Huda Akil

Born:

Damascus, Syria
May 19, 1945

Education:

American University of Beirut, Lebanon, BA (1966)
American University of Beirut, Lebanon, MA (1968)
University of California, Los Angeles, CA, PhD, (1972)
Stanford University, Palo Alto, CA, Postdoctoral Fellowship (1974–1977)

Appointments:

Adjunct Assistant Professor, Psychology Department, Stanford University (1974–1978)
Assistant Professor of Psychiatry, University of Michigan (1978–1981)
Associate Professor of Psychiatry, University of Michigan (1981–1987)
Professor and Senior Research Professor, Department of Psychiatry and Molecular & Behavioral Neuroscience Institute (MBNI), University of Michigan (1987–present)
Co-Director (with S. Watson), MBNI, University of Michigan (1995–present)

Honors And Awards:

Rockefeller Scholarship, American Univ. of Beirut, 1963–1968
Penrose Award, American University of Beirut, 1968
Sloan Foundation Postdoctoral Fellowship for the Neurosciences, 1974–1976
NIDA Pacesetter Award, 1993
Member, Institute of Medicine of the National Academy of Sciences, 1994
Robert J. and Claire Pasarow Foundation Award for Neuropsychiatric Research, 1994 (with S. J. Watson)
Bristol Myers Squibb Award for Distinguished Achievement in Neuroscience, 1998
President, American College of Neuropsychopharmacology, 1998
Fellow, American Association for the Advancement of Science (AAAS), 2000
President Elect, President, and Past President, Society for Neuroscience, 2001–2004
Named as one of the most highly cited researchers in neuroscience by the Institute for Scientific Information (ISI), 2002
Member, American Academy of Arts and Sciences, 2004
Henry Russel Lecturer Award, University of Michigan, 2006
John P. McGovern Award in Behavioral Sciences, AAAS, 2006
Mika Salpeter Lifetime Achievement Award, Society for Neuroscience, 2007
Goldman-Rakic Prize for Achievement in Cognitive Neuroscience, National Alliance for Research in Schizophrenia and Affective Disorders (NARSAD), 2007
Council, Institute of Medicine, National Academy of Sciences, 2006–2012
American College of Neuropsychopharmacology, Paul Hoch Award, 2010
New York Academy of Medicine, Thomas W. Salmon Award (with S. Watson), 2010
Member, National Academy of Sciences, 2011
Rhoda and Bernard Sarnat International Prize in Mental Health, 2012 (with S. Watson)

Stanley J. Watson, Jr.

Born:

Henrietta, Oklahoma
May 30, 1943

Education:

University of Southern Mississippi, Hattiesburgh, MS, BS (1965)
University of Iowa, Iowa City, IA, PhD (1970)
Tulane Medical School, New Orleans, LA, MD (1974)
Stanford University, Palo Alto, CA, Postdoctoral Fellowship (1974–1977)

Appointments:

Internship, Pacific Presbyterian Medical Center, San Francisco (1974)
Psychiatric Internship and Residency, Stanford University School of Medicine (1974–1977)
Assistant Professor and Assistant Research Scientist, University of Michigan (1978–1981)
Associate Professor and Associate Research Scientist, University of Michigan (1981–1987)
Associate Director, Mental Health Research Institute, University of Michigan (1984–1995)
Research Professor, Mental Health Research Institute, University of Michigan (1987–1993)
Associate Chair for Research, Department of Psychiatry, University of Michigan (1993–2002)
Co-Director (with H. Akil) and Research Professor, MBNI, University of Michigan (1995–present)

Awards:

National Merit Scholarship Finalist, 1960
Academic Honors Scholarship at Tulane and University of Southern Mississippi, 1961–1964
Member Psi Chi (Psychology Honor Scholarship), graduated with honors, 1964
Merck Award, Tulane Medical School, 1974
Bank of America—Giannini Fellowship in Biomedical Research, 1977
McAlpin Award Winner (with Dr. Floyd Bloom), 1980
Grass Foundation Lecturer, University of Pittsburgh, 1984
Visiting Professor, University of Hawaii, 1985
University of Michigan Senior Research Scientist Lectureship Award, 1988
Theophile Raphael Professor of Neurosciences in Psychiatry, 1993
Pfizer Visiