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Solomon Snyder identified receptors for opiates and neurotransmitters and elucidated mechanisms of drug action. He characterized messenger systems including IP_3 receptors and inositol pyrophosphates. He identified novel neurotransmitters including nitric oxide, carbon monoxide and D-serine.

Solomon H. Snyder

Like most other people, I am the product of my parents. Hence, a brief review of their lives may provide insight into my own. Similarly, the lives of my siblings may be informative. My dad was born in 1911 in Baltimore, the fifth of seven children. His father moved to Washington when he was 2 years old to open a small grocery store a block away from the butcher shop operated by Al Jolson's father. Like his father and most of his siblings, Dad was musical and for many years was a semiprofessional saxophonist in dance bands, though his greatest love was classical music. Graduating high school in 1929, he meandered among clerical jobs at Federal agencies in the depths of the depression. Soon after marrying Mom in 1935, Dad became the 10th employee of a tiny government agency which emerged as the National Security Agency (NSA). Throughout World War II he led a team of a few hundred cryptanalysts addressing various Japanese codes. At the end of the war modern computers were invented and Dad was assigned to "find out if these machines might help the code breaking effort." Within a couple of years he led an effort that made NSA the largest computer installation on earth. He became so enamored with computer programming, that when I was 10 years old he taught me to program computers in "machine language" which incorporated the binary number system. Though not technically a scientist, Dad greatly admired science and often spoke with me about science as the highest activity of mankind. However, Dad was very easy going and never ordered or even strongly urged that any of we five kids pursue particular avenues of personal development.

Mom was complex. Born in New Haven, Connecticut, she came to Washington during the depression to find work with the Federal Government. She had a decided entrepreneurial flair. Thus, when my sister Elaine and I were 4 and 2 years old, respectively, and Washington was flooded with lonely young government workers arriving from all over the country, she decided to "do something." With Dad's assistance she organized "The Carefree Circle," a social club. Within a year the organization overflowed our tiny house and attracted the attention of the city's newspapers, the *Washington Post* and the *Washington Star*. The Carefree Circle spawned a semi-professional sandlot baseball team. My mother knew nothing about baseball but appointed herself "manager." Her main contribution was to introduce a female pitcher despite the fact that there had never been a single female sandlot player in the history of the city. As projected, this gimmick attracted further news media coverage.

After World War II, with four small kids at home, Mom perceived the enormous pent-up demand for housing (no homes were built during the war) and founded Snyder Realty Company. Soon she had a sales manager and 15 salesmen. Once the real estate boon wound down, Mom became interested in the emerging world of contesting, for example, "Write in 25 words or less why you like Ivory Soap." Soon she emerged as one of the top contesters in the country winning several major competitions including the "Stop the Music" jackpot which yielded $10,000 (in 1952 money), a trip to Jamaica, wardrobes of the finest men's and women's clothes, a kitchen full of appliances and other prizes. Her creative flair led to massive numbers of innovative jingles or short essays, while Dad's organizational abilities eventuated in an operation generating hundreds of copies of each entry (years before Xerox machines existed) as well as collating myriad box tops from diverse products that were required for entry submission. Perhaps a combination of Mom's and Dad's genes impacted my scientific career. One might speculate that free-floating creative associations coupled with clear, well-organized conceptualization make up the qualities that make for success in science.

Science has never been much evidenced in our family. My late older sister Elaine was an artistic prodigy who could draw almost perfect likenesses of human faces when she was 5 years old. At Mom's behest she developed an act in which she would sing a song which she would simultaneously illustrate. After winning various talent contests sponsored by Washington television stations, she appeared on network television. She did all the show business stuff under pressure from Mom, a notorious stage mother. Wed to an eminent entomologist, Elaine became one of the country's leading natural science illustrators with a major retrospective of her work upon her retirement from the Smithsonian Institution. My younger sister Carolyn also performed as a singer and dancer. A natural beauty, she was a finalist in the Miss America contest, Washington, D.C. division, and ultimately became a psychiatric nurse. My younger brother Irving, though also musical, never became involved in show business and is presently a psychiatric social worker. Joel, "the baby" of the family, 15 years younger than I and 17 years younger than Elaine, has been an actor since he was 7 years old. He continues to perform semiprofessionally though he became an arts administrator to support a wife and daughter who is a professional actress.

Both sets of my grandparents immigrated to the United States in the first decade of the twentieth century. I was closest to my maternal grandfather who lived with us for much of my childhood years. In Vitebsk, Russia, he played balalaika and the mandolin and, when I was age 9, gifted me his extra mandolin. Having played the piano since I was age 5 and the clarinet for a few years, I had some musical background and glommed on to the mandolin, rapidly assimilating everything my grandfather knew. Seeking a mandolin teacher for me, Dad encountered Sophocles Papas, the leading

teacher of the classic guitar in the United States and Andres Segovia's clos-
est friend, who also taught the mandolin. After 3 years of mandolin lessons,
I switched to the guitar. Although I enjoyed the mandolin, I adored the gui-
tar which was soon a consuming passion. Before I graduated high school
I was giving public recitals and seriously considered spending a summer at
master classes in Siena, Italy, with Segovia and then pursuing a musical
career. However, a desire to be "one of the guys" supervened so that, like
most of my friends, I went to college as a premed.

I differed from the other guys in that I had no particular interest in med-
icine, biology, or science. Instead, I loved philosophy and speculating about
origins of the universe and the raison d'etre of life. Much of this may have
come from the first 9 years of my schooling in a Jewish day school where we
studied Torah every morning and participated in group prayer services. What-
ever the underlying conscious or subconscious motivations, I loved to think
about "big questions." I rationalized that if I could stomach the science courses
of college and medical school, I might become a psychiatrist that, to my naive
way of thinking, wouldn't differ too much from a life in philosophy.

As foolish as most of this reasoning may have been, it largely came to
pass. I attended Georgetown College for 3 years (in those days you didn't need
a bachelor's degree to enter many medical schools) and then Georgetown
Medical School. I did become a psychiatrist and my life has been devoted to
brainstorming about what might be construed as "big questions" though
with a far more molecular emphasis then I would have anticipated.

In college I did well in most subjects but was particularly strong in
English and philosophy. My success in writing was surprising considering
that Calvin Coolidge public high school, which I attended after the Hebrew
Academy, was replete with lazy faculty who never assigned essays simply
because they were not willing to read and grade them. Freshman English
was a year-long course in writing essays, a new 500-word essay each week.
I recall vividly the first essay I wrote, "The Case for the Classic Guitar,"
somewhat plagiarized from my high school term paper, the only piece of
writing we ever did in high school. This English course was designed to
shock the students into an appreciation of their weaknesses and to inspire
some intellectual discipline. Every single fellow in the class (Georgetown
was an all-boys college) received no higher than a C+ except for a single A+
which was me. Our English professor, the faculty advisor for the George-
town Literary Magazine, arranged to have the essay published. Over the
course of freshman year others of my essays, all on music, were published in
the magazine.

In the summers following freshman and sophomore year of college my
father arranged for jobs for me at NSA (even though the top-secret code
word security clearance cost the government $10,000 each summer versus
the $500 that I earned). In the first year, he had me work with the IBM sorters,
collators, and printers that were the predecessors of computers, whereas in

the second summer I worked directly with computers. Although I was suitably impressed with the elegance of the logic involved in computer programming, computers never "turned me on." I had negligible mathematical aptitude and, while fascinated by the challenges of cryptography, I clearly lacked any gift for code breaking.

Throughout college I maintained an interest in the guitar, practicing regularly and working every Saturday at my teacher's guitar shop, minding the shop, keeping the books, and giving lessons. I helped organize events for the local guitar society, especially when Andres Segovia performed in Washington, D.C. On one of these occasions Mr. Papas had me perform for the maestro. By this time, a junior in college, I was somewhat out of practice and knew that it would be foolhardy to attempt to impress with my virtuosity. Instead, I surprised Segovia by playing two fantasies that he had himself composed and published years earlier in a guitar magazine. They were deeply expressive pieces but with no major technical challenges, and I adored them. Segovia evidently liked the performance—at least he complimented me on my "expressive soul."

The same year on a Saturday afternoon a young physician entered the shop and inquired about the possibility of guitar lessons with Mr. Papas. When I told him the rates, he shuddered and asked whether anybody else taught and charged less. I indicated that he could take lessons with me for a lower fee. He then asked whether I would charge still less if I came to his apartment, not far from my own home, and could thus pocket the entire fee myself. My new guitar student, Don Brown, was then in the first Research Associate class at the NIH and subsequently essentially founded the field of molecular embryology. Don and I became lifelong friends as well as teacher and student. For the summer after my junior year, just before I started medical school, Don asked whether I might work with him as a technician. Although I had no particular interest in scientific research, I thought it would be interesting to learn a little bit about the biologic underpinnings of medicine. Thus commenced a romance with the NIH. Throughout medical school I spent every summer and elective period at the NIH. This led to my time with Julie Axelrod and everything thereafter.

Medical School

The summer before medical school I worked with Don developing techniques to monitor the metabolism of histidine in animals and humans by administering [^{14}C]histidine, then fractionating and identifying urinary metabolites. Why study amino acid metabolism? Don was doing his military service obligation in the Laboratory of Clinical Science at National Institute of Mental Health (NIMH), whose director Seymour Kety was fascinated by reports of abnormal biogenic amines in the urine of schizophrenics suggesting some metabolic abnormality in the precursor amino acids. Each Research Associate

was supposed to select an amino acid and develop techniques that would ultimately be employed to compare normals and schizophrenics in the NIMH metabolic wards. Don selected histidine and subsequently showed there were no differences in the metabolism between normal controls (Mennonite conscientious objectors) and schizophrenics. In the process he identified novel metabolic pathways such as the formation of hydantoin propionic acid and developed evidence that the pathway from histidine to glutamic acid proceeded through an unstable intermediate, imidazolone propionic acid. After my first summer at the National Institutes of Health (NIH), Don completed his military service and departed for Paris to learn modern molecular biology with Jacques Monod. He designed for me a well-articulated project, namely an attempt to identify and purify the enzyme that would convert imidazolone propionic acid to formimino-glutamic acid, which then donated its forminino group to tetrahydrofolate ultimately leading to the critical methyl group of S-adenosylmethionine. I labored for a couple of summers and elective periods trying to characterize and purify the enzyme. For the majority of the time, I was working with an artefact, as imidazolone propionic acid was so unstable that it was decomposing nonenzymatically. I finally stabilized the substance by exhaustively eliminating all oxygen from the sealed test tubes, characterized and purified the enzyme about 50-fold and wrote by myself a full-length paper for the *Journal of Biological Chemistry* that was accepted with no revisions (Snyder et al., 1961b).

My single original contribution in the lab came when Marian Kies, the Laboratory Director, received a letter from a pediatrician in Milwaukee, Stanley Berlow, who had read publications of the laboratory about histidine metabolism. He was treating a mentally retarded 10-year-old girl whose urine was positive in the ferric chloride test for phenylketonuria, whereas paper chromatography revealed normal levels of phenylalamine but a massive histidine spot. To seek the patient's metabolic abnormality, I journeyed to Milwaukee with a bottle of [14C]histidine. I fed her large amounts of [14C]histidine (there was no institutional review board) and personally collected her urine for a day. Back in Bethesda I fractionated her urine, just as I had done previously with monkeys and rats and, from the urinary metabolite pattern, was able to conclude that she was missing histidase, the initial enzyme in the pathway from histidine to glutamate. Thus, she suffered from a condition which, though rare, occurs in fairly substantial numbers of children and is designated histidinemia (Snyder et al., 1963). In an amazing coincidence, another group at the NIH, led by Bert LaDu and Leon Rosenberg, had been characterizing histidase and found that, besides the liver, it was localized to the stratum corneum of the skin so that one could assay the enzyme in scrapings from the underside of fingernails. When they encountered a local patient with high urinary histidine levels, they simply assayed for the enzyme in the patient and her extended family, elegantly delineating the enzyme deficiency and its genetic distribution.

Psychiatry crept into my school time research. My sister Elaine's first husband was a Ph.D. psychologist who had developed simple paper-and-pencil tests to elucidate Gestalt phenomena such as "closing gaps." Because there was a schizophrenia research award closely adjacent to our laboratory, I obtained permission from Dr. Kety to administer these tests to the patients, under the supervision of the distinguished psychologist David Rosenthal. The pilot studies with a few patients at the NIH were so promising that I was dispatched to St. Elizabeth's to test larger numbers. Chronic schizophrenics displayed less "perceptual closure" than normals. Perceptual closure of normals involves closing gaps, hence copying figures inaccurately so that in this instance schizophrenics could be conceptualized as doing better than normal individuals. The studies also revealed another paradox. In many test paradigms schizophrenics are far more variable than normals. Yet for perceptual closure their variability was notably less.

The perceptual closure work resulted in two papers in the *Journal of Abnormal Psychology* (Snyder et al., 1961a; Taylor et al., 1963), and one in the *Archives of General Psychiatry* (Snyder, 1961). For me, more important than the research was the opportunity to encounter psychotic patients. Most medical students are distinctly uncomfortable in confronting people who behave bizarrely. I enjoyed sitting quietly with them, trying to absorb their "essence" and to fathom what was underlying their disordered behavior. During junior year in our medical school psychiatry rotation, we were assigned patients to "treat" in a psychotherapy setting one-on-one in a private office. I savored the experience so that my ill-formed plans to become a psychiatrist now had a foothold in reality.

Julie

Knowing I wanted to be a psychiatrist, my next challenge was how to cope with the doctor draft. In the early 1960s every male medical school graduate had to pursue 2 years of military service or figure some "way out" such as joining the Reserves or National Guard, which were perennially oversubscribed. As a component of the Public Health Service, the NIH was "military." To attract young physicians into science, the NIH had developed a Research Associate program, essentially a 2-year postdoctoral position with "military" appointment at the equivalent level of Major. Initially, I had in mind a similar position, Clinical Associate, involving 2 years at the NIH doing clinical research as well as caring for psychiatric patients. Besides providing an entrée into academic psychiatry, the 2 years at the NIH would count for a year of psychiatric residency, shortening what seemed like an overly long period of training. In this model, I would be coming to the NIH after 2 years of psychiatric residency so there was no hurry. These elegant plans were disrupted by romance. In senior year of medical school Elaine Borko and I began dating and were engaged about Christmas time. I was set on interning

in San Francisco as, for lack of funds, I had lived at home all the way through college and medical school and wanted to get far, far away. However, if we were to marry when I graduated medical school, Elaine would have another year of college, which required residency in the Washington area to fulfill her practice teaching requirements.

With the new pressure to return to Washington after a single year interning in San Francisco, I scoured the NIH seeking any laboratory with an opening for one year hence. Unfortunately, all labs were filled, because the Research Associate positions were allocated by a match program that had already closed. After a prolonged and fruitless exploration, my salvation turned out to be directly across the hall from the laboratory of Marian Kies where I had worked all through medical school—Julius Axelrod. I had met Julie during my summers working on histidine metabolism. In those days Julie was discovering one methylating enzyme after another. Because Don Brown had been working on histidine, Julie suggested that they collaborate to seek a histamine-methylating enzyme that they successfully identified. When I approached Julie, he commented that most of the applicants he interviewed were "valedictorians from Harvard or Yale." However, the Harvard student who had matched with Julie had abruptly cancelled so there was a vacancy.

Research Associate Years

The year in San Francisco, where I interned at the Kaiser Hospital on Geary Blvd., was perhaps the happiest of my life. Newlywed, Elaine and I explored San Francisco and its environs and made close friends we have retained through the years. Whereas interns at East Coast academic hospitals worked every other night all night, we were typically on call only every fourth or fifth night and even then would get 4 or 5 hours sleep. I even had a small bit of exposure to science. On Julie's recommendation, I spent my one month elective working in the laboratory of Alan Burkhalter at the University of California San Francisco Medical Center in the Pharmacology Department, where he had developed a novel fluoremetric assay for tissue histamine. I carried out a few experiments but mostly enjoyed the gorgeous view of San Francisco from high atop Parnassus Avenue.

Julie began all of his students with a carefully structured project to ensure positive feedback, often taking advantage of some unique feature of a student's prior training. For instance, Dick Wurtman had spent his elective periods in Harvard Medical School working on the biology of the pineal gland whose extracts inhibited estrus and lowered ovarian weight. Julie suggested that he seek the pineal gland's "hormone" that turned out to be melatonin. Because of my background in histidine metabolism and my brief exposure to Burkhalter's histamine assay, Julie recommended that I monitor the disposition of exogenous histamine using the same techniques he had

employed to discover norepinephrine uptake. I administered radiolabeled histamine to rats and monitored its disposition in various organs. In contrast to the experience with norepinephrine, there was no pronounced accumulation of histamine in any tissue. Instead, I found large amounts of the metabolic product imidazoleacetic acid as a riboside, raising questions about its potential biologic role (Snyder et al., 1964). Because of my sloppiness, I dismissed as artefact a prominent radioactive band at the origin of my paper chromatograms that was shown by Jack Peter Green at Yale (together with his postdoctoral fellow David Fram, my classmate from kindergarten through high school) to be imidazoleacetic acid ribotide, a novel metabolite. Yet another lesson in avoiding my inherently hasty and slovenly approach to experiments.

The real excitement in Julie's lab at the time lay in the series of breakthroughs Dick Wurtman was making regarding melatonin acting as a pineal hormone with its biosynthesis being influenced by light exposure. Melatonin is formed from serotonin which is acetylated. N-acetylserotonin is then methylated by hydroxindole-O-methyltransferase (HIOMT), an enzyme discovered by Julie, to form melatonin. Julie showed me a paper he had noticed in a chemical journal reporting that heating serotonin with ninhydrin, a standard chemical stain for proteins, led to an intensely fluorescent product whose fluorescence was about 10 times that of serotonin itself in strong acid solution, the standard assay for serotonin. Within a week I had developed an organic solvent extraction system that permitted an assay for tissue serotonin utilizing the ninhydrin technique (Snyder et al., 1965a). With this assay, we could monitor serotonin levels in as few as two to four rat pineal glands, each weighing 1 mg. In an heroic study, consuming about 400 rats, Wilbur Quay had reported a dramatic diurnal rhythm in serotonin levels with peaks at noon of 100 µg/g (100 times brain levels of serotonin), about 10 times higher than nocturnal troughs. With far fewer rats, I was able to replicate Quay's finding. Because relatively few rats needed to be consumed for each experiment, I was able to evaluate various experimental conditions. To prevent the effects of light, I enucleated the eyes of rats or maintained them in constant darkness. To my amazement, the serotonin rhythm persisted (Snyder et al., 1965b). I remember exclaiming to Julie, "We've discovered a biological clock." Julie was even more excited than I until a brief library search the next day revealed that endogenous diurnal rhythms, biological clocks, had been well characterized in mammals since the 1920s. We learned a good deal about regulation of the circadian serotonin rhythm which we now know to reflect an opposing rhythm in serotonin N-acetyltransferase, the rate-limiting enzyme in melatonin formation, whose augmented night-time activity depletes serotonin from the pineal.

Thirty years later, I returned to the pineal gland. Jimo Borjigin, a new postdoctoral fellow, had done her doctoral work with Jeremy Nathans on the molecular biology of vision. She regarded vision as a mature area of

research and wanted to explore related but relatively unmined areas. She was fascinated by the pineal gland that, in some species, is a "third eye." It was well accepted that serotonin N-acetyltransferase (NAT) is the regulatory enzyme in melatonin formation, but no one had ever isolated or cloned it. Purifying such a protein from such a small organ seemed hopeless. Instead, Jimo decided to employ subtractive hybridization. She knew that NAT expression was vastly higher at night than during the day. Accordingly, she collected large numbers of rat pineal glands during the day and at midnight, seeking messages expressed selectively at midnight. One of these turned out to be NAT (Borjigin et al., 1995). Independently, David Klein at the NIH, who had first discovered diurnal rhythms in NAT almost thirty years earlier, cloned the same enzyme (Coon et al., 1995).

Julie allowed, indeed encouraged, his students to carry out "flyers" on their own, for which he did not assume any authorship. Thus, during my 2 years at the NIH I collaborated with Martin Reivich administering LSD to monkeys, dissecting many small brain regions and discovering marked variations in LSD levels. The paper, published in *Nature* (Snyder and Reivich, 1966), attracted much attention; but why LSD should display regional variations was puzzling. We now know what was going on. LSD was binding to the serotonin 1A receptors that mediate actions of psychedelic drugs. Hence, this study was the first demonstration of a serotonin receptor in an intact organism.

As part of my fascination with psychedelic drugs, I noted in a short book by the Nobel Laureate Albert Szent-Gyorgi a comment that LSD had remarkable charge transfer capacities. My medical school classmate Carl Merril was also at the NIH in a laboratory where computers were being applied to molecular orbital calculations. Together we carried out computations on a fairly extensive series of psychedelic drugs and showed a correlation between their charge transfer capacities and their psychotropic potencies (Snyder and Merril, 1965). This was my first foray into the adventure of divining how drugs exert their pharmacologic actions.

I also collaborated with Arthur Michaelson in subcellular fractionation studies. Arthur had recently completed a postdoctoral period in Cambridge, England, with Victor Whittaker participating in the pioneering subcellular fractionation techniques that permitted isolation of pinched-off nerve terminals, synaptosomes. Arthur was likely the only American scientist with expertise in the arcane sucrose gradients required for such fractionation. Together, we used these techniques to purify norepinephrine storage granules from the heart and to identify synaptosomal fractions in the brain following labeling with [³H]norepinephrine (Snyder et al., 1964). Labeling synaptosomes with [³H]norepinephrine, was done in our lab together with my good friend Jacques Glowinski, then also a postdoctoral fellow with Julie (Glowinski et al., 1966). Jacques had perfected technology for intraventricular injections of [³H]norepinephrine permitting these studies as well as the

important experiments in which Jacques and Julie showed that the relative potencies of antidepressants in inhibiting norepinephrine accumulation into the brain paralleled their antidepressant efficacy. Jacques and I were together with Julie for the same 2 years, while our "third musketeer," Leslie Iversen, was in Julie's lab only for a year, spending the second year of his Harkness fellowship at Harvard with Steve Kuffler.

First Years at Johns Hopkins

The 2 years with Julie were exhilarating. Like all Julie's students, I learned the joy of brainstorming new ideas, conceptualizing experiments that one could carry out in a day, digesting the results that evening, and planning the next day's experiments. Although many experiments failed, a good number succeeded, and Julie was constantly encouraging even during the fallow periods. All of this imbued me with the science bug. Nonetheless, I never lost my desire to become a clinical psychiatrist. While I was at the NIH, two other aspiring research psychiatrists who were a couple of years ahead of me, Ernie Noble and Jack Barchas, had worked out a remarkable arrangement in the Stanford Psychiatry Department. The departmental chair, David Hamburg, was trying to build up a department with strong research psychiatrists. He offered Ernie and Jack "research residencies" during which they would be paid salaries comparable to junior faculty and would direct their own laboratories.

During a visit with Dr. Hamburg we came to a handshake agreement that he would do the same for me. At a late stage, too late for additional residency applications, the arrangement fell through, Hamburg lacking the facilities to provide a third research residency slot. I was crushed. Julie said that I could continue in the lab for another year or more. I met with Seymour Kety who indicated that his friend Joel Elkes had recently become Director of Psychiatry at Johns Hopkins and, with the associated turnover of personnel, it was likely that there were residency vacancies. Baltimore wasn't Palo Alto, but Dr. Kety indicated, "Beggars can't be choosers." Elkes offered only a conventional residency which was distressing, because residents in those days were paid $250 a month. Elaine and I had been married throughout internship and the 2 years at NIH. If she had to continue working to support me throughout a 3-year residency, we would have been married 5 years before being able to have children, a seeming eternity by 1965 standards.

Just about that time Julie lectured at Case Western Reserve Pharmacology Department where the Chair, Nick Carter, was recruiting new faculty. Julie mentioned my availability but indicated that I wanted to do a psychiatry residency. Nick countered that the Dean of the medical school, Douglas Bond, was a psychiatrist and might be able to help. I visited Cleveland and was well received. Doctors Carter and Bond worked out an even more attractive

arrangement than the one at Stanford. I would be appointed a full-time Assistant Professor of Pharmacology with a salary better than Stanford and a larger, better funded lab all while doing a psychiatry residency. When I phoned Dr. Elkes to decline Hopkins, he advised that he had already heard about the Cleveland arrangement from Dr. Bond and that Hopkins was prepared to do yet better.

I arrived at Johns Hopkins for Psychiatry residency July 1, 1965, under an arrangement in which I spent the first year as a full-time resident (at $260/month) but beginning with the second year, I was a full-time Assistant Professor of Pharmacology. Elaine was able to stop working, and on October 30, 1966, our first daughter Judith Rhea was born. She was joined 4 years later by her sister Deborah Lynn.

For me, clinical psychiatry was energizing and anxiety provoking. American psychiatry in the 1960s was totally dominated by psychoanalysis. Although Hopkins was far more eclectic than most other university departments, we residents still devoted the vast bulk of our time to one-on-one psychotherapy even with hospitalized schizophrenics. Sitting for an hour with patients and just listening was initially loaded with stress, as we were often dealing with agitated patients, hoping to "do something" for them. Listening didn't seem to be accomplishing very much. With the assistance of some wise supervisors, I calmed down and learned how to balance activity and passivity in therapeutic settings. Those were also the days of the "therapeutic community" in which doctors, nurses, and patients met regularly as a group with everyone being "equal" in coming to decisions about how to run the ward. I assimilated fairly rapidly the art of handling such complicated group dynamics, lessons which served me well over the years in coping with my faculty as well as board members of our condominium, synagogue, the Society for Neuroscience, and various biotech companies. This was the era of "love and trust" as exemplified by the ongoing tumult in the Haight-Ashbury district of San Francisco replete with LSD, STP, and every other conceivable psychedelic agent. In those days, medical insurance was far more generous than today with policies for federal workers, who abounded in Baltimore and Washington, covering up to a year or more of psychiatric hospitalization. Of course, most adults couldn't take off such amounts of time from work. Accordingly, Phipps Clinic, the Hopkins psychiatric hospital, was typically filled with college-aged boys and girls. Some were rebellious while others were more accommodating. One notably recalcitrant teenager, T.M., responded to group therapy by becoming far more community minded, taking over responsibility for maintaining the planters that were beautifying the ward. Every day he carefully pruned the lovely green, leafy plants. Only after 1 or 2 months did one of the aides consult a gardening text and report, "Dr. Snyder those are marijuana plants."

During the second 2 years of residency, as Assistant Professor of Pharmacology, I devoted about half my time to the laboratory. Paul Talalay, my

Chair, outfitted my laboratory and paid for a technician and my first postdoctoral fellow. I even lectured to second-year medical students on psychopharmacology. Two members of the class asked to work in my laboratory that first summer and both stayed with me throughout the remainder of medical school. Both became psychiatrists. One of them, Alan Green, now chairs the Psychiatry Department at Dartmouth, while another, Joe Coyle, formerly chaired the Harvard Psychiatry Department and is now a professor there.

In my new lab at Hopkins, I felt it important to avoid the trap of just continuing with my postdoctoral research. Hence, I terminated all work on the pineal gland, which had been my principal NIH focus. At the NIH, independently of Julie, Joe Fischer, another Research Associate with surgical training at Massachusetts General Hospital, and I had collaborated in a study demonstrating dramatic increases in the activity of histidine decarboxylase, the histamine synthesizing enzyme, in rat stomach after portocaval shunt, a surgical procedure that clinically causes gastric hyperacidity (Fischer and Snyder, 1965). At Hopkins I decided to pursue the dynamic regulation of histidine decarboxylase in the stomach and worked out the rapid turnover of this enzyme following gastrin stimulation of acid secretion, consistent with histamine being the key mediator of acid secretion (Snyder and Epps, 1968). This was of interest, because in those days, prior to the discovery of histamine H2 receptors and their acid-blocking antagonists, cimetidine and ranitidine, it was thought that gastrin rather than histamine was the final common mediator of acid secretion.

When Joe Coyle entered the lab, I was becoming somewhat bored with histamine, as virtually no one else in the world seemed to care about it, all the "action" being with the catecholamines. Up till that time, the reuptake inactivation of norepinephrine had only been studied in intact organs. When people tried to monitor uptake into isolated synaptosomes in sucrose, there was no uptake because the transporter required sodium ions. Homogenizing the brain in salt solutions disrupted synaptosomes. Joe stumbled on a simple-minded approach wherein he homogenized the brain in sucrose and then added salt-containing buffers, the sucrose protecting the synaptosomes from disruption. Utilizing a relatively crude preparation, we could monitor about 50 samples at a time, varying concentrations and working out kinetics of the uptake process (Coyle and Snyder, 1969b). In studying dopamine uptake in the corpus striatum, Joe discovered that a number of widely used anti-Parkinsonian drugs, thought to act exclusively as anticholinergics, were rather potent inhibitors of dopamine reuptake which thus may contribute to their clinical effects (Coyle and Snyder, 1969a). Joe was remarkably innovative and technically skilled. Most of his key experiments were conducted during a 4-month minisabbatical that Elaine and I and our 2½ year old daughter Judy enjoyed in London, Joe and I communicating regularly by letter.

This high throughput screening for neurotransmitter uptake was soon exploited for therapeutic ends. In 1970 I received the John Jacob Abel Award

of the American Pharmacologic Society. Award winners were expected to give a lecture at Lilly, the sponsor of the award. I described our work, which by that time involved synaptosomal uptake of serotonin, norepinephrine, dopamine, and numerous other neurotransmitters. Many years later, in reading Peter Kramer's volume "Listening to Prozac" I learned of the consequences of my visit. Ray Fuller, a Lilly neuropharmacologist, was impressed with the utility of our assays for pharmaceutical-level screening of candidate drugs and recommended that his colleague David Wong explore the matter. There followed a search for serotonin-specific uptake inhibitors with one of these, fluoxetine (Prozac) coming to super-successful fruition.

While a psychiatry resident, I continued my involvement with psychedelic drugs. Elliot Richelson, a classmate of Alan's and Joe's, worked with me making molecular models of various psychedelic drugs and showing commonalities between phenethylamines such as mescaline and indoles such as LSD and psilocybin (Snyder and Richelson, 1968). This led to my most memorable and scary episode at a scientific meeting. I was invited to a meeting at the Salk Institute where all of the eminent "Salk Associates" were assembled. When I presented the Richelson model, the world renowned chemist Leslie Orgel skewered me, remarking that the conformations I proposed were surely not the "favored" ones. I felt humiliated and froze, unable to respond. Suddenly, Francis Crick stood up and admonished, "Leslie, you are an old fuddy duddy. Don't you realize that in biological solutions other forces may arise to induce conformations not favored in pure solution. This 'boy' may well be onto something important."

I even became involved in clinical studies of psychedelic drugs. Dr. Elkes had been invited by the Dow Chemical Company research labs in Walnut Creek near San Francisco to serve as a consultant regarding the following dilemma. Alexander Shulgin, their star chemist, had been synthesizing methoxyamphetamines, derivatives of mescaline, some of which he maintained could elicit enhanced self-awareness at lower doses than those that were psychotomimetic, hence might be useful in facilitating psychotherapy. At this time, 1967, the Haight-Ashbury district of San Francisco was attracting national attention, its streets replete with "acid heads." Besides LSD, the hippies ingested multiple drugs. The most notorious, designated STP (serenity, tranquility, peace) was said to elicit an overwhelming psychedelic effect lasting 3 days. Dow wanted to terminate the chemical program and ascertain whether anything of clinical benefit might be salvaged. Dr. Elkes dispatched me to California in his stead. I reviewed the "clinical" data that Shulgin had obtained largely by testing progressively increasing doses of the various methoxamphetamines on himself, his wife, and his son. The laboratory notebooks were impeccable, and I thought he might be on to something important.

Dow agreed to fund clinical studies at Hopkins. Accordingly, I administered low doses of DOET (2,5-diethoxyamphetamine) to Hopkins undergraduate

students (clinical research standards were rather lax in those days). Just as Shulgin attested, my students reported a subjective sense of enhanced self-awareness with no psychotomimetic effects, an effect resembling low doses of marijuana. As we were completing the study, I received a phone call from federal narcotic agents indicating that they had solved the structure of STP and it was 2,5-dimethoxyamphetamine (DOM), remarkably similar to the agent I was studying.

I convinced the officials that I had nothing to do with STP. They then asked whether I would help them with the following challenge. They knew the source of all of San Francisco's illicit LSD and STP, a skilled chemist named Audsley Stanley. Stanley couldn't be arrested for making STP, because they had no proof that it was a psychotomimetic. Might I agree to demonstrate such effects with DOM (STP) in my student population? They offered a notably generous contract, quadruple the size of my NIH grant. I used these funds and a similar grant from Dow to outfit my lab.

Within a few weeks we gave the students increasing doses of DOM that was indeed psychotomimetic at a high-enough dose. This episode gave rise to my most successful publishing experience, a paper in *Science* published with record-making alacrity (Snyder et al., 1967). The top brass at the NIH as well as at the Federal Narcotics Bureau wanted the results of our study promulgated widely and rapidly. They put me in touch with John Ringle, one of *Science*'s senior editors, who said, "Dr. Snyder, if you provide a manuscript to me with a table but no figures I can guarantee publication in two weeks including referee evaluation." And, indeed, in about 2 weeks the paper was published.

After completing psychiatry residency in 1968, I was promoted to associate professor and given a larger lab so that I was able to recruit additional students. My first official postdoctoral fellow was the extraordinarily energetic Diane Russell who kept rigid 8:30–5:00 hours, because she was raising two small daughters at the same time. I asked her to address a seemingly arcane issue in histamine metabolism. The Swedish physiologist George Kahlson had demonstrated massive levels of histidine decarboxylase in fetal rat liver suggesting a link to rapid tissue growth. However, in regenerating adult rat liver, the classic model for rapid tissue growth, there was no change. Histamine is a diamine. I wonder whether other diamines such as putrescine, formed by the decarboxylation of ornithine, might be involved. In short order, Diane mastered the technique of extirpating two-thirds of the rat liver, which grew back in about a week. We ordered [^{14}C]carboxyl-labeled forms of about 10 amino acids and monitored decarboxylation. I vividly recall that first experiment. For histidine and eight other amino acids, counts were hardly above background. For ornithine decarboxylase, the counter seemed to explode with at least a 50-fold elevation of enzyme activity. This led to an opus showing a role for ornithine decarboxylase and the polyamines which it produces in tissue growth and cancer (Russell and Snyder, 1968).

The extremely rapid massive increase in enzyme activity suggested that ornithine decarboxylase must be a very rapidly turning over enzyme. By monitoring its decline following inhibition of protein synthesis with cyclo-heximide or puromycin, Diane demonstrated that ornithine decarboxylase was the most rapidly turning over known mammalian enzyme, with a half-life of 10 to 15 minutes (Russell and Snyder, 1969). By contrast, enzymes such as tyrosine transaminase, then the height of fashion as dramatically inducible proteins, had half-lives of 1 to 2 hours.

In the late 1960s *gamma-aminobutyric acid* (GABA) was just being accepted as a neurotransmitter, and there were faint hints that glutamate and glycine might be excitatory and inhibitory neurotransmitters, respectively. I wondered whether the techniques that Joe Coyle developed to monitor synaptosomal uptake of neurotransmitters might be applicable to amino acid transmitters. If reuptake inactivation was "the rule" for terminating activities of neurotransmitters, then the amino acids that were neurotransmitters might display high affinity, sodium requiring uptake in contrast to classic amino acid transporters which were rather low affinity. Bill Logan, a neurologist in the lab, and Jim Bennett, an M.D./Ph.D. student, carried out the principal studies showing that in the cerebral cortex glutamate displayed high affinity sodium-requiring uptake with only low affinity systems evident for the other amino acids (Logan and Snyder, 1971). Interestingly, neuro-physiologists had shown that glycine is likely an inhibitory transmitter in the spinal cord and lower brainstem but not in the cerebral cortex. We detected high-affinity uptake for glycine in the spinal cord but not in the cerebral cortex. We also showed that the accumulated radiolabeled amino acids could be released from brain slices by depolarization in a calcium dependent fashion whereas nontransmitter amino acids were not released in this fashion (Bennett et al., 1972).

Opiate Receptors

In 1970 I was promoted to full professor and given more lab space permitting assumption of additional projects. One of the most exciting events in the neurotransmitter world at that time was the identification in several laboratories of the nicotinic cholinergic receptor in the electric organ of electric fish utilizing ^{125}I-labeled versions of the remarkably potent and pseudo-irreversible alpha-bungarotoxin. A major portion of the success of this heroic opus lay in the fact that up to 20% of the protein of the electric organ of certain fish comprised the cholinergic receptor. By contrast, armchair calculations told us that typical neurotransmitter receptors should only be about one-millionth by weight of the brain. I recall conversations with my friend Leslie Iversen and others in which we concluded that the very success of the cholinergic receptor effort told us that brain receptors would probably never be identified in our lifetime.

At about this time, the news media reported frantically that thousands of American soldiers in Vietnam were heroin addicts. In the United States, New York, and many other major cities were experiencing the worst epidemics of heroin abuse in history. President Nixon declared "war on heroin" and appointed a drug czar, Jerome Jaffe. Jerry had authority to commandeer whatever he needed of the billions of drug abuse dollars in the Defense Department, NIH, and other agencies to solve the problem. Jerry, a Psychiatry Professor at the University of Chicago, was an old friend and called me about the challenges he was facing. Because of government bureaucracy, he had a negligible staff but he did have the authority to "draft" anybody from another government agency. I pointed out that Alan Green, my former medical student, was now a Research Associate at the NIH working with Erminio Costa. Alan had a long-time interest in civic affairs and had even aspired to someday be Senator from Connecticut. Within 24 hours Alan was ensconced with Jerry in a mansion directly opposite the White House and was in charge of all drug abuse research in the United States. Arnie Mandell, then Chair of Psychiatry at University of California/San Diego (UCSD), and I talked about how to ensure that these vast sums of money could in some small way be devoted to quality research. We hatched a proposal to create Drug Abuse Research Centers under the aegis of William (Biff) Bunney, then in charge of the drug abuse effort of NIMH, a division that would subsequently become the National Institute on Drug Abuse. Soon a national competition for such centers was initiated. Johns Hopkins and UCSD were among the recipients.

What was I to do? I didn't know morphine from marijuana. I had read a paper by Avram Goldstein attempting to label opiate receptors by the binding of radiolabeled levorphanol seeking stereospecific binding. He found such binding but it was only 2% of the total binding and subsequently was shown to involve a lipid, cerebroside sulfate. Had there existed opiate receptors, Goldstein's experiments wouldn't have identified them. The specific radioactivity of his levorphanol was so low that he needed to employ high concentrations of the drug that would have greatly exceeded the presumed affinity constant for a potent drug interacting with its receptor. In my application to the NIH for the Center Grant, I had suggested novel binding strategies, but the study section poo-pooed that portion of the application, instead favoring our second proposal to study catecholamines and amphetamines, an area in which I already had ample experience.

At that time the only receptor sites that had been labeled biochemically were those involving peptide hormones such as insulin. Pedro Cuatrecasas had been one of the first to identify insulin receptors by the binding of ^{125}I-insulin. He had recently joined our Pharmacology Department at Hopkins, and his lab was adjacent to mine. I saw a paper in *Science* reporting the amino acid sequence of nerve growth factor and showing marked similarities to proinsulin. As I had a new postdoctoral fellow, Shailesh Banerjee,

joining the lab, I suggested to Pedro that we collaborate with Shailesh doing the experimental work while Pedro would teach us how to utilize his custom-made vacuum-linked filtration manifold and other gimmicks involved in the receptor art. Shailesh soon identified nerve growth factor receptors in sympathetic ganglia (Banerjee et al., 1973). He even succeeded in solubilizing and characterizing purified receptors, a remarkable achievement in those days when few membrane proteins had ever been solubilized in a functional state (Banerjee et al., 1976).

Candace Pert, a graduate student who had been working on high affinity choline uptake, which my postdoc Hank Yamamura had shown to label selectively cholinergic neurons (Yamamura and Snyder, 1972), was getting bored with the project. I suggested that we apply the strategies we had been imbibing from Pedro to a hunt for opiate receptors. The only commercially available radiolabeled opiate was [³H]dihydromorphine. We tried it and found no binding. In retrospect we know that dihydromorphine is light sensitive, and we had failed to turn off the lab lights. Instead, we reasoned that only antagonists would display receptor interactions. Accordingly, I splurged on a custom preparation by New England Nuclear Corporation of [³H]naloxone. Within a week Candace had identified receptor binding in the brain and the guinea pig intestine (Pert and Snyder, 1973). The binding was robust with specific binding exceeding nonspecific blank levels by several fold enabling us to characterize rapidly many properties of the receptor. My technician Adele Snowman (who continues as a lab manager for me today) had gifted hands and soon could conduct 500 receptor assays in a day. Lars Terenius at the University of Uppsala (Terenius, 1973) and Eric Simon (Simon et al., 1973) at New York University also detected opiate binding to brain membranes. Details of the opiate receptor story are described in a book I authored *Brainstorming* (Snyder, 1989).

We were able to answer all sorts of questions in short order. Neither codeine nor heroin bound to opiate receptors, because the phenolic hydroxyl of morphine, which must be unsubstituted to bind receptors, is methylated and acetylated respectively in codeine and heroin. This fit with the pharmacologic actions of these drugs. Thus, codeine (O-methyl-morphine) is slow in onset, because it must first be demethylated in the liver to enter the brain as morphine. Heroin is diacetyl-morphine. The acetyl groups permit far more rapid penetration into the brain than is the case for morphine. Within the brain, the acetyl group connected to the benzene ring rapidly falls off in a nonenzymatic fashion. Because heroin "rushes" into the brain far more rapidly than morphine, it is a more pronounced euphoriant.

In collaboration with Michael Kuhar, my first graduate student but by this time a faculty member, we dissected many small areas of the monkey brain. We unearthed dramatic differences in densities of opiate receptors that could explain diverse pharmacologic effects (Kuhar et al., 1973). Thus, discrete portions of the thalamus, involved in mediating the deep, achy pain

that is relieved by morphine, were enriched with receptors, whereas thalamic regions mediating pin-prick sensations were not. Numerous areas of the limbic system were loaded with receptors, which could readily explain the euphoric actions of the drugs. Midbrain nuclei that regulate pupillary diameter had high densities of receptors that can account for the pinpoint pupils of opiate addicts.

One important question that eluded our initial studies had to do with the differentiation of agonists and antagonists. Minor variations, such as changing an N-methyl to an N-allyl group, transformed morphine into the antagonist nalorphine. Of particular interest were the mixed agonist–antagonists that offered promise as less addicting analgesics but that were not readily detected by conventional tests in intact animals. In our initial experiments matched agonists and antagonists displayed identical affinities and displacement curve slopes. A breakthrough came when we were studying the effects of ions. Gavril Pasternak, an M.D./Ph.D. student, found that sodium decreased receptor binding, while Candace said that it increased or didn't affect binding. Adele Snowman agreed to conduct experiments to resolve this dispute. The answer is that both were right. Gavril was working with the agonist dihydromorphine while Candace was using as a ligand the antagonist naloxone. We quickly developed a means of screening large numbers of drugs for the "sodium effect" by measuring their potencies for inhibiting [^{3}H]naloxone binding in the presence or absence of sodium. Agonists became up to 40 times less potent in the presence of sodium, while pure antagonists were unaffected and the mixed agonist–antagonists behaved in an intermediate fashion.

To this day we don't know exactly what the "sodium effect" represents. It clearly was telling us that in our ligand binding experiments we were not only monitoring the recognition site for the drug but also mechanisms, which we now know to involve G proteins, that linked receptors to second-messenger systems inside the cell. By its effects on G proteins, guanosine 5'-triphosphate (GTP) similarly differentiates agonists and antagonists, while sodium and GTP synergize in this action.

Man was not born with morphine in him. Why do we have opiate receptors? Might there be an endogenous opiate-like substance, a pain/affect regulating neurotransmitter? In our lab Gavril Pasternak discovered an activity in protein-free brain extracts that competed for the binding of [3H]naloxone to receptors and whose density varied markedly throughout the brain in parallel with variations in opiate receptor concentration (Pasternak et al., 1975). This ensured that we were not dealing with some nonspecific inhibitory substance. In Aberdeen, Scotland, John Hughes and Hans Kosterlitz demonstrated in brain extracts a substance that mimicked morphine's inhibition of electrically induced contractions of the mouse vas deferens and whose effects were blocked by naloxone (Hughes, 1975). Both labs proceeded to purify the substance. Gavril finished his thesis work and returned to the

clinic, while an Israeli postdoctoral fellow, Rabi Simantov, took up the challenge of purifying the active ingredient. In 6 months he had what appeared to be a single small peptide and had done some sequencing when Hughes and Kosterlitz reported their isolation and sequencing of the two enkephalin peptides, which differed only in the C terminal amino acid, leucine for one and methionine for the other (Hughes et al., 1975). Rabi completed the sequencing of the same two peptides about 4 weeks later (Simantov and Snyder, 1976).

Characterizing the disposition of the enkephalins proceeded with extraordinary speed. With antibodies raised against the enkephalins, Rabi mapped their localization at a microscopic level (Simantov et al., 1977). At about the same time Mike Kuhar had developed autoradiographic techniques enabling him to map the localization of opiate receptor microscopically (Pert et al., 1976). The two maps coincided with considerable precision. This provided the most compelling evidence that the enkephalins were indeed the physiologic neurotransmitters for the opiate receptors. This conclusion was of importance, as numerous other larger peptides that incorporated the enkephalin sequence were being identified about this time; but, in general, these had somewhat different localizations. With the appreciation of multiple subtypes of opiate receptors whose localizations more or less matched those of different opioid peptides, the situation became somewhat muddy.

Other Receptors

The New England Nuclear Company made large sums of money marketing tritiated versions of various opiates. Accordingly, they were willing to provide complimentary radiolabeled versions of any drug I might suggest as a potential tool to identify neurotransmitter receptors. One of the first was [³H]strychnine as a ligand for glycine receptors, because strychnine was well known to block the synaptic actions of glycine. My M.D./Ph.D. student Anne Young initiated this effort, which presented far greater challenges than the opiate receptor work. For a long period of time she could not identify receptor binding. We subsequently appreciated that though strychnine had high affinity for the glycine receptor, it dissociated very rapidly from the receptor. Its high affinity reflected a correspondingly rapid association rate. After overcoming these technical hurdles, Anne characterized the receptor (Young and Snyder, 1973) and uncovered an important physiologic correlate (Young and Snyder, 1974). Glycine exerts its inhibitory synaptic effects by opening chloride ion channels. Neurophysiologists had established the relative potencies of various anions in permeating the glycine-associated chloride channel. Anne observed inhibition of strychnine binding by chloride and by other anions in proportion to their ability to pass through the channel. Thus, as with the opiate receptor, ligand binding was being modulated by a second messenger system, in this case the associated ion channel.

Rapid dissociation popped up again in Anne Young's second project, a search for the benzodiazepine receptor. Valium (diazepam) was then the best-selling drug on earth. No one had any idea just what sort of neurotransmitter it was mimicking or blocking so that speculation was rampant that there might exist an "endogenous Valium" comparable to the enkephalins. In her first experiments examining the binding of [^{3}H]diazepam Anne saw robust, saturable binding with some indications of drug specificity. However, try as she might, using all the tricks of the trade she had employed with strychnine, she could not obtain reproducible enough binding to complete the study. Moreover, it was time for her to return to her clinical training so the project was dropped. Subsequently two European groups led respectively by Hans Mohler and Claus Braestrup obtained saturable binding of [^{3}H]diazepam and elegantly characterized the receptors. They succeeded simply by conducting the binding experiments at low temperatures to slow down dissociation, whereas Anne had restricted herself to 37°. As for the endogenous ligand, John Tallman at the NIH subsequently showed that the benzodiazepine receptor is simply an allosteric site on GABA-A receptors.

Hank Yamamura, a postdoctoral fellow, joined our lab to identify muscarinic cholinergic receptors following a semibizarre interaction. Shortly after our initial work on the opiate receptor, I was giving a seminar at Yale and visiting my close friend George Aghajanian. George suggested the muscarinic receptor as a target based on his experiences during military service at Edgewood Arsenal close to Baltimore. Edgewood Arsenal was, in part, devoted to the chemical warfare effort with concerns that the Russians might spray mind-altering drugs over U.S. cities or on our troops in the battlefield. Accordingly, George participated in experiments administrating LSD and related agents to soldier "volunteers." One of these agents was an extremely potent muscarinic anticholinergic drug, quinuclidinyl benzilate (QNB). He assumed that QNB was still classified and not readily available. I was at that time in the process of recruiting Hank who was doing his military service at Edgewood Arsenal. I phoned Hank and asked what he knew of QNB. After a long pause, he nervously replied, "How did you know about that?" I told him about the conversation with George. A few months later when Hank reported to Hopkins, he brought along a small bottle of QNB. I asked no questions. [^{3}H]QNB labeled muscarinic receptors impeccably with extremely low levels of nonspecific binding (Yamamura and Snyder, 1974). Used by the pharmaceutical industry to screen for drugs with potential anticholinergic side effects, [^{3}H]QNB is likely the most widely and successfully employed neurotransmitter receptor ligand. Muscarinic receptor studies answered some important questions about neuroleptic antipsychotic drug action. As discussed below, neuroleptic drugs elicit antipsychotic actions and Parkinsonian, extrapyramidal side effects by blocking dopamine receptors. Though neuroleptics vary widely in their affinities for dopamine receptors, at therapeutically effective doses they all occupy about 50% of

receptor sites. Assuming that receptors mediating therapeutic effects are similar to those mediating extrapyramidal side effects, then at comparable therapeutic doses the incidence of such side effects should be the same for all drugs. Yet we knew that there were major differences. Some drugs, such as clozapine, elicit few such effects, whereas a majority of patients receiving haloperidol suffered these influences. The answer came when we evaluated the affinities of various neuroleptics for muscarinic receptors (Snyder et al., 1974). Since the days of the French neurologist Charcot in the 1870s, antimuscarinic agents had been used to lessen Parkinsonian symptoms. We found that the drugs, such as clozapine, with the least incidence of Parkinsonian side effects displayed the greatest anticholinergic activity. Thus, neuroleptics tend to elicit extrapyramidal side effects by blocking dopamine receptors and relieve the same effects by blocking muscarinic receptors with the ratio of affinities for dopamine and muscarinic receptors determining the incidence of side-effects.

Our initial receptor successes had used radiolabeled antagonists whose dissociation constants for receptor binding were 1 to 5 nanomolar (nM) that seemed to be the affinity range necessary to obtain binding that would be stable enough to withstand the vigorous washing necessary to remove non-specific binding. We assumed that neurotransmitters themselves, agonists, would have affinities in the micromolar range and so would not be useful ligands. This prejudice was erased when Anne, together with a medical student Steve Zukin, successfully labeled GABA receptors with [^{3}H]GABA (Zukin et al., 1974). This study opened a minor floodgate of new receptor research, as tritiated versions of most neurotransmitters were readily available commercially eliminating the burden of designing novel ligands and enabling our labeling serotonin receptors with [^{3}H]serotonin (Bennett and Snyder, 1976b) and α and β-adrenergic receptors with [^{3}H]norepinephrine or [^{3}H]epinephrine (U'Prichard and Snyder, 1977).

David Burt, a postdoctoral fellow who had just provided our first identification of peptide receptors utilizing [^{3}H]TRH (thyrotropin releasing hormone) (Burt and Snyder, 1975), had some free time and successfully labeled dopamine receptors with [^{3}H]dopamine (Burt et al., 1975). Ian Creese, a new postdoc in the lab, had done his Ph.D. thesis with Susan Iversen characterizing behavioral roles of dopamine and was eager to join the dopamine team. By this time we had obtained [^{3}H]haloperidol to investigate antagonist binding to dopamine receptors (Creese et al., 1975). To our surprise, the drug specificities for dopamine receptors labeled with agonists and antagonists, respectively, were quite different. Most strikingly, butyrophenones, such as haloperidol and spiperone, were extremely potent at sites labeled by [^{3}H]haloperidol but about a thousand-fold weaker at sites labeled with [^{3}H]dopamine. Spiperone was the champion in terms of receptor potency, with a dissociation constant of 0.3 nM. How might we explain this discrepancy? The research showing that sodium differentiates agonists and antagonists at

opiate receptors had suggested that opiate receptors exist in distinct, inter-convertible conformations respectively preferring agonists and antagonists. Hence, we initially supposed that what we were observing were simply distinct agonist and antagonist preferring conformations of dopamine receptors. Work from numerous labs, especially John Kebabian's, clarified that we were studying two separate receptor proteins that are now designated D1, for the dopamine preferring form, and D2, for the butyrophenone-preferring form.

Identifying dopamine receptors permitted us to test the hypothesis of Arvid Carlsson that antipsychotic neuroleptic drugs act by blocking dopamine receptors. His hypothesis was based on the augmentation of dopamine metabolites in rats treated with neuroleptics. Arvid speculated that neuroleptics block dopamine receptors leading to a feedback causing dopamine neurons to fire more rapidly and generate larger amounts of metabolites. While we were carrying out our initial dopamine receptor studies, Paul Greengard published a paper describing a dopamine-sensitive adenylate cyclase, presumably associated with a dopamine receptor, whose activity was blocked by neuroleptic drugs. Butyrophenones were quite weak as inhibitors of the cyclase, so we presumed that his enzyme activity reflected the receptor sites labeled with [³H]dopamine. Moreover, because butyrophenones are far and away the most potent antipsychotic drugs, these findings suggested that blocking the adenylate cyclase linked dopamine receptors was not the mechanism of antipsychotic drug effects. We examined the relative potencies of an extensive series of drugs in competing for [³H]dopamine and [³H]haloperidol binding sites. The correlation of clinical potencies with affinity for the [³H]haloperidol sites was extraordinarily high, with a correlation coefficient of about 0.9 (Creese et al., 1976). Such a correlation was particularly remarkable considering that the clinical potencies reflected effective doses in human patients, values separated from receptor affinities by drug absorption, metabolism, and penetration into the brain. Yet, over an extensive series of drugs, these factors evidently equalized out. Independently, Philip Seeman in Toronto also labeled dopamine receptors with [³H]haloperidol (Seeman et al., 1975) and observed similar influences of neuroleptic drugs (Seeman et al., 1976).

Dopamine receptors enabled us to characterize dynamic changes in receptor number/sensitivity. Ian had devoted his Ph.D. thesis to selectively lesioning dopamine neurons unilaterally and monitoring the behavioral consequences in terms of circling behavior, reflecting a unilateral loss of the dopamine regulation of motor activity. This process reflected receptor supersensitivity on the lesioned side, as such rotation was elicited by administering dopamine agonist drugs such as apomorphine. One could quantify the extent of receptor supersensitivity simply by monitoring the number of rotations. Following such lesions Ian observed a notable increase in numbers of dopamine receptor binding sites on the lesioned side (Creese et al., 1977).

The increased number of receptors correlated closely with the increased rotational behavior in individual rats establishing that increased receptor number accounted for behavioral supersensitivity.

The notion that receptor supersensitivity was determined by altered numbers of receptors enabled us to address the question of tardive dyskinesia. Patients treated for long durations with high doses of neuroleptics develop abnormal movements that can be so severe that they interfere with eating. Clinical features suggested that dopamine receptor supersensitivity was involved. Thus, the abnormal movements resembled the side-effects of high doses of L-dihydroxyphenylalanine (L-DOPA), were worsened when neuroleptic drug administration was stopped, and were improved by increasing the doses of neuroleptics. We created an animal model of tardive dyskinesia by administering neuroleptics for a month or more leading to significant increases in numbers of dopamine receptors that could account for receptor supersensitivity in tardive dyskinesia (Burt et al., 1977).

Receptor research elucidated other side effects of neuroleptics. Thus, David U'Prichard, Steve Peroutka, and David Greenberg showed that the sedating effects of neuroleptics correlate well with their blockade of alpha-adrenergic receptors (U'Prichard et al., 1978). For many years thereafter, we didn't work much on neuroleptics. Recently, the psychiatric community has been distressed by the sometimes massive weight gain caused by atypical neuroleptics such as olanzapine (Zyprexa) and clozapine. Sangwon Kim, a postdoctoral fellow, discovered that these drugs very potently stimulate hypothalamic adenosine monophosphate (AMP) kinase, an enzyme that regulates the body's response to altered energy states (Kim et al., 2007). Thus, when energy consumption depletes adenosine triphosphate (ATP), AMP levels are elevated to activate AMP kinase. In supraoptic and paraventricular nuclei of the hypothalamus, which are eating centers, leptin that decreases eating behavior, depresses AMP activity, while orexigenic agents stimulate AMP kinase. Orexigenic neuroleptics very potently activate hypothalamic AMP kinase, whereas those that don't increase appetite are without effect. I was puzzled by the extraordinary potency of these drugs in stimulating enzyme activity, something one rarely sees with enzymes. I wondered whether the effects on AMP kinase might be secondary to blockade of some receptor, as nanomolar effects of receptor antagonists are commonplace. Sangwon showed that the orexigenic neuroleptics are extremely potent inhibitors of histamine H1 receptors whose blockade increases AMP kinase activity. All of this made good sense as there was already a substantial literature about the importance of neuronal histamine in regulation of hypothalamic eating centers. These findings may provide a way to develop safer, more effective neuroleptics.

Definitive evidence that binding sites we were labeling with agonists and antagonists reflected two different receptors came from experiments of Steve Peroutka and David Greenberg, M.D./Ph.D. students, and David U'Prichard, a postdoctoral fellow, investigating alpha-adrenergic receptors.

We could label these sites with an alpha antagonist [³H]WB4101, agonists such as [³H]epinephrine, [³H]norepinephrine, [³H]clonidine or the ergot derivative [³H]dihydroergokryptine, a mixed agonist-antagonist (Peroutka et al., 1978). Numbers of agonist and antagonist labeled sites differed markedly in various brain regions. Moreover, we could abolish binding of [³H]antagonists with unlabeled antagonists while completely preserving [³H]agonist binding and vice versa. These and other experimental results provided compelling evidence that we were labeling two distinct alpha-adrenergic receptors which are now designated alpha1 for the antagonist-preferring sites and alpha2 for the agonist-preferring ones.

Discrimination of two distinct receptors with different physiologic functions came with Steve's elegant studies of serotonin receptors (Peroutka et al., 1981). These could be labeled with [³H]serotonin, [³H]LSD, a mixed agonist–antagonist, or [³H]spiperone, an antagonist. Interestingly, in cerebral cortical membranes we could label serotonin receptors exclusively with [³H]spiperone, while in the corpus striatum the same ligand labeled only dopamine receptors. Steve monitored the behavioral "serotonin syndrome" in rats and its blockade by various drugs. Drug potencies in blocking the serotonin syndrome closely paralleled their potencies at the [³H]spiperone sites which we designated serotonin-2 (5-HT-2) receptors. Regulation of [³H]serotonin binding by GTP and other properties suggested that these sites that we designated 5HT1 receptors, reflected the known serotonin-stimulated adenylate cyclase. We now know of about 12 distinct serotonin receptor subtypes whose differentiation has led to important new drug classes such as the antimigraine triptans and numerous atypical neuroleptics.

Receptor studies that most "turned me on" were those that might explain the therapeutic actions of drugs and/or were decidedly atypical. For instance, Fred Bruns, a postdoctoral fellow, in collaboration with John Daly at the NIH, identified two populations of adenosine receptors labeled with agonists and antagonists, respectively (Bruns et al., 1980). Adenosine was well known to be generated in large amounts from ATP during hypoxia. No one had ever considered a role for adenosine as a potential neurotransmitter in the brain. Karen Braas, a postdoctoral fellow, immunohistochemically mapped adenosine to populations of large neuronal cells with relatively few adenosine containing nerve terminals (Braas et al., 1986). Ted Rall at the University of Virginia had monitored adenosine effects on cyclic AMP and reported blockade by caffeine (Sattin and Rall, 1970), and John Daly had characterized such effects in considerable detail (Smellie et al., 1979). At that time pharmacology textbooks attributed the stimulant effects of caffeine to inhibition of phosphodiesterase, but such effects required concentrations 100 times higher than those that occur in the brain following coffee ingestion. Jefferson Katims, a student working in the lab while applying to medical school, monitored the relative stimulant effects of a variety of methylxanthines and compared their behavioral potencies to affinities for adenosine receptors. There was a close correlation with the adenosine receptors

labeled by a xanthine derivative and which we designated "A2" (Snyder et al., 1981). Numerous studies had shown that caffeine facilitates psychomotor performance so that derivatives lacking the cardiac effects of the drug might be useful therapeutic agents. Accordingly, we carried out structure-activity analysis leading to xanthine derivatives 100,000 times more potent than caffeine in blocking adenosine receptors (Bruns et al., 1983). It is now accepted that the stimulant effects of caffeine derive from blockade of adenosine receptors. It is interesting that it took so many years to come up with a mechanism of action for the most widely ingested psychoactive agent in the world.

In terms of atypical receptors, we had much fun seeking odorant receptors and coming up with the odorant binding protein. The project arose as a product of a dinner hosted by the Neuroscience Research Program. My dinner partner was Hank Walters, CEO of International Flavors and Fragrances (IFF) the largest manufacturer of odorants and a devotee of neuroscience. He commented, "Sol, all the receptors you guys study in the brain aren't nearly as sensitive as those in my dog's nose. Why isn't anybody looking for odorant receptors?" I explained that the NIH focuses on major diseases, whereas nobody ever died because he or she can't smell. Hank retorted, "I'll put my money where my nose is." For the next 10 years he supported our laboratory generously to study olfaction. To seek odorant receptors, IFF prepared for us a series of tritiated odorants. I asked a newly arrived graduate student, Jonathan Pevsner, to work on the project, which turned out to be fortuitous, because, unbeknown to me, Jonathan since birth had been totally anosmic, unable to smell, a secret he had hid from everyone including his parents. Jonathan did discover high affinity binding of [^{3}H]odorants to olfactory tissue with no such binding evident in any other organ (Pevsner et al., 1985). Further investigation revealed that we weren't dealing with physiologic odorant receptors, as the binding involved a small soluble protein, which Jonathan purified and cloned with the help of Randy Reed, a molecular biologist (the first foray of our laboratory into molecular biology) (Pevsner et al., 1988b). The odorant binding protein (OBP) bound a wide range of odorants of greatly varying structure. We showed that it is made in the lateral nasal gland whose secretions are dispersed from the nose to the outside world in an atomizer-like spray. Jonathan developed evidence that the function of OBP is to collect odorants in the ambient air and whisk them back to the odorant receptors in the back part of the nose (Pevsner et al., 1988a). Many years later Linda Buck, in her Nobel Prize oration described how reading our papers on OBP motivated her successful quest for the true odorant receptors.

