamate for cysteine and a primary mechanism for the synthesis of the antioxidant, glutathione (Zaczek et al. 1987a, 1987b). Tim Murphy, a graduate of St Mary's College and world-class wind-surfer, pursued this issue further as his thesis project. He showed that blocking the antiporter caused oxidative stress that could be mitigated by treatment with antioxidants or by drugs that induce antioxidant defenses. Immature neurons were particularly vulnerable (Murphy et al. 1989, 1991). Tim subsequently became professor of psychiatry at the University of British Columbia.

With Nancy Simonian, MD, a postdoctoral fellow and clinically trained neurologist, we explored whether oxidative mechanisms might also explain excitotoxicity. We had hints of this as Masa Miyamoto, PhD, a visiting scientist in my laboratory from Takeda Pharmaceuticals, found that idebenone, a potent centrally active antioxidant, protected against kainite toxicity in the striatum (Miyamoto and Coyle 1990). Nancy, who later became the chief scientific officer at Millennium Pharmaceuticals, showed that kainite caused neuronal apoptosis secondary to oxidative stress (Simonian et al. 1996). In 1991, after I was elected president of the Society for Neuroscience (after serving as program chair, treasurer, and councilor), I was invited by *Science* to write a review article on a topic of my interest. Given our findings and those of others, I chose to review oxidative stress in neurodegenerative disorders, emphasizing the role of glutamate (Coyle and Puttfarcken 1993). This article proved to be the most highly cited (more than 2,900 times) in my career because it is still relevant after more than 25 years.

NMDA Receptor Modulation

Although understanding the pathophysiology of schizophrenia seemed to me to be the "great white whale" of psychiatric research, it took some fortuitous events to draw me into this focus during the latter third of my research career. Because the structural determinants of kainite receptor binding involved an unsaturated side chain extending beyond the primary glutamate structure (London and Coyle 1979), we hypothesized that the endogenous ligand might be a polypeptide containing glutamate. Rob Zaczek proceeded to purify acidic peptides from rat brain by ion exchange and high-performance liquid chromatography using the Tris-HCL buffer [3H]-glutamate binding assay ("quisqualate receptor"). He isolated N-acetyl-aspartyl-glutamate (NAAG), a peptide previously described by Italian neurochemists in horse brain (Zaczek et al. 1983). Randy Blakely, the first PhD candidate in the nascent neuroscience doctoral program at Hopkins, was carrying out his thesis research in my laboratory. While N-acetylaspartyl-[3H]glutamate appeared to bind to the quisqualate receptor, Randy showed that in fact the [3H]NAAG was cleaved by a quisquale-sensitive peptidase to [3H]glutamate, which was then efficiently concentrated into glutamatergic synaptosomes (Blakely et al. 1988a, 1988b). Randy developed highly specific antiserum to NAAG linked to bovine serum albumin by carbodiimide and showed that NAAG was concentrated in forebrain glutamatergic neurons (Blakely et al. 1987; Ory-Lavollée et al. 1987). Subsequent studies revealed high concentrations of NAAG in the locus coeruleus, NBM, compacta dopaminergic neurons, and motor neurons (Tsai et al. 1993). Randy is now head of the Florida Atlantic University Brain Institute.

Mike Robinson, PhD, a postdoctoral fellow, who later became a professor of pediatrics at University of Pennsylvania, working with a doctoral student, Barbara Stauch (Slusher), purified the peptidase to homogeneity from rat brain (Robinson et al. 1987). We then raised an antiserum for immunocytochemistry and were surprised to find that it was expressed on the surface of astrocytes (Slusher et al. 1992; Berger et al. 1999). Ruth Carter (Luhti), who grew up in Kansas and is now a professor of neuroscience at Leicester University in England, moved to Harvard with me in 1991 to carry out her thesis research on the cloning and molecular characterization of rat N-acetyl-L-aspartyl-L-glutamate peptidase (NAALADase). This was new territory for the laboratory, but she succeeded with our rat antibody to identify a clone. Sequencing revealed that the human homologue to human prostate-specific membrane antigen (PMSA), a protein highly expressed on the surface of metastatic prostate cancer cells, was the same as NAALADase (Carter et al. 1996). Then, Chuck Halstead at the University of California, San Francisco, contacted us because the amino acid sequence of our protein matched the sequence of a protein fragment of folate hydrolase 1, a gut enzyme that cleaves off the polyglutamate tail

from dietary folate for absorption (Halstead et al. 1998). The gene is now known as *FOLH1*.

Emil Tsai, MD, a Taiwanese psychiatrist, was pursuing his Hopkins PhD thesis in neuroscience in my laboratory. He had been working on the NAAG project and decided to carry out a postmortem study on NAAG, glutamate, and NAALADase in the brains of subjects who died with schizophrenia and matched controls because clinical pharmacologic studies suggested that NMDAR antagonists produce a syndrome resembling schizophrenia, including the cognitive and negative symptoms not seen in stimulant-induced psychosis (Krystal et al. 1994). He found reduced NAALAdase activity in the cortex and hippocampus, reduced glutamate levels, and elevated NAAG in the hippocampus (Tsai et al. 1995). Indirect evidence suggested that NAAG was an NMDAR antagonist, a proposal that was supported by elegant electrophysiologic studies performed by my former postdoctoral fellow, Richard Bergeron, MD, PhD, and currently professor of psychiatry at the University of Ottawa (Khacho et al. 2015).

My Clinical Life

Isolating my clinical experiences from my research career is artificial but may be less confusing to the reader. For, at my core, it is difficult to separate my interest in how the brain works (albeit focusing primarily on one corner: glutamaterigc mechanisms) and how it doesn't work in neuropsychiatric disorders. Both roles stimulated research questions that, not surprisingly, overlapped.

The 1969–1970 pediatric internship at Hopkins went by in a blur with the brutal three-day rotation: 24 hours on, next day on until the care of your assigned patients is complete (typically around 8:00 P.M.) and then off at 5:00 P.M. on the third day. Every other weekend, we were on duty. Exhaustion was a constant, so that I would fall asleep whenever I sat down (once in the bathtub). There was no phlebotomy team then. The intern would arrive before rounds to draw bloods for the necessary tests. The residents one to two years removed in experience supervised an intern, who was clinically quite naïf. The motto was "watch one, do one, teach one." Nevertheless, with the layers of oversight from intern, to assistant resident, to chief resident, and finally to attending faculty, I think that the care was excellent.

Certain clinical experiences stick with me. On the neonatal intensive care unit (ICU), we were treating a 1,000 gm premature infant born to a 14-year-old girl. Although he had been in the ICU for weeks, no family member had visited. The infant had been successfully resuscitated from several cardiac arrests but clearly sustained major brain damage. Given the circumstances of likely lifelong disability and no family support, the attending recommended a "Do not resuscitate" order and a "DNR" sign

on the incubator. Several days later, the chairman of pediatrics, Robert Cooke, MD, made rounds on the floor, saw the DNR sign, and became quite angry, saying, "Take down that sign. At Hopkins we treat children, we don't kill them."

At the time, the treatment of childhood cancers was quite primitive. Children three to six years old would be admitted with leukemia, looking normal, and then would die from an overwhelming infection weeks later. One of my patients was a 10-year-old boy, who had no recorded visitors since his admission but was dying of end-stage lymphoma. I sat with him as he expired so he wouldn't be alone. Another young girl presented in the emergency room with a painful leg mass and a note from her local physician ("I can do nothing"). She soon died of metastatic osteosarcoma. All of these conditions are now curable. These deaths of children were a reality hard to accept but an impetus for some of my fellow interns to take a deep dive into cancer research. After internship, to keep up my clinical skills and to obtain the patient contact that I enjoyed, I volunteered at an outpatient clinic in Anacostia, a poor African American community in southeast Washington, DC, while I was a postdoctoral fellow in the Axelrod laboratory at NIH.

My three-year residency in psychiatry at Hopkins presented some challenges because I also joined the faculty as an assistant professor of pharmacology in the second year. While I was at NIH, the American Board of Psychiatry and Neurology changed the requirements for psychiatry, ruling that an internship was no longer needed because "psychiatry was so far removed from medicine." To me, this was the nadir for American psychiatry as it became "brainless" in the words of Leon Eisenberg (1986). Being four years older and much more clinically experienced than my fellow first-year residents, I found that I could accomplish my clinical responsibilities more efficiently so that I was able to free up time to set up my laboratory.

I did not shirk my clinical training. One of the highlights for me that stood me in good stead throughout my career was the T-group run by Kenneth Artis, MD, who supervised therapeutic groups for the clinical staff at the National Cancer Institute (thereby reducing burnout and suicide). The seminar was built around reading theoretical articles on group dynamics; and our resident discussion group served as an incubator where the group dynamics played out in real life. Another memorable experience was my long-term, insight-oriented psychotherapy patient for which I received intense supervision from a psychoanalyst. She was a single mother, who was employed as a court reporter, a position that paid well. In my first year of residency, she started weekly hour-long psychotherapy sessions with me for panic attacks. Her attacks increased progressively in intensity and frequency. She developed agoraphobia, causing missed work and leading to her termination. As a consequence of her unemployment, she had to apply for welfare to support herself and her daughter. In spite of her worsening condition, she

was committed to the psychotherapy, and she and my supervisor felt that she was making progress with insights into the causes of her anxiety. Then, I discovered a recently published paper demonstrating the efficiency of imipramine for treating panic attacks and agoraphobia (Zitrin et al., 1978). My supervisor opposed treatment with imipramine as he believed that it would disrupt the therapeutic alliance. My patient however, agreed to try the drug, and within a few weeks her symptoms began to melt away.

As I was completing my residency, I was involved in the initial evaluation and treatment of a young woman experiencing her first psychotic episode of schizophrenia. A bright, athletic, and a quite attractive adolescent, she was a senior in a private high school. But, her thinking was very disturbed, and she was experiencing auditory hallucinations. I treated her as an outpatient, except for an occasional crisis requiring hospitalization, for the next 15 years. She responded reasonably well to antipsychotic drugs, but her hallucinations did not disappear. She simply ignored them when doing well. It was distressing to observe the gradual coarsening of her appearance and the erosion of her social skills as negative and cognitive symptoms emerged over time, components of schizophrenia unresponsive to neuroleptics and little noted at the time. Long-term treatment relationships reveal the true course of disease, not cross-sectional "snap-shots."

After completing residency in 1976, I became an assistant professor of psychiatry in addition to my pharmacology appointment. My laboratory was up and running with two postdoctoral fellows, a technician, an R01, and a Career Development Award. Paul McHugh, the chairman of psychiatry, required all psychiatry faculty to take on a clinical responsibility. I chose to run the Chronic Treatment Clinic (CTC), in which residents provide outpatient treatment for schizophrenic patients. This resident-run clinic had no faculty oversight because these patients were not viewed as very intellectually challenging. To me, they were the essence of psychiatry: individuals struggling with symptoms that disrupted their emotions, cognition, and relationships with others, who needed the best treatment that we could provide.

With the recent formulation of the dopamine hypothesis for schizophrenia (Snyder 1976), I felt that by using neuroscientific methods we could optimize antipsychotic treatment. We exploited the ability of drugs in serum to compete with and inhibit [3H]-ligand binding to a receptor, based on the affinity of the drug for the receptor of therapeutic interest, regardless of structure, to measure serum drug levels. Serum antipsychotic levels were measured using [3H]spiroperdol as the ligand for the D_2 dopamine receptor (Tune et al. 1980a). Anticholinergic drug levels were measured by their displacement of [3H]quincyclindyl benzoate from muscarinic acetylcholine receptors (Tune and Coyle 1980). Although we were able to optimize neuroleptic doses, with some caveats (Tune et al. 1980b), and minimize cognitive impairments, most patients remained severely impaired, estranged from their families and unemployed.

In 1981, Paul McHugh, the chairman of psychiatry, asked me to become the director of the Division of Child Psychiatry, which had languished since its last director, Leon Eisenberg moved to Harvard several years before. Because virtually all child psychiatry programs in the country were dominated by psychoanalytic theory, I thought that by building a program based on developmental neurobiology with a focus on serious mental disorders in children, we could transform the field. Richard Ross, MD, the dean at the time, raised substantial funds in support of my vision. Over the next several years, we created two inpatient units for severely ill children and adolescents (13 beds each) designed with the assistance of James Harris, MD, to provide the optimal therapeutic environment. Our residency expanded from a couple of foreign medical graduates per year to attracting top American medical school graduates, many of whom went on to academic careers, such as Joseph Piven, MD, who is now the Thomas E. Castelloe Distinguished Professor of Psychiatry, Pediatrics, and Psychology, and Director of the Carolina Institute for Developmental Disabilities at the University of North Carolina; and Maryland Pao, MD, the deputy director of the NIMH Intramural Research Program.

By the end of the 1980s, my career seemed to be going quite well. I was elected president of SfN (effective 1991), elected to the Institute of Medicine of the National Academy of Sciences, and was named nationally as "Top Doc" in both child psychiatry and geriatric psychiatry. The laboratory was well funded with productive projects on the neurobiology of AD, Down syndrome, and excitotoxicty. However, clouds were gathering on the horizon. Managed care, which ravaged psychiatry from the late 1980s through the late 1990s, severely cut income for clinical services. This income underwrote the academic mission of the division. Maryland Medicaid drastically reduced reimbursement to $15.00 per day per patient for clinical care on our inpatient units in spite of reimbursing the hospital for its entire "expenses" (about $1,000/day). So, in spite of our nationally recognized success in developing a scientifically grounded program in child psychiatry and a growing NIH grant portfolio, the hospital refused to offer assistance, unlike most academic hospitals that reimbursed psychiatry departments for managing inpatient units because they were quite profitable to the hospital. After I left, the hospital agreed to subsidize the salaries of the psychiatrists on the inpatient services for the next director.

Dan Tosteson, MD, the dean at Harvard Medical School, had approached me on a couple of occasions to head a subdepartment of psychiatry at Harvard Medical School (historically, clinical departments were managed by their affiliated hospitals at Harvard). I demurred because of the almost comical rivalry among the subdepartments evident during residency recruitment, which undermined the credibility of their programs in the eyes of Hopkins applicants. In 1991, he enticed me to serve as the chairman of the Consolidated Department of Psychiatry, bringing together the academic

programs in the nine clinical departments of psychiatry affiliated with Harvard Medical School. This was underwritten with a $20 million commitment over a decade by the participating hospitals to support the academic mission.

With this commitment, we reorganized the residency training, condensing six competing adult residency-training programs into three thematically differentiated programs with a single application form. The six child psychiatry residencies were merged into three with a single core curriculum. The medical student curriculum, historically dependent on the idiosyncrasies of the nine hospital departments, was reorganized with clear objectives and evidence-based teaching so that all students could be subjected to the same test of their knowledge. Over the decade, external research funding to psychiatry grew nearly fourfold to more than $65 million per year with faculty members affiliated with different hospitals collaborating for the first time. These educational and research initiatives are still in place to this day.

I managed to maintain my laboratory, which continued to compete successfully for NIH grants, but I knew that after 10 years of major administrative responsibilities I had lost a few steps: I was the master of 20th-century neuroscience, but we were now in the 21st century where molecular approaches reigned supreme. I signed up for the Woods Hole Neurobiology Course, a nine-week course divided into three-week trimesters focused on molecular biology, electrophysiology, and imaging. The course leaders were incredulous (acceptance by postdoctoral fellows was highly competitive), because they assumed that I just wanted to kick back and enjoy the Cape. I swore that I would be a "full" student and would not miss any classes; and they accepted me. It was a very intense experience, starting with three hours of seminars in the morning followed by labs that extended to 10:00 P.M. or later; and it was a bit humbling living in a dormitory room (but I snuck my wife in on weekends). The 11 other students were extremely talented and diverse. We still stay in contact. And the faculty was unbelievable in their generosity; tops in their fields but spending hours providing hands-on supervision to this small group of students. This experience gave me the energy, experience, and confidence to move to the next stage of my scientific career that took a much more molecular approach.

With the freedom to pursue research on a full-time basis after finishing my administrative responsibilities for an academic department containing more than 1,600 members, I did want to maintain a clinical "presence." Cathy DeAngelis, a long-time friend from my Hopkins days, was the editor of *JAMA* and was also responsible for the *Archives* family of journals. She called me in some panic in October 2001, because the current editor of the *Archives of General Psychiatry* (*AGP)* had tendered his resignation, and she needed a replacement as soon as possible. *AGP* had been the "journal of record" in psychiatry for decades, maintaining the highest five-year citation impact in the field because it published substantive articles "with legs" as

they were cited long after the two-year window of the Institute for Scientific Information's rankings. I joined the editorial board in the 1980s when Danny Freedman, MD, was the editor. I saw this as another opportunity in which the neuroscience perspective could be brought to bear on this important journal, although I was scientifically omnivorous about psychiatry, recognizing the importance of epidemiology, health services research, and medical anthropology. I accepted Cathy's offer and started January 1, 2002.

The journal was still being run entirely with paper. So, in late December, we received about 25 cartons of old manuscripts under review, unprocessed recent submissions, and correspondence. My editorial assistant, Fran MacNeil, worked tirelessly to organize the materials so we could proceed. I decided that until I fully grasped the process, I would assign all reviewers (e-mailing invitations to about six possible reviewers and going with the first four) and evaluate all reviews for the editorial decision. *AGP* could publish only about 140 manuscripts a year because of the fixed number of pages, meaning that our acceptance rate was less than 15 percent of submissions. As time went on, I tried to identify important trends. I think that we were ahead of the curve on the reassessment of the prevalence of autism and its genetic and environmental determinants, inflammation and psychiatric illness, and late-life depression as a risk factor for dementia and biomarkers for AD.

JAMA had a long tradition of having art on its cover whereas *AGP* had the table of contents for the issue, which was informative but not visually attractive. I enlisted my long-time friend and child psychiatrist from Hopkins, James Harris, MD, to do the covers. He created *Art and Images in Psychiatry*; the cover of each issue had a reproduction of a painting and an essay linking the art to issues relevant to psychiatry. For the associate editor, I selected Stephan Heckers, MD, whom I had recruited into our psychiatric residency while he was doing a postdoctoral fellowship with neuroanatomist Marcel Mesulam, MD, PhD. Stephan applied his neuroanatomic expertise to brain imaging and had made some significant discoveries. We were complementary. Using Isaiah Berlin's metaphor, Stephan was the hedgehog digging down to get the facts right, and I was the fox looking over the landscape for something new and transformative. The partnership worked very well, and he ultimately replaced me as editor.

Closing in on Schizophrenia

Given our findings of an elevated endogenous NMDAR antagonist, NAAG, in postmortem schizophrenic brains (Tsai et al. 1995), findings of elevated kynurenic acid levels in their brains (Schwarcz et al. 2001), and the clinical observations that dissociative anesthetics like ketamine reproduced the full spectrum of symptoms of schizophrenia (Krystal et al. 1994), I finally felt like I had a "hook" on the fundamental pathophysiology of the disorder. Free of administrative responsibilities, I decided that the most effective

way to proceed was to organize a group of basic and clinical investigators with shared interests in the "NMDA receptor hypothesis of schizophrenia" to apply for a NIMH Conte Center, a new grant mechanism that fostered translational and interdisciplinary research. We focused on the NMDA receptor and the hippocampus. The first attempt did not receive a fundable priority score because it was "too diffuse" and "lacked sufficient expertise in hippocampal physiology." Our resubmission included Stephan Heckers, MD, for hippocampal imaging; Robbie Greene, MD, PhD, for hippocampal NMDA receptors; Don Goff, MD, for NMDA receptor clinical trials; Howard Eichenbaum, PhD, for hippocampal memory; Mike Hasselmo, PhD, to head a computational core; John Lisman, PhD, for hippocampal physiology; and myself. NMDA receptor modulation was successful, and the Conte Center was ultimately funded at $20 million for 10 years.

Instead of the typical scientific advisory board, I requested that we use the funds for an annual retreat at which we would focus on a particular aspect of the hypothesis and bring in outside experts to present and to participate in discussions. John Lisman hosted these retreats at Brandeis and, on occasion, had the dinners at his lovely condo overlooking the Charles River. With his infectious curiosity and gregarious personality, John evolved into the "soul" of the Conte Center and worked to bring together the disparate findings into a consistent model. This led to his leading a consensus article on a circuit-based approach for understanding neurotransmitter and risk gene interactions in schizophrenia that was published in *Trends in Neuroscience* and has been cited more than 500 times (Lisman et al. 2008).

I had reservations about pharmacologic models of schizophrenia, such as chronic ketamine treatment (the brain adapts), MAM lesion (GABAergic neurons are down-regulated and not lost), or postnatal stress (a nonspecific risk factor for depression, anxiety disorders, and schizophrenia). Therefore, I decided to use a molecular approach by genetically silencing serine racemase (SR), the enzyme that synthesizes D-serine (Basu et al. 2009). Previous studies indicated that D-serine was a coagonist at forebrain NMDARs and was synthesized in astrocytes, being the prototypic "gliotransmitter" (Schell et al. 1995). Thus, genetically silencing SR should result in forebrain NMDA receptor hypofunction.

The meandering path on which this research took us reaffirmed the power of Julie's admonition: "follow the results." Using commercially available antibodies against SR, we confirmed its forebrain localization and the loss of SR immunoreactivity in SR-/- mice (Basu et al. 2009). I assigned my new postdoctoral fellow, Mike Benneyworth, PhD, to use a conditional knockout strategy to ascertain the relative contribution of neurons and glia to the expression of SR. Given the abundant literature on the glial localization of SR, we chose the inducible glial knockout strategy first. Over several months, in spite of increasing doses of tamoxifen and varied durations of treatment, the inducible knockout of SR in astrocytes produced simply

small to negligible effects on SR levels. Reluctantly, we turned to the CamK-promoter-driven Cre recombinase, which was neuron selective. We found a 65 percent reduction in SR (Benneyworth et al. 2012), indicating that SR was expressed primarily in neurons.

We found an overlooked article from Mori's laboratory that used SR knockout mice to control for immune specificity and demonstrated that SR was expressed in forebrain glutamatergic and GABAergic neurons but not in astrocytes (Miya et al. 2008). We replicated their findings on SR expression with our SR-/- controls and found that astrocytic "D-serine immunoreactivity" was intense in the SR-/- mice under published conditions for blocking L-serine cross-reactivity when the levels of D-serine were less than 10 percent of WT. Increasing the concentrations of blocking L-serine hundredfold revealed D-serine staining only in neurons as it vanished from astrocytes (Balu et al. 2014).

In a very productive collaboration with Dan Liebl, PhD, we were able to show that SR was indeed expressed in astrocytes but in A1 reactive or toxic astrocytes (Liddelow et al. 2017) that appear after traumatic brain injury (TBI). TBI results in cognitive impairment by seven days after the insult when the SR-expressing astrocytes are prominent in the hippocampus (Perez et al. 2017). Using our inducible astrocyte selective knockout, we showed that the cognitive impairment resulted from D-serine release from the A1 astrocyte, because silencing SR expression only in astrocytes prevented the cognitive impairment. Thus, SR was indeed expressed in astrocytes, but not in resting astrocytes in the normal brain.

Articles continued to appear in high-impact journals concluding that astrocytes were the primary source of D-serine. Together with Herman Wolosker, a visiting scientist from Technion Institute, who originally cloned SR (Wolosker et al. 1999), and Darrick Balu, we published a perspective in *Trends in Neuroscience* that described the many artifacts that led scientists to the misperception that D-serine is a gliotransmitter (Wolosker et al. 2016, 2017).

Postmortem studies in schizophrenia have revealed consistent brain pathology, including atrophy of cortical pyramidal neurons, down-regulation of the parvalbumin-positive (PV+) GABAergic neurons, oxidative stress, and reduction in neurotrophic pathways (Steullet et al. 2017). Darrick led a series of studies to characterize the phenotype of the SR-/- mouse, focusing on the males, which were more severely affected, consistent with the lower severity and later onset of schizophrenia in females. These studies showed a reduction in dendritic complexity, total dendritic length, and spine density of pyramidal neurons in the frontal cortex, primary sensory cortex, and hippocampus that would result in a reduction of approximately 30 percent of cortical glutamatergic synapses (Balu et al. 2012, 2013). The actual degree of cortical and hippocampal volume loss was significant but small (about 4 percent), comparable to what is observed in schizophrenia. It is surprising

how this subtle volume loss masks the serious degree of disconnection first predicted by Seymour Kety (Coyle et al. 2016). The SR-/- mice also showed the stigmata of oxidative stress and the down-regulation of the cortical PV+GABAergic neurons similar to schizophrenia (Steullet et al. 2017). The SR-/- mice exhibited memory deficits in several domains. Intracranial self-stimulation studies indicated a profound reduction in hedonic state. In vivo dialysis showed a significantly elevated basal release of dopamine in the ventral striatum, consistent with the hyperdopaminergic state associated with psychosis. Thus, the parallels in the phenotype of the SR-/- mouse and pathology of schizophrenia were striking.