Peptide Research

The identification of the enkephalins led to an explosion of research characterizing numerous peptides as putative neurotransmitters. The emergence

of immunohistochemistry of peptides in the brain, led notably by the efforts of Tomas Hokfelt, showed distinct neuronal pathways for individual neuropeptides. For instance, substance P, recently isolated by Susan Leeman, was highly localized to unmyelinated sensory nerve fibers suggesting a role in pain perception. We investigated a number of neuropeptides (Snyder, 1980). Bob Innis, an M.D./Ph.D. student, mapped cholecystokinin (CCK) neurons in the brain (Innis et al., 1979). He also characterized CCK receptors demonstrating two distinct subtypes, which subsequently have had important pharmacologic and therapeutic relevance (Innis and Snyder, 1980). Another M.D./Ph.D. student, George Uhl, identified neurotensin receptors (Uhl et al., 1977a) and mapped novel neurotensin pathways in the brain (Uhl et al., 1977b). Jim Bennett characterized angiotensin receptors (Bennett and Snyder, 1976a).

Bob Innis and Don Manning, a graduate student who subsequently also earned a Hopkins M.D. degree, identified receptors for bradykinin, not known primarily as a neurotransmitter but rather as a presumed inflammatory mediator (Innis et al., 1981). Working together with Larry Steranka at the Nova Pharmaceutical Company, Don localized bradykinin receptors to the terminals of sensory neurons and demonstrated analgesic actions of bradykinin-blocking drugs (Steranka et al., 1988). This spawned major efforts in the pharmaceutical industry to develop therapeutically useful bradykinin antagonists.

Ken Murphy, an M.D./Ph.D. student, and Robert Gould, a postdoctoral fellow, characterized receptors for the dihydropyridine calcium antagonist drugs employing [^{3}H]nitrendipine. As with the glycine receptor, they were able to identify linkages of the drug recognition site to the physiologic calcium channel, as [^{3}H]nitrendipine binding was absolutely dependent on the presence of calcium, stimulated by cations that mimic calcium and inhibited by cations that block calcium channels (Gould et al., 1982).

Other atypical receptors included those for neurotransmitter transporters that several labs, including our own, labeled with [^{3}H]antidepressants. Chi-Ming Lee, a postdoctoral fellow, identified norepinephrine transporters with [^{3}H]desipramine (Lee and Snyder, 1981). A fascinating adventure labeling transporters and enzymes involved the neurotoxin 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the early 1980s MPTP attracted attention when it was shown to be a contaminant of crude synthetic opiates that elicited devastating Parkinson's disease in young drug users. Others had shown that monoamine oxidase converted MPTP to 1-methyl-4-phenylpyridinium (MPP)+ which in animals models appeared to be the active ingredient in destroying dopamine neurons. Mysteriously, MPTP/MPP+ in rather low doses selectively destroyed only dopamine neurons. Jonathan Javitch, an M.D./Ph.D. student, attacked this problem by monitoring high-affinity binding of [^{3}H]MPTP that appeared to label monoamine oxidase-B (Javitch et al., 1984). This afforded a means of studying the MPTP–MPP+ conversion but didn't explain why dopamine neurons were selectively damaged.

Using preparations of monoamine oxidase, Jonathan synthesized [³H]MPP+ and discovered that it mimicked dopamine at transporter sites and could explain the unique dopamine-specific toxicity of MPTP (Javitch et al., 1985). Jonathan showed that MPP+ is concentrated in dopamine neurons several thousand fold over the external media. At these high concentrations, MPP+, a free radical, simply burned out dopamine nerve terminals. But this didn't account for the long-term destruction of dopamine cell bodies. Another M.D./Ph.D. student, Bob D'Amato, discovered that MPP+ binds to neuromelanin with high affinity. Thus, the neuromelanin that is greatly enriched in dopamine cells serves as a depot for MPP+, releasing it continuously until it destroys the cells. Proof that neuromelanin binding of MPP+ mediates neurotoxicity came from Bob's experiments with the antimalarial drug chloroquine (D'Amato et al., 1987). Bob discovered that chloroquine, which has high affinity for melanin, blocked MPP+ binding to neuromelanin binding and, in monkeys treated with MPTP, protected them from dopamine neuronal destruction and Parkinsonian motor abnormalities.

Inositol Phosphates

In the mid-1980s inositol 1,4,5-trisphosphate (IP3) was identified as a major second messenger generated by neurotransmitter-hormone stimulation of phospholipase C and that released intracellular calcium. It was assumed that inside cells small sacs of endoplasmic reticulum loaded with calcium possessed sites on their surface that responded to IP3. Efforts by other labs to identify IP3 receptors by ligand binding revealed only small amounts of saturable binding that might be associated with receptors. To identify an enriched source, Jay Baraban, a psychiatrist, and Paul Worley, a neurologist, both doing postdoctoral training in our lab, took advantage of our facilities for radioligand autoradiography. They found enormous amounts of [³H]IP3 binding sites in the cerebellum, virtually exclusively associated with Purkinje cells (Worley et al., 1987a). Cerebellar membranes provided an abundant source for characterizing the receptor. One of the earliest observations was that modest increases of calcium above physiologic intracellular levels led to inhibition of the receptor, which is now appreciated as a major regulatory mechanism (Worley et al., 1987b). An M.D./Ph.D. student, Surachai Supattapone, successfully solubilized IP3 receptors and was able to purify them to homogeneity (Supattapone et al., 1988). This enabled us to address a major question, did the IP3 binding protein we had isolated represent only the IP3 recognition apparatus or did this single protein also contain the relevant calcium ion channel? We approached this question by collaborating with Rick Huganir, who, as a Ph.D. student with Ephraim Racker, had reconstituted the acetylcholine receptor into lipid vesicles loaded with radioactive sodium and demonstrated that the isolated receptor included a sodium channel. Chris Ferris, an M.D./Ph.D. student, carried out the studies reconstituting

IP3 receptor protein into lipid vesicles loaded with radioactive calcium (Ferris et al., 1989). IP3 released calcium from the vesicles with an inositol phosphate specificity identical to that of IP3 binding sites. Chris was able to use the reconstituted receptors to learn a great deal about their regulation. For instance, he demonstrated a potent activation of calcium release by low concentrations of ATP that diminished as ATP was increased to physiologic levels (Ferris et al., 1990). This may provide a physiologic mechanism to regulate calcium release coincident with the filling of calcium stores by the calcium activated ATPase pump. He also showed that IP3 receptors are regulated by phosphorylation via numerous kinases and that the receptor autophosphorylates (Ferris et al., 1992).

Upon reading our papers on IP3 receptors, Katzuhiko Mikoshiba realized that the IP3 receptor might be identical to a protein he had purified years ago when he was working as a postdoctoral fellow with Jean-Pierre Changeux in Paris and discovered a cerebellar/Purkinje cell enriched protein which he was now cloning. The cloned IP3 receptor turned out to be a very large protein with a small IP3 recognition site at the N-terminus and a small calcium channel domain at the C-terminus with more than 1,000 amino acids of unknown function in the intervening area (Furuichi et al., 1989). In recent years, Randen Patterson and Damian van Rossum, postdoctoral fellows in our lab, utilizing yeast two hybrid methodology, discovered other proteins that bind IP3 receptors such as RACK1 (Patterson et al., 2004) and DANGER (van Rossum et al., 2006). A postdoctoral fellow, Darren Boehning, together with Randen and Damian, identified cytochrome C as an IP3 receptor binding protein (Boehning et al., 2003a, 2003b). Darren showed how this interaction mediates calcium-dependent apoptosis. There is a vast body of literature indicating an important role for calcium release in apoptotic cell death thought largely to be mediated by calcium-dependent proteases. There was a separate large literature on cytochrome C being released from mitochondria by apoptotic processes. Because IP3 mediated calcium release takes place in the endoplasmic reticulum and cytochrome C functions in mitochondria, no one had ever linked the two systems. Darren discovered that as little as 1 nM cytochrome C blocks the feedback system whereby released calcium inhibits further release from IP3 receptors. Other workers had established that the external membranes of mitochondria are closely juxtaposed to endoplasmic reticulum. Darren showed that cytochrome C, released from mitochondria, enters the endoplasmic reticulum to block the calcium inhibitory feedback so that larger amounts of calcium are released from the endoplasmic reticulum to enter mitochondria and trigger further release of cytochrome C in a feed-forward vicious cycle that is amplified throughout the cell to initiate apoptosis.

Concentrations of IP3 are only 1 μM, while substantially higher levels of other inositol phosphates exist in most tissues. For instance, levels of IP6 can be 100 times greater than those of IP3. As inositol has only six hydroxyl

groups, most people assumed that IP6 would be the "highest" inositol phosphate. Accordingly, I was amazed to read papers by Steven Shears at the National Institute of Environmental Sciences (NIH) in North Carolina and Len Stephens in England identifying IP7 and IP8 in which certain hydroxyls of the inositol ring contained two attached phosphates, forming inositol pyrophosphates. I wondered whether these energetic groups might carry out functions similar to the pyrophosphates of ATP, such as phosphorylating proteins. It was clear that the only way to make progress in this field would be to find the biosynthetic enzymes. Susan Voglmaier, an M.D./Ph.D. student, embarked on what she assumed would be a project of a few months, purifying and then cloning IP6 kinase. The protein turned out to be extraordinarily labile and nonabundant. After three years full of frustration she purified to homogeneity IP6 kinase (Voglmaier et al., 1996) and then returned to the clinics. A new postdoctoral fellow, Adolfo Saiardi, prepared large batches of IP6 kinase enabling him to obtain partial amino acid sequence and clone what turned out to be a family of related enzymes (Saiardi et al., 1999). He discovered three IP6 kinases and a fourth enzyme that could phosphorylate multiple inositol phosphates so that we called it inositol polyphosphate multikinase (IPMK). In what turned out to be an extraordinarily arduous undertaking, Adolfo successfully employed IP6 kinase to manufacture [32P]IP7 and demonstrated that it phosphorylates proteins to a similar extent as ATP with almost as many targets (Saiardi et al., 2004). Phosphorylation by IP7 is nonenzymatic even though it displays many of the same properties as ATP phosphorylation, such as requiring magnesium. In this way it resembles other nonenzymatic post-translational modifications such as S-nitrosylation by nitric oxide. Why should the body utilize a second mode of protein phosphorylation when ATP phosphorylation was doing quite well? The answer came in experiments of Rashna Bhandari, a postdoctoral fellow, who demonstrated that IP7 doesn't simply phosphorylate proteins, it pyrophosphorylates them (Bhandari et al., 2007). Although the IP7 mediated pyrophosphorylation is more labile to chemical insults than ATP phosphorylation, it resists the many phosphatases that degrade ATP-phosphorylation. Hence, in intact organisms IP7 pyrophosphorylation may be more stable.

What might be the physiologic role of this pyrophosphorylation of proteins? We attacked this question in yeast with deletion of IP6 kinase. Vesicular endocytosis is markedly distorted in yeast lacking IP6 kinase (Saiardi et al., 2002), and ribosomal function is aberrant (Saiardi et al., 2000). These findings fit nicely with evidence that the best substrates for IP7 pyrophosphorylation are ribosome-associated proteins and clathrin-related proteins involved in vesicular endocytosis.

Inositol pyrophosphates play a role in cell death. Robert Luo and Anutosh Chakbraborty, postdoctoral fellows in the lab, have provided insight into this arena. Daniel Lindner at the Cleveland Clinic had screened the effect of antisense libraries on cell death of ovarian carcinoma cells seeking

new apoptotic molecules. He uncovered IP6 kinase-II (Morrison et al., 2001), one of the enzymes that Adolfo had cloned. Robert Luo and Eiichiro Nagata, postdoctoral fellows in our lab, established that IP6 kinase-II selectively mediates cell death, as antisense to this enzyme but not to its two isoforms prevents apoptosis in multiple cell lines (Nagata et al., 2005). Anutosh Chakraborty, a more recent postdoctoral fellow, has shown how the system works (Chakraborty et al., 2008). Under basal conditions, IP6 kinase-II is maintained in the cytoplasm bound to the heat shock protein HSP-90 that sequesters IP6 kinase-II in an inactive form. Apoptotic stimuli block the binding of the two proteins with IP6 kinase-II translocating to the nucleus and killing cells. Anticancer drugs such as cisplatin, at therapeutic concentrations, block the binding, and lose their apoptotic effects when IP6 kinase-II is depleted from cells. Hence, the anticancer effects of such drugs may reflect inhibition of IP kinase II-HSP 90 binding more than deoxyribonucleic acid (DNA) damage. Selective inhibitors of the binding may afford less toxic anticancer drugs.

IPMK is the principal enzyme generating IP5 in cells. Adam Resnick, a graduate student, together with Adolfo Saiardi, discovered a novel function for IPMK, as a phosphoinositide-3-kinase (PI-3-kinase) (Resnick et al., 2005). PI-3-kinase had been discovered in the early 1990s by Lewis Cantley as an enzyme that adds a phosphate to the #3 position of phospholipids generating phosphatidylinositol(3,4,5)-trisphosphate (PIP3). PIP3 in turn activates the kinase Akt that stimulates protein synthesis and elicits other anti-apoptotic effects. Only a single PI-3-kinase was thought to exist, but IPMK is just as robust in mediating this function as the classic enzyme. The relative roles of the two enzymes are a hot area of inquiry in our lab these days.

Immunophilins

Why should a psychiatrist explore the immune system? Immunophilins are a family of proteins discovered as receptors for the classic immunosuppressant drugs that have made organ transplantation possible. The cyclophilins were identified as small soluble proteins that bound the first important immunosuppressant drug cyclosporin. The other prominent immunosuppressant, FK506, binds to a group of proteins called FK506 binding proteins (FKBPs). Although I knew nothing about immunosuppressants, I was entranced by the publication in *Nature* reporting the isolation of the first and most prominent FKBP, a 12 kilodalton protein designated FKBP-12, which bound [³H]FK-506. Since the early days of receptor binding, I had remained a consultant to New England Nuclear and thought that [³H]FK-506 would be a splendid addition to their catalog. The company asked me to test whether the product they manufactured was biologically active. Besides checking out conventional immune tissues such as lymphocytes, I asked my

new postdoctoral fellow Joe Steiner to screen a wide range of tissues—for good measure. He was amazed to find massive levels of binding in the brain, about 50 to 100 times greater than immune tissues (Steiner et al., 1992). Together with Ted Dawson, a neurologist doing postdoctoral training in our lab, he noted an association of the binding sites with growth cones and other sites relevant to nerve growth. Ted and his wife Valina, along with a graduate student Ernie Lyons, examined influences of FK-506 upon the extension of nerve processes from the neuronal-like PC12 cell line (Lyons et al., 1994). FK-506 stimulated neurite extension from these cells and was even more potent in enhancing the outgrowth of neuronal processes from sympathetic ganglia, with effects in the low nanomolar range. In intact animals FK-506 enhanced the regrowth of damaged facial and sciatic nerves. Most impressive was the ability of the drug to restore dopamine neurons following treatment with the neurotoxin MPTP. FK-506 was neurotrophic and neuroprotective, preventing the loss of dopamine neurons if administered prior to MPTP.