After much of this research had been completed, the results of the largest genome-wide association study (GWAS) in schizophrenia comprising 110,000 controls and nearly 40,000 subjects with schizophrenia were published (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). The results supported the hypothesis that schizophrenia was a disorder of complex genetics with multiple risk alleles of modest effect interacting with the environment, thereby causing the disorder (in terms of individualized medicine, schizophrenia consists of multiple gene-based disorders with overlapping phenotypes).

I was not surprised that only a few of the "usual subjects," candidate genes for the dopamine hypothesis, showed up in the GWAS. This failure of the candidate gene approach, popular in the first decade of the 21st century, resulted from underpowered studies, yielding misleading results (Johnson et al. 2017). However, as was pointed out in the analysis of genes localized to highly penetrant copy number variants (Kirov et al. 2012), genes encoding proteins associated with the glutamatergic synapse were quite well represented in the GWAS. We showed that eight genes encoding proteins like SR either modulated NMDA receptor function or mediated down-stream consequences of NMDA receptor activation (Balu and Coyle 2015). The impact of epistatic interactions of gene products adversely affecting glutamatergic neurotransmission is not difficult to envision, resulting in the schizophrenic phenotype.

Comment

The convergence of neuroscientifically generated hypotheses based on clinical and postmortem findings and the GWAS results sparks new confidence that the field is on the right track for deciphering the many etiologies of this disorder that we call schizophrenia and related serious mental disorders. However, I have been disabused of the belief that answers will emerge quickly. My early involvement in HD and AD research filled me with optimism that once we found the risk genes, effective treatments would rapidly emerge. Contrary to this assumption, 25 years after identifying risk genes for HD, AD, and amyotrophic lateral sclerosis (from which my editorial

assistant, Fran, died), we still have no treatments. If the "low-hanging fruit" of autosomal dominant mutations are not amenable to rapid solutions, the common psychiatric disorders with their complex genetics face log-unit more challenges. Perhaps, as in oncological research, a "tipping point" in understanding will be reached that results in a burst of therapeutic advances.

At the close of my career, I should hazard some insights to guide the next generation if they happen upon this autobiography. After nearly 50 years, I still find Julie Axelrod's insights particularly helpful. I believe that his admonition to pay particular attention to the finding that is inconsistent with your hypothesis to be particularly valuable, and one that I have emphasized to my trainees. Rob Zaczek's deconstructing the "quisqualate receptor," which undermined the conclusions of many of ours and others' publications, led us to the discovery of *FOLH1*. More important, Rob did not feel constrained by a "must be right theme" that dominates many laboratories. Julie's admonition comports nicely with the fundamental argument of Karl Popper in *The Logic of Scientific Discovery* that the function of research is to disprove hypotheses and thus get closer to an approximation of reality.

When I started on my career in research, I assumed that science was transparent and self-correcting. I have learned that there is, in fact, a complex social culture of science that determines to a great extent what appears to be important and correct at the time (see Thomas Kuhn, *The Structure of a Scientific Revolution*). My most recent experience with this phenomenon is the controversy over the localization of serine racemase and its product, D-serine. Several years after we have demonstrated both to be localized to neurons using rigorous immunocytochemical (Balu et al. 2014) and molecular techniques (Benneyworth et al. 2012), high-impact journals continue to publish studies claiming to demonstrate a purported glial localization based on flawed methods (for review, Wolosker et al. 2016).

I feel extraordinarily blessed that society has granted me the resources to pursue a career in scientific research and to gratify my need to explore the unknown. I believe that I have created useful knowledge that will ultimately lead to improved treatments of brain disorders. But, as I grow older, I have come to appreciate the complexity of these conditions and fear that meaningful help is still years to come. I know that my thirst for new knowledge, and new insights into how the brain works, will not be dimmed for a while, but I will miss having the tools and the resources to directly participate in the process of discovery, the wonderful "ah-ha" experience that I first felt 50 years ago.

Finally, the really important issues: Genevieve and I are celebrating the 50th anniversary of our marriage. She retired after a nearly 40-year career as a psychotherapist. She has now become a skilled sculler, an accomplished potter, and a political advocate. We continue to be surprised by each other. Our sons are married and are pursuing satisfying careers of their own. And, we have two beautiful grandchildren.

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