The obvious therapeutic potential of such drug actions was somewhat muted by concerns about administering immunosuppressant drugs to neurologic patients. Immunosuppressant actions of cyclosporin and FK-506 involve the following mechanism. The drug-immunophilin complex binds to the calcium-activated phosphatase calcineurin inhibiting it and preventing the nuclear translocation of the transcription factor NFAT which normally turns on interleukin-2 synthesis in the nucleus. Certain drugs could bind to immunophilins but, for some unknown reason, the drug-immunophilin complex failed to interact with calcineurin so that these agents were not immunosuppressants. We found that nonimmunosuppressant derivatives of FK-506 and cyclosporin were just as neurotrophic/neuroprotective as the immunosuppressant derivatives (Steiner et al., 1997). Johns Hopkins licensed its patents on these discoveries to Guilford Pharmaceuticals, a neuroscience-biotech company I had cofounded. Guilford chemists were able to fabricate derivatives of FK-506 which were much smaller and more "drug-like" yet quite potent in neurotrophic/neuroprotective animal models. In monkeys with MPTP-induced Parkinsonism, regrowth of dopamine neurons and clinical improvement with these drugs were dazzling.

The third classic immunosuppressant drug, rapamycin, acts somewhat differently than the first two. It binds with extremely high affinity to FKBP-12. However, the drug-immunophilin complex does not bind to calcineurin. In a search for a "target of rapamycin," my M.D./Ph.D. student David Sabatini discovered a large protein that binds to the rapamycin/FKBP-12 complex which he purified, cloned and designated RAFT (*Ra*pamycin and *F*KBP-12 *T*arget) (Sabatini et al., 1994). Independently, two other groups identified this protein which is now designated mTOR (*M*ammalian *T*arget *o*f *R*apamycin). mTOR has become one of the hottest areas of molecular biologic research as it transmits information about amino acid availability to the protein synthetic machinery. We have recently developed new insights into

how the system senses amino acids with a key component being our friend from the inositol phosphate world, IPMK. IPMK was identified in yeast some 20 years ago as a gene whose deletion alters the influence of amino acids, especially arginine, upon yeast. David Maag, a postdoctoral fellow, has found that deleting the gene for IPMK impairs mTOR responses to altered nutrient status. This signaling cascade involves the binding of IPMK to Akt which in turn signals to mTOR.

Our separate efforts on immunophilins and IP3 receptors converged when Andy Cameron, an M.D.-Ph.D. student discovered that IP3 receptors bind FKBP12 that regulates the receptor's calcium flux (Cameron et al., 1995).

Gases as Neurotransmitters

In the mid-late 1980s ligand binding to neurotransmitter receptors was becoming a "mature" field. Subtleties of drug actions at subtypes of receptors was of interest and being exploited by the drug industry for novel therapeutic agents, but many of the big questions had already been answered. There was fun in applying ligand binding to novel targets such as the IP3 receptor and odorant binding proteins, but I was ready for new challenges. I read a magnificent paper in *Nature* by Salvador Moncada identifying the gas nitric oxide (NO) as endothelial derived relaxing factor (EDRF). I had vaguely heard of EDRF and was fascinated that such a strange molecule as NO, a noxious free radical, should turn out to have a biological function. There were even hints, from a publication by John Garthwaite, that an NO-like substance is formed in the brain. I discussed all of this with a new M.D./Ph.D. student in the lab, David Bredt. We decided to seek a brain function for NO. It was already known that NO relaxes blood vessels by stimulating guanylyl cyclase to form cyclic guanosine monophosphate (cGMP). In the brain glutamate, acting through N-methyl-D-aspartate (NMDA) receptors, stimulates cGMP formation in the cerebellum. Arginine derivatives, such as N-methylarginine, which block the conversion of arginine to NO, were readily available. David soon established that the stimulation by glutamate of cGMP in the brain could be blocked by N-methylarginine (Bredt and Snyder, 1989), findings obtained independently by Moncada and Garthwaite (Garthwaite et al., 1989). This convinced us that NO was worth exploring as a potential neurotransmitter.

The only way to really understand NO functions would be to find the enzyme that generates it. Numerous groups had tried to purify the putative NO synthase (NOS), which would convert arginine to NO, but the enzyme seemed to be terribly labile. In his initial efforts, David also found a total loss of enzyme activity whenever he poured brain extracts over a column. He couldn't believe that any protein could be so incredibly labile and suspected that the column purification was separating out some cofactor.

Recombining fractions restored enzyme activity, supporting this notion. Based on hints in the literature that calcium was involved in NO formation, he tried adding calmodulin back to extracts and obtained total restoration of enzyme activity (Bredt and Snyder, 1990). If NO were a neurotransmitter, calcium–calmodulin activation would make sense. Classic neurotransmitters are stored in synaptic vesicles with large storage pools of excess vesicles available for release upon neuronal depolarization. A gas can't be stored in vesicles. Accordingly, each successive nerve impulse must regenerate NO. Neuronal depolarization leads to calcium influx which can activate calmodulin and NOS.

In short order David purified NOS to homogeneity and then cloned the relevant gene (Bredt et al., 1991). It turned out that there are three forms of NOS. The first that we cloned is the neuronal form, nNOS, whereas the blood vessels have a distinct form, endothelial NOS (eNOS), and all tissues, especially those involved in inflammation, possess an inducible form, inducible NOS (iNOS). Charlie Lowenstein, a cardiologist working in our lab, collaborated with David to clone iNOS (Lowenstein, Glatt, Bredt, Snyder, 1992), while other labs, using our nNOS sequence as a template, also cloned iNOS and identified eNOS.

With the purified enzyme protein, David raised antibodies and demonstrated strikingly selective neuronal localizations throughout the brain and the peripheral nervous system (Bredt et al., 1990). The autonomic nervous system proved far more useful than the brain for establishing neurotransmitter function. Thus, David found nNOS highly localized to the innervation of the penis. We collaborated with Arthur (Bud) Burnett in the Hopkins Urology Department showing that penile erection elicited by nerve stimulation was abolished by NOS inhibitors (Burnett et al., 1992). These findings established that NO is the neurotransmitter of penile erection. Utilizing similar techniques, several laboratories established that NO is a transmitter of nonadrenergic, noncholinergic transmission in the gut.

Years later we learned that our work on NO and penile erection affected the development of an important clinical drug. At the press conference launching Viagra (sildenafil), the research director of Pfizer explained that sildenafil is an inhibitor of phosphodiesterase-5, which elevates levels of cyclic GMP that then relaxes smooth muscle. Pfizer sought a drug to relax coronary arteries for use in angina but the drug failed in clinical trials. Moreover, it elicited a peculiar side effect, unwanted penile erections. Sildenafil was thus buried until Pfizer scientists read our 1992 *Science* paper on NO and penile erection and decided to conduct clinical trials in erectile dysfunction. Although Johns Hopkins had filed for patent protection covering NO and penile erection, the patents didn't extend to cyclic GMP—so it goes.

Abundant literature indicates that vascular stroke damage stems in large part from a massive release of glutamate from stressed glia with the glutamate overactivating NMDA receptors to cause neuronal damage.

NMDA neurotoxicity is readily demonstrable in brain cultures. Moreover, NMDA antagonists markedly reduce stroke damage. We wondered whether NO might mediate the neurotoxic actions of NMDA receptor activation. Ted and Valina Dawson, together with David, showed that NMDA neurotoxicity is greatly reduced by NOS inhibitors (Dawson et al., 1991). Others showed that such drugs prevent stroke damage.

To seek additional functions of neural NO, we collaborated with Paul Huang and Mark Fishman at Massachusetts General Hospital in generating nNOS knock-out mice (Huang et al., 1993). Initially, we were distressed at the absence of any obvious phenotype. Then, Ted noticed that in the cages housing male nNOS knock-outs and wild-type littermates, he often found dead mice, invariably the wild-type animals, who displayed all manner of scars and torn hair. To investigate further, we collaborated with Randy Nelson in the Psychology Department. Randy demonstrated an incredible increase in aggressive behavior in the nNOS deleted mice (Nelson et al., 1995). Within seconds of placement together of a male nNOS knock-out and a wild-type animal, the knock-out would attack and often kill his cage partner. Increased aggressive behavior in some gene knock-out mice had been previously described, but nothing remotely approaching this level of violent behavior had ever been seen previously in mice, at least to our knowledge.

Randy discovered another notable behavior. When male mice are together with females, the male will initially mount the female. If she is not in estrus, she emits a clue and the male retreats. Not so with the male nNOS knock-outs. They would mount the females repeatedly despite loud squeals, "Rape! rape!" of the females. Such dramatic sexual aggression appears to be unprecedented in mice.

Prior to these behavioral forays, the only obvious phenotype of the knock-outs was an enlarged stomach. nNOS neurons innervating the pyloric sphincter provide relaxation so that the knock-outs were displaying pyloric stenosis with associated gastric dilation. Chris Ferris, who had completed his M.D./Ph.D. training and residency in medicine, was pursuing a gastroenterology fellowship and had returned to our lab. He noted a similarity of the nNOS knock-out stomachs to what happens in diabetic gastroparesis, a common complication of diabetes. Crystal Watkins, an M.D./Ph.D. student, collaborated with Chris to show that diabetic rodents display enlarged stomachs with slowed gastric emptying much like the nNOS knock-outs (Watkins et al., 2000). They also evinced a virtual abolition of nNOS neuronal staining in the pyloric area. We first assumed we were witnessing an extension to the stomach of diabetic neuropathy with the thin, unmyelinated nNOS neurons degenerating like so many others in diabetics. However, other staining techniques showed that the neurons were still there but simply lacked nNOS. Moreover, treatment with insulin restored the staining. The NO signaling in the stomach that regulates gastric propulsion involves cyclic GMP, as treating diabetic rodents with sildenafil alleviated diabetic gastroparesis.

NO does not signal only through cyclic GMP. At Duke, Jonathan Stamler showed that NO, being chemically reactive, can nitrosylate cysteines in various proteins (Hess et al., 2005). Because nitrosylation is rapidly reversible, it was difficult to ascertain whether nitrosylation was a normal event occurring under basal conditions with physiologic levels of NO that are far less than those resulting from addition of large concentrations of conventional NO donors. Samie Jaffrey, an M.D./Ph.D. student, developed a novel chemical technique, the biotin-switch assay, that detects nitrosylation of individual protein bands (Jaffrey et al., 2001). He showed that many prominent proteins are nitrosylated in the brain under basal conditions. Moreover, such nitrosylation vanishes in nNOS knock-out mice, establishing that this modification derives from physiologically formed and released neuronal NO.

Neurotransmitters come in chemical classes such as biogenic amines, amino acids and peptides. Might NO not be the only gaseous neurotransmitter? My M.D./Ph.D. student Ajay Verma asked whether carbon monoxide (CO) might function like NO. He noted that CO was already known to be formed in mammalian tissues, something of which I had been unaware. Heme oxygenase (HO), which degrades the heme released from hemoglobin in aging red blood cells, cleaves the ring to form biliverdin and at the same time releases a one carbon fragment as CO. The best characterized subtype of HO is an inducible form, highly concentrated in the spleen where aging red blood cells reside, and is designated H01. In the process of purifying H01, Mahin Maines at the University of Rochester found another form of the enzyme which she designated H02. H02 didn't seem to be physiologically relevant, at least to the known roles of heme in degrading hemoglobin, as it was concentrated only in the brain and testes. Ajay showed that H02 is localized to discrete neuronal populations in the brain closely resembling the localizations of guanylyl cyclase which it activates similarly to NO (Verma et al., 1993). Moreover, he showed that CO physiologically regulates cyclic GMP in the retina. Randa Zakhary, an M.D./Ph.D. student, then established a neurotransmitter role for CO (Zakhary et al., 1997). She showed that NANC neurotransmission, which underlines normal intestinal peristalsis, is reduced by about 50% in nNOS knock-out mice and by the same proportion in H02 knock-out mice. Moreover, H02 and nNOS are localized in the same populations of neurons in the myenteric plexus of the gut suggesting that they may function as co-neurotransmitters. In analogy with NO, we asked, "How might CO be regenerated with each new nerve impulse to support neurotransmission?" Darren Boehning showed that, like nNOS, H02 is physiologically stimulated by calcium-calmodulin (Boehning et al., 2004) as well as being regulated by casein kinase-2 (CK2) phosphorylation (Boehning et al., 2003a). Masao Takahashi, a postdoctoral fellow, found that H02 also binds APP, the precursor of the Aβ42 peptide that occurs in Alzheimer's plaques and mediates neurotoxicity (Takahashi et al., 2000). A-beta peptide precursor protein (APP) regulates H02 activity with Alzheimer mutant APP markedly diminishing H02 activity.

What about the other product of HO, biliverdin? Biliverdin readily accumulates in mammalian tissues that all contain an abundance of biliverdin reductase, that rapidly reduces biliverdin to bilirubin. This yellow pigment is generally regarded as the end product of heme metabolism, as it is conjugated to glucuronide and excreted. But this didn't make any sense, because biliverdin is more readily excreted. Why would nature create two extra enzymes and, in the process, create bilirubin that in high concentrations deposits in the brain to cause kernicteric damage? An answer to these questions came in the studies of my postdoctoral fellow Sylvain Doré (Doré et al., 1999). He discovered that brain cultures from H02 knock-out mice are much more sensitive to all forms of neurotoxic insult than wild-type specimens and that the H02 mutants display substantially greater stroke damage. He wondered whether the loss of any product of H02 accounted for the neurotoxicity. Adding CO did not reverse the toxicity in brain cultures but low nanomolar concentrations of bilirubin were markedly neuroprotective. This was puzzling, because Sylvain was eliciting neural damage by adding to the cultures 100 μM concentrations of the oxidant hydrogen peroxide. It was well known that bilirubin is antioxidant. But how could minute concentrations of this antioxidant protect against 10,000 times higher concentrations of an oxidant? We thought of a possible explanation. Whenever a molecule of bilirubin acts as an antioxidant, it is itself oxidized to biliverdin. Perhaps the abundant tissue concentrations of biliverdin reductase regenerate bilirubin. Such an enzymatic amplification could readily enable bilirubin to cope with 10,000 times higher concentrations of hydrogen peroxide. A M.D./Ph.D. student David Baranano proved that this hypothesis is correct (Baranano et al., 2002). He showed that depletion by ribonucleic acid (RNA) interference of biliverdin reductase prevents the neuroprotective actions of bilirubin and also worsens the neurotoxic effects of various agents.

All of these findings suggested that bilirubin serves as an endogenous antioxidant cytoprotectant. Biliverdin reductase would provide an elegant means for nature to make use of bilirubin but maintain low endogenous concentrations, as higher levels of bilirubin are toxic to the brain and other tissues. Clinical data support this notion. Gilbert's syndrome is a condition in which individuals have a defect in the bilirubin glucuronidation process and so display modestly elevated serum levels of bilirubin. The prevalence of ischemic heart disease in these individuals is about a sixth of control levels. Multiple studies in "normal" populations show less atherosclerosis in individuals with elevated bilirubin.

Glutathione is a well-known antioxidant that is an endogenous cytoprotectant. Why do we need bilirubin? One possibility lies in the markedly different chemical properties of the two molecules. Glutathione is a water soluble tripeptide, whereas bilirubin is an extremely lipophilic molecule. Perhaps glutathione primarily protects water soluble proteins, whereas bilirubin would prevent peroxidation of membrane lipids. To test this concept, Tom Sedlak, a psychiatrist in our lab, monitored soluble protein oxidation

as well as oxidation of lipids (Sedlak and Snyder, 2006). He showed that bilirubin selectively protects lipids, whereas glutathione protects the proteins. He depleted glutathione with an agent that inhibits its biosynthesis and depleted bilirubin reductase by RNA interference. Loss of glutathione led to a greater increase in protein oxidation than lipid oxidation and the reverse transpired with the loss of bilirubin.

The heme oxygenase-biliverdin reductase story provides yet one more example of the beauty with which nature sculpts the body. As Julie Axelrod always emphasized, "When nature finds a good molecule, he/she uses it again and again in different contexts."

D-Serine

The history of neuroransmitters is filled with "laws" that are repeatedly overturned. Acetylcholine was the first neurotransmitter and formed the paradigm for "proper" transmitters. One rule was that a "neurotransmitter must be inactivated by a specific synaptic enzyme." Julie's work with norepinephrine reuptake inactivation overturned that notion. Peptides are not inactivated by enzymes or uptake and to this day don't display any unique inactivating system—they probably just diffuse away from synapses. To ensure specificity, it was assumed that nature created molecules that were highly specialized to be neurotransmitters. Amino acids such as glutamate and glycine dispensed with that concept. Far more radical were the gases. They were not stored in synaptic vesicles nor released by exocytosis, nor did they act upon receptors on adjacent neuronal membranes. D-amino acids, especially D-serine, are even more bizarre.

I had been intrigued by a little-noticed paper from the laboratory of Professor Toru Nishikawa who was developing a prodrug of D-serine as a nonmetabolized glycine analogue for administration to schizophrenics. He was testing the "NMDA hypothesis of schizophrenia" based on the similarity to schizophrenia of the psychosis elicited by phencyclidine, which blocks NMDA receptors so that stimulating the "glycine site" of the NMDA receptor should be therapeutic. To assess whether the prodrug delivered D-serine to the brain, his postdoctoral fellow Atsushi Hashimoto developed a high performance liquid chromatography (HPLC) system to separate the isomers. Remarkably, in placebo-treated rodents the brain contained D-serine at levels about a third those of L-serine, while there were no other D-amino acids detectable except for some D-aspartate. My graduate student Michael Schell tried different means of assaying D-serine with little success till our faculty colleague Mark Molliver suggested generating an antibody. This succeeded magnificently and was followed soon by an antibody to citrulline, the coproduct of NOS action, which was used to monitor NOS activity in the brain by immunohistochemistry (Eliasson et al., 1997).

The D-serine antibody revealed notable surprises (Schell et al., 1995). D-serine was highly localized to areas of the brain enriched in NMDA receptors. This was tantalizing, because D-serine was known to be substantially more potent than glycine at the so-called glycine site of the NMDA receptor. The classic work of Phillipe Ascher had established that NMDA receptor activation requires another agonist and that glycine satisfied this requirement. It was assumed that the NMDA receptor was unique in requiring two agonists, because its overstimulation could be neurotoxic. Because glutamate is a dietary amino acid, eating a steak dinner might cause a stroke. The requirement for a second neurotransmitter would provide a fail-safe mechanism—two keys required to open the lock. However, this didn't make sense, as glycine was also an abundant dietary amino acid. We felt that D-serine, a rare molecule formed only in the vicinity of NMDA synapses might make better sense. Jean-Pierre Mothet, a postdoctoral fellow, carried out the critical experiment to test this possibility (Mothet et al., 2000). In 1935 the great Hans Krebs had discovered a novel enzyme, D-amino acid oxidase, which surprisingly degraded only D-amino acids. We showed that at physiologic pH the enzyme is rather selective for D-serine and, when added to brain extracts, it can totally degrade D-serine without influencing levels of any other amino acid, especially glycine. Adding D-amino acid oxidase to brain slices or cultures greatly reduced NMDA neurotransmission despite completely normal levels of glycine. Hence, it appeared likely that D-serine is the predominant coagonist with glutamate at NMDA receptors. Very recently, in collaboration with my former student Joe Coyle, we have found alterations of NMDA transmission as well as long-term potentiation (LTP) in mice with knock-out of serine racemase.

The next surprise came with localizations. Herman Wolosker, a postdoctoral fellow, undertook the task of seeking an enzyme that physiologically generates D-serine. After some heroic biochemistry, he successfully purified and then cloned serine racemase, which converts L- to D-serine (Wolosker et al., 1999). The immunohistochemical localizations of serine racemase and D-serine were the same, both in the vicinity of NMDA synapses. However, both were highly concentrated in astrocytic glia that ensheath the synapse. Hence, D-serine appeared to overturn an unspoken but clearly fundamental rule of neurotransmission—a neurotransmitter should be in neurons. Subsequently, following his move to a faculty position at the Technion in Israel, Herman has shown that serine racemase and D-serine also occur in neurons but a variety of evidence indicates that glial D-serine mediates neurotransmission.

GAPDH and Cell Death

In recent years our laboratory has addressed signaling systems that are cytotoxic or cytoprotective. These include the IP3 receptor-cytochrome C

interaction, NO mediating glutamate neurotoxicity, IP6 kinase-II killing cells, the neuroprotective actions of drugs influencing immunophilins, and bilirubin serving as a cytoprotectant.

One of the most striking of these signaling cascades involves glyceraldehyde-3-phosphate dehydrogenase (GAPDH). GAPDH is a well known glycolytic enzyme whose generation of ATP is critical for cells in various contexts. I became interested in GAPDH upon reading a paper by De Maw Chaung utilizing antisense technology to identify potentially neurotoxic proteins (Ishitani and Chuang, 1996). He found that antisense to GAPDH blocked neurotoxicity elicited by an anticancer drug in cerebellar cultures. Akira Sawa, an M.D./Ph.D. psychiatrist doing postdoctoral work in our lab, attempted to confirm and extend this finding. Akira showed that antisense to GAPDH protects against toxicity elicited by multiple stimuli in a wide range of cell cultures (Sawa et al., 1997). He then noticed that with all these apoptotic stimuli about 4% of cellular GAPDH translocated to the nucleus. Although antisense treatment had little effect on total cellular levels of GAPDH, it depleted the nuclear pool, which presumably turned over more rapidly.

We wondered how GAPDH, which lacks a nuclear localization signal, enters the nucleus. Akira utilized yeast two-hybrid technology to look for binding partners with nuclear localization signals and detected Siah, a ubiquitin-3-ligase. By a selective mutational analysis Akira established that Siah is responsible for the translocation of GAPDH to the nucleus following apoptotic stimuli. But how would such stimuli cause GAPDH to bind to Siah? A graduate student Makoto Hara established the following signaling cascade (Hara et al., 2005). Following any cell stressor iNOS is induced. The generated NO nitrosylates GAPDH at cysteine-150, which is critical to catalytic activity. Although abolishing catalytic activity, nitrosylation confers upon GAPDH the ability to bind to Siah. In the brain neurotoxic stimuli elicit glutamate release which, via NMDA receptors, generates NO to nitrosylate GAPDH.

Once in the nucleus, how does GAPDH kill the cell? Makoto and Nilkantha Sen, a postdoctoral fellow, obtained insight by showing that nitrosylated GAPDH in the nucleus binds to the protein acetylase p300/CBP which then acetylates GAPDH enabling it to activate p300/CBP by augmenting its autoacetylation. p300/CBP then acetylates and activates p53, the well-known tumor suppressor whose ability to kill cells is well established.

Extracurricular Activities

Music has long been a passion. I began piano lessons when I was just 5 years old and, before I was 6, I performed on a local radio talent show "Uncle Bud's Amateur Hour"—a reflection of my Mom's "stage mother" proclivities. When I was 8 years old, our piano was sold—possibly because of a clash about practicing. As detailed above, I ended up playing the classic guitar and

continue to practice more or less regularly as time permits. I try to help the guitar community in my capacity as a board member of the Peabody Conservatory where I have adopted the Guitar Department as my special focus. For instance, we sponsor the top guitar student each year or two in a Carnegie Hall recital. I also serve as a trustee of the Shriver Hall Chamber Music Concert Series, one of the most prominent in the country, and have successfully lobbied for guitar recitals.

My most extensive civic commitment in the music world involves the Baltimore Symphony Orchestra (BSO). As one of the principal cultural organizations of the city, which derives half its $30 million budget from philanthropy, the board has typically been dominated by local business leaders whose corporations are a mainstay of support. An apocryphal but true story deals with the CEO of Baltimore's premier bank. When asked to join the BSO board, he responded, "Okay, just so long as I never have to go to a concert." Hence, when a friend of mine on the nominating committee advocated for my membership, she argued, "Shouldn't we have at least one person on the board who cares about music?" Soon after joining the board in 1992 I became chair of the Music Committee, a position that I maintain and cherish today. Why should a symphony orchestra need a music committee composed of trustees? What have they to offer the music director? One of my passions is the commissioning of new symphonic works. I argue to the board that in the laboratory we don't constantly repeat the experiments of Pasteur—hence, let's encourage new symphonic works.

To raise commissioning funds, I have sought links to events that appeal to appropriate donors. One of my first activities was to commission a new concerto for the guitar that I funded by soliciting contributions from former students of my teacher Sophocles Papas. I uncovered a zionistically motivated donor to support a commission in honor of the 50th anniversary of the state of Israel. Baltimore's was the only major symphony orchestra sponsoring such a commission. The biggest challenge was a concerto that our music director David Zinman had conceptualized for two left-handed pianists, Leon Fleisher and Gary Graffman, both of whom suffered from focal dystonia that incapacitated their right hand. For a rather large fee, the U.S. composer William Bolcom accepted the challenge. He realized that it would be rare to have two left-handed pianists in the same concert hall on the same day. Hence, he elaborated two separate concertos for the left hand that could also be played together, hence three distinct concertos. A fund-raising breakthrough arrived when we realized that a distinguished Baltimore hand surgeon, recently deceased, had treated Fleisher and Graffman, and his hospital was fund-raising for a new hand surgery building. The world premiere of the concerto was a sold-out fund-raiser for the hospital, raising ample funds for the commission and for the new building.

Participating in our synagogue has been rewarding. My religious roots go back to when I was five years old. Although our family was reform, I was

sent to a newly founded modern orthodox Hebrew day school, largely because there was bus service and a hot lunch. I remained at the Hebrew Academy of Washington till high school but thereafter was little involved with religion. When our older daughter Judy was school age, we joined a liberal, unaffiliated synagogue populated in large measure by academics, lawyers, and physicians. I accidentally attended a board meeting and soon was on the board and a few years later was president. I accepted the position after being assured that, "the synagogue runs itself. You'll have little to do between the monthly board meetings." Within the first two months of my tenure our custodian fell from a ladder in the lobby, bumped his head, and died. Then our beloved cantor underwent surgery for a presumed herniated lumbar disc and emerged paraplegic. A new rabbi arrived. We were a bare-bones congregation with no secretary, just an administrator who couldn't type.

Despite all the chaos, my tenure was much fun. As Hopkins is the leading hospital in Baltimore, where many of our ill congregants were treated, I took to making regular hospital visits, complementing the pastoral activities of the rabbi, an enterprise that was personally enriching. Of course, the president ought to attend synagogue every Saturday. To make this a meaningful experience, I encouraged our tradition of a full Shabbat lunch for all the congregants. Besides being a time when I could transact most synagogue business, interpersonal interactions at synagogue were rewarding. Rather than finding weekly attendance a chore, it became an addiction and to this day I attend synagogue regularly on Saturday mornings.

I never thought of myself as a scientist but rather a physician/psychiatrist who happens to do some research. I wanted to be a psychiatrist long before I had any interest in science. I still devote a good bit of time to helping people in distress find appropriate referrals. Although I have maintained a faculty appointment in the psychiatry department at Johns Hopkins and for years continued to supervise residents in psychotherapy, I was never involved in departmental administration. To get a feel for the big picture of psychiatry, I agreed to serve on the board of the Sheppard Pratt Hospital, the largest private psychiatric hospital in Maryland. Learning the economics of a large hospital's administration, its delicate interplay with governmental politics and bureaucracy, and somehow keeping the hospital out of bankruptcy are remarkable challenges. Somehow they concatenate in a bizarre mixture which has worked well—at least for our hospital. One of my pet efforts on the board has been to help launch a museum of art with mental health themes. In the new hospital building, with inviting public spaces, the art attracts the local community. Thus, the edifice is not regarded as an "insane asylum" but as an important communal gathering place.

Scientific Public Life

The life of a biomedical researcher can be great fun—especially if the work is going well and is well funded. I recall Julie giving a public address soon

after winning the Nobel Prize and saying, "I never cease wondering at my good fortune to be in a job that is so much fun that I would be doing it even if there were no pay." Research can be an all-consuming 24/7 enterprise. If so, who minds the store? Someone needs to chair departments, serve as Dean, edit journals, and organize scientific societies. I have always been relatively well organized and so have been drafted into various civic endeavors.

My first encounter in the civic life of the scientific community involved efforts to honor Julie. It started with a scientific meeting where Leslie Iversen, Jacques Glowinski, Lincoln Potter, Hans Thoenen, and other former students of Julie were assembled. We talked about "doing something" for Julie. Somehow I ended up responsible for organizing "something" for what I thought would be Julie's 60th birthday in 1971—it turned out to be his 59th. In August 1970 I asked the powers of the American Pharmacology Society, ASPET, to allow us a slot during the ASPET banquet to be held in April 1971 at the Federation of American Societies for Experimental Biology (FASEB) gathering in Chicago. I was told, "So many of our colleagues have birthdays that we can't single out any single individual for special treatment." Irv Kopin, who was serving as president of the Catecholamine Club, which held dinner meetings at FASEB, agreed to a program of Julie's former students. Raising money from drug companies to subsidize travel expenses seemed hopeless. Then, in October 1970 Julie's receipt of the Nobel Prize was announced. Money from drug companies flowed in. I received a phone call from the ASPET president eager to include us on his program— I declined. The Catecholamine Club event was much fun and emotionally moving. Oxford University Press put out an elegant volume incorporating chapters from all the speakers.

Another challenge came in the early 1970s. Every 6 years since the late 1950s catecholamine researchers had gathered for a major meeting. I was drafted to chair the Catecholamine Conference to be held in Strasbourg in 1972. I was soon initiated into the world of fund-raising. Although just a 32-year-old twirp, I was obliged to toady up to major drug company VPs seeking donations. Somehow, we raised enough money to support the travel of the 120 invited speakers and to provide amenities for the 500 to 600 participants.

One of the principal sources for funding was Robert Maxwell, the notorious, now-deceased founder of Pergamon Press. Normally scientific publishers do not fund meetings or publications, merely providing modest royalties on sales of the volume. At that time Maxwell had just returned to leadership of Pergamon after a hiatus during which the British government found him "not fit to run a public company." He was eager to resurrect the scientific image of Pergamon and appeared willing to pay for the privilege of publishing our volume. I recall vividly meeting with him more than a year before the meeting when he invited me to his enormous suite in the San Francisco Hilton Hotel at the time of the International Pharmacology Congress. Of the six rooms in his "presidential" suite, one was a cocktail

lounge where he exerted his famous charismatic charm. I was rather flum-
moxed, me a nobody, being wooed by this famous man. However, I knew
what I wanted, a large advance on royalties, something that was in those
days unprecedented for scientific books, especially for proceedings of scien-
tific meetings. I sold him on the "massive" interest by the biomedical com-
munity in catecholamines and walked away with a handsome advance.
Although I subsequently obtained gifts from the major drug companies,
Maxwell's was the largest contribution.

Another unique feature of the Strasbourg meeting was its "opening to
China." The conference took place in June 1973 soon after Richard Nixon's
trip to China. We had sent pro forma letters of invitation to officials of the
Chinese Academy of Sciences expecting nothing in return. Remarkably, we
received a delegation of top Chinese biomedical researchers. They were
warm and friendly individuals, most of whom had only recently been resur-
rected from their exile to the countryside during the Cultural Revolution.
When I returned to Baltimore I had visits from the FBI and the CIA asking
about my sojourn in Strasbourg. The agents revealed that Chinese atten-
dance at a catecholamine meeting was no accident but a calculated effort to
learn new research that might benefit Mao Tse-tung's Parkinson's disease.

I was elected president of the Society for Neuroscience for the 1980 year,
highlighted by some interesting challenges. The Society had been launched
in 1970 with a few hundred members and had grown to about 7,000 when I
took office. People were complaining that the annual meeting was so crowded
that "one couldn't be with one's own friends." There was an incipient move-
ment to fracture the always tentative union between the molecular oriented
"wets" and the neurophysiologic "drys." I argued that the raison d'etre of
the Society was to bring together these two streams of neuroscience. More-
over, I noted that membership size was plateauing—a false prediction for a
society which now numbers about 37,000 members. The union held.

In a single year as president, one can't accomplish too much. I felt it
important to select a special focus. Ours was then, and still is, the largest
biomedical research society, yet was alone in not publishing a society spon-
sored journal. Members of Council resisted, "There are already too many
journals." However, I thought there were not enough journals of distinction
in the neurosciences. If we could make subscription to the society journal a
component of member dues, we would launch the journal with 7,000 sub-
scribers, substantially more than almost any other basic biomedical journal.
With such a proposition, we could probably obtain far more favorable terms
from a publisher than the usual 50/50 split of the "profits," which too often
evaporated with accounting legerdemain. As successful journals generally
run a 40% operating profit, I proposed that the publisher pay us 20% of
gross revenue, which ought to correspond to half the profits, and editorial
office expenses. Max Cowan agreed to be the first Editor-in-Chief. We inter-
viewed a series of publishers and set up an auction that attracted impressive

bids from about five companies. Williams and Wilkins, the respected publisher of the *Journal of Biological Chemistry, the Journal of Pharmacology,* and *Experimental Therapeutics* and others, provided the best offer. Thus was the *Journal of Neuroscience* born.

Years later I returned to service on behalf of the *Journal of Neuroscience.* In the mid-1990s the Internet was beginning to affect scientific publishing, with a few journals developing online editions while others resisted the expense and chaos of this "passing fad." I was asked to chair the Committee on Publications. It became evident to me that online publishing was the future and that laggards would be losers. Following some struggle with Council, we collaborated with Stanford University's HiWire operation to launch an online version of our journal. Ours was the second major basic biomedical online publication following the *Journal of Biological Chemistry*, a pioneer from which we gleaned precious wisdom. Today a favorite cocktail party competition is guessing the date when hard copies of biomedical journals will vanish.

That year, 1980, was a busy one. Joshua Lederberg had assumed the presidency of Rockefeller University and had a single "professorship" open. He had long had a fascination with the brain and psychiatry—his wife is a psychiatrist. Josh courted me aggressively, indicating that I could bring with me two other faculty, my colleagues Joe Coyle and Mike Kuhar. Rockefeller provided munificent support for faculty, so much that one almost didn't need to apply to the NIH for research grants. Fully intending to leave Johns Hopkins, I visited Dean Richard Ross. He said that large amounts of "hard" money for a professor were out of the question. However, many people had advocated that Hopkins develop a department focused on the brain. He proposed designating Joe, Mike, and myself as the Department of Neuroscience. He would provide us more money than Rockefeller offered with no more responsibilities than directing the medical student freshman course in neuroscience. The law of inertia prevailed, I remained in Baltimore, and the Neuroscience Department was launched as a tiny group of three faculty charged with coordinating activities for neuroscientists throughout the medical school.

Our group didn't remain tiny for long. Howard Hughes chose to "get into" the neuroscience game at University of California/San Francisco (UCSF), Mass General, Columbia, and Hopkins. Hughes funding permitted us to recruit four new faculty. Then Vernon Mountcastle and all the other neurophysiologists in the Physiology Department elected to move into our department as did Mark Molliver and other neuroanatomists in the Anatomy Department. The construction of a new basic science building about this time enabled all of us to congregate in contiguous space. Before long we were the largest basic science department at Hopkins. My faculty have all been civic minded so that chairing the department was never onerous and rarely occupied more than 20% of my time. I found that recruiting new

faculty and nurturing their development was much akin to developing the careers of young postdoctoral fellows and of parenting children. If you make your children, students, and faculty your number one priority, they'll rapidly wean, become independent successes, and bring you joy.

In 2006, after some 26 years directing the Department, I stepped down and Rick Huganir assumed leadership. In 1987 Rick was one of our first Howard Hughes recruits into neuroscience, till then a senior associate of Paul Greengard's at Yale. It has been particularly gratifying to witness his growth over two decades to a world-class neuroscientist and respected administrator.

Industry

Ever since taking the pharmacology course in medical school, I have been fascinated by drugs. It was fortuitous that my research training was with Julie Axelrod, likely the greatest pharmacologist of his era. Much of the early work in my laboratory at Hopkins involved drugs such as amphetamines and psychedelic drugs. However, my interactions with the drug industry had been limited to begging for financial support for scientific meetings. Receptor research changed all of that. Until the advent of ligand binding for neurotransmitter receptors, drug development in the pharmaceutical industry required screening agents in intact animals, demanding chemical engineering feats to deliver many grams of drug to the pharmacologist. If one chemical was more potent than another, there was no way of determining whether it had greater affinity for the putative receptor, was metabolized less, or penetrated more readily to the target organ. Thus, intelligent structure-activity analysis was impossible. Receptor binding changed all of this. Even with the relatively primitive binding apparatus in our laboratory, we could screen thousands of chemicals a day.

Soon I was a consultant to a substantial number of leading pharmaceutical companies including Sandoz (now Novartis), Burroughs-Wellcome, Warner-Lambert, Dupont, and others. One of the first and most productive relationships was with Sandoz. A little more than a year after publication of the opiate receptor paper, I was visited by Stephan Guttmann, head of chemistry at Sandoz. He grasped the potential importance of receptor binding for drug development and also saw it as an opportunity to incorporate biology into the chemistry division to mitigate his dependence on the Sandoz pharmacologists. I became a consultant to the company, visiting Basle four to six times a year and hosting chemists from Sandoz in our laboratory where they learned receptor technology.

Visiting the laboratories at Sandoz and other companies was illuminating, teaching me much about the psychology of industry scientists. Chemists were typically horrified when I advocated screening their large libraries of chemicals at random to seek "hits" that could then be further refined to

secure greater potency. They took umbrage that I would be treating chemists as automotons doing blind screening "like monkeys." After much effort, I convinced most that high-throughput screening would provide greater intellectual stimulation for the chemists. They might uncover totally unexpected structures that were uniquely active at particular receptors. My classic argument utilized the opiate receptor as a paradigm. What if enkephalin were a known neurotransmitter and one wished to find a drug to mimic it? Molecular modeling with the most advanced computers would never lead to morphine, whereas a simple screen of plant extracts would hit pay dirt rapidly. Enkephalin is a useful example, as finding small molecules to activate or block receptors for peptides is a particularly major challenge that, over recent decades has been successfully addressed.

Receptor screening has been particularly useful in sculpting drugs to avoid side effects. Muscarinic cholinergic actions have bedeviled many psychotropic drugs including most neuroleptics and antidepressants. Although Prozac was heralded for introducing the class of serotonin-specific uptake inhibitors (SSRIs), its principal clinical benefit has been the absence of anticholinergic side effects based on screening candidates for effects on [^{3}H]QNB binding to muscarinic sites.

In 1980 Genentech went public, and the biotech boom emerged. In late 1982 I was approached by two young brothers David and Isaac Blech. Utilizing their meager savings from Bar Mitzvah gifts and borrowings from friends, they had launched Hybritech, the first biotech company to focus upon making monoclonal antibodies. Within a year they had founded several biotech companies. The dozens of biotech companies then extant largely did very similar things, cloning genes for proteins such as insulin or making monoclonal antibodies. The Blechs asked their advisors whether there existed any other biomedical technology that would be relevant to the pharmaceutical industry. They spoke to my former M.D./Ph.D. student Gavril Pasternak at Cornell, who pointed out the obvious relevance of receptors and sent them to me. The brothers journeyed to Baltimore, we had lunch at Danny's, a fancy restaurant near the train station, agreed that a receptor-based company made good sense, shook hands, and within a few months launched Nova Pharmaceutical Corporation.

There is a formula for developing new companies, especially in high tech areas. One begins with seed funding to hire a handful of people and get some sort of "proof of principle." Then comes venture capital funding at substantially greater levels and finally, many years later, a public offering, affording financial liquidity. Nova overturned all these rules. In the summer of 1983 Nova had no labs and no products. The only employee was Don Stark, former president of the American division of Sandoz and an expert in marketing drugs, but no knowledge of science. I recruited David U'Prichard, my former postdoctoral fellow and then a faculty member at Northwestern University, as our Research VP. David had no industrial experience. Biotech was

extremely hot that summer. The Blechs wanted to catch the frenzied market optimism before it dissipated. Hence, with nothing but a dream, Nova went public. The stock offering was highly successful, with the share price increasing almost 50% in a day.

One of my first challenges was ensuring that my activities with Nova didn't interfere with my obligations to Johns Hopkins. Nova never funded any of my Johns Hopkins research. In terms of time commitments, I strived to honor the University guidelines that faculty shouldn't devote more than 20% of their effort to outside activities. Accordingly, I declined the great majority of invitations to give talks at other universities and participate in scientific meetings, except for those that provided unique intellectual rewards. I set up regular monthly meetings with the head of research and with laboratory researchers and, of course, was available for phone calls.

Nova thrived. I enjoyed the availability of an outlet whereby new findings in our lab with potential therapeutic benefit could be exploited. For instance, bradykinin was well recognized as an important peptide mediator of pain and inflammation. Hence, our identification of bradykinin receptors (Innis et al., 1981) might have therapeutic relevance if it were only possible to make bradykinin antagonists. Our collaborator, John Stewart at the University of Colorado, had made modifications in the bradykinin peptide structure that conveyed antagonist properties. Patents from the University of Colorado were licensed to Nova whose peptide chemists soon came up with potent and selective bradykinin antagonists. In a collaborative scientific investigation between Nova and ourselves, we showed that the bradykinin antagonists had analgesic properties in rodents leading to a drug development enterprise at Nova (Steranka et al., 1988). As there was a literature on a role for bradykinin in mediating the symptoms of the common cold, Hans Mueller, Nova's CEO, conducted an informal clinical trial on his own nose and decided that the bradykinin antagonists "obliterated all my symptoms." More extensive clinical studies were less promising. Other companies subsequently came up with even more potent bradykinin antagonists, some of which are still being explored for anti-asthmatic actions.

The biotech industry has long endured an exhilarating/panicky seesaw existence on Wall Street with 1991 a time of exuberance. All public biotech companies were able to raise substantial amounts of cash, and mergers became popular. Nova merged with Scios, a California-based company about the same size as Nova. I remained on the Scios board and followed closely the up-down meanderings of the company culminating in its highly successful sale in 2004 to Johnson and Johnson.

As Scios focused on cardiovascular products, neuroscience didn't make much business sense. I convinced the CEO Rich Casey to spin off the neuroscience efforts into a new company which we dubbed Guilford Pharmaceuticals. The name was my wife Elaine's brainchild, reflecting the section of Baltimore in which we reside.

Guilford afforded new potentials for drug development. Henry Brem, then a young neurosurgeon at Hopkins, had worked with the eminent chemical engineer Robert Langer in developing a novel treatment for primary brain tumors. The classic anticancer drug carmustine was incorporated into a biodegradable polymer and implanted in the brains of patients at the site where the surgeon had removed their tumor. As carmustine is an alkylating agent, it would not diffuse away from the site of implantation so that patients could receive, at the site of tumor regrowth, concentrations of the drug more than 1,000 times what would be possible by conventional routes of administration. The resultant product Gliadel had been under development by Nova since 1985, but the medical chief of Scios thought it was "silly" and declined to carry the product forward even though Phase III clinical trials had already been completed. I successfully inveigled Rich Casey, the Scios CEO, to gift the project to Guilford—we sealed it with a handshake in the men's room at a Scios retreat.

Besides Gliadel, Guilford developed potential neuroprotective drugs based on the immunophilin research in our lab. Phase II trials of the lead agent GPI1485 showed promise in slowing the progression of Parkinson's disease. Particularly striking was our use of radiolabeled ligands of dopamine neuronal transporters to image dopamine neurons and directly demonstrate a retardation of their loss following drug treatment. Technical problems regarding the drug's bioavailability have hampered progress.

What have I learned from my experiences with industry? Some argue that academics should confine themselves to basic research and let drug companies learn about their findings from publications. I think differently. The NIH doesn't fund biomedical research because science is beautiful. Rather, every dollar of our grant support is intended to find causes and, more importantly, treatments for disease. The increasing sophistication of molecular approaches to biomedical science brings new basic findings far closer to therapeutic application than in past years. Yet there remains a gulf between the two. Drug development in large pharmaceutical organizations is driven in substantial part by the marketing divisions which too often advocate "me-too" approaches to capture 10% to 20% of market share of some other company's multibillion dollar blockbuster. If a university scientist approaches a large company with an idea based on his or her newly discovered receptor/enzyme, the retort will be, "We have long lists of great 'inhouse' ideas already which we don't have time to pursue. Moreover, we have no guarantee that a drug acting on your new receptor/enzyme will be effective, as there is not yet already a drug acting at this target." Small companies founded by university scientists, the mainstay of the biotech industry, can bridge this chasm.

Family

Elaine and I were married a week after I graduated medical school. Our honeymoon comprised hoisting our worldly goods into my Volkswagen and

driving from Washington, D.C., to San Francisco where I interned at the Kaiser Hospital. Most internships are grueling affairs that, in the 1960s on the East Coast of the United States, involved working every other night all night. By contrast, medical life was much less stark in San Francisco with the on-call schedule at Kaiser generally being every fourth or fifth night. Coupled with the lack of need to study for exams, internship was the most relaxed period of my life since beginning college. Elaine and I made many close friends, some of whom we have retained throughout the years. My first foray into songwriting occurred when a former intern lured me into collaborating on a musical show satirizing medicine. Elaine and I developed an interest in art collecting, making purchases of some original prints, which we couldn't afford as each of us was earning about $250 a month. Such an idyllic year formed a fruitful beginning to a marriage that has happily endured till the present.

Our first-born daughter Judith, like typical first borns, was always well behaved and grew up to realize her parents' aspirations. From the time she was 5 years old she knew, more or less, that she would be a physician. However, to avoid becoming a "grind" Judy majored in art history at Princeton even helping Elaine and I in our collecting activities.

Judy loved every specialty in medical school, especially pediatrics. She knew that she would never wish to "compete with dad" and so eschewed even considering psychiatry. However, she fell in love with the discipline as soon as she began her psychiatry clerkship and now is in private practice of psychiatry in Philadelphia.

Judy married Stephen Kastenberg while she was still a medical student. During her psychiatry residency she gave birth to Abigail, 2 years later to Emily, and 5 years later to Leo. The grandchildren have become a most important part of our life. As Philadelphia is only 1 to 2 hours by car from Baltimore, we see the kids every 2 to 3 weeks.

All grandparents repeat the same mantra, "Nothing is so wonderful. It's positively spiritual." For me, the grandchildren released a new burst of creative fervor, especially in music. As soon as Abigail was born, I wrote a song, "Abigail I Love You." Now original songs with lyrics and chords emerge at the birthdays of all the kids as well as on numerous other occasions, such as the departure of guys and girls from the lab, the birth of their children, special birthdays of friends, and numerous other occasions. Grandchildren are a fitting capstone to anyone's life.

Deborah emerged 4 years after Judy, very different in temperament. She always marched to her own drummer and was remarkably creative from the outset. By the time she was 7 years old, Debbie was involved in school theatre. In high school she joined a American-Russian musical troupe whose production "Peace Child" toured the Soviet Union, Japan, and other countries on multiple occasions. Besides becoming fluent in Russian, the Peace Child experience fostered Debbie's pre disposition for the theatre. Thus, in

college she majored in theatre and participated in every aspect, writing, directing, and performing. Her playwriting gift soon became apparent so she is now a New York—Los Angeles playwright—screenwriter. Courtesy of the California Supreme Court's ruling legalizing gay marriage, Debby and her partner Sonora Chase, a talented actress, were wed in August 2008.

Bottom Line

What is it all about? What I've tried to convey in this essay is that, for me, life works best if one incorporates a diversity of interests. Participation in the arts enhances fecundity in scientific discovery. Parenting children involves the same practices as mentoring students, faculty, and other professional colleagues. Doing "deals" in the business world augments one's acumen for meandering the jungle of modern science. Most of all, all of these activities should be fun. If not, why bother?

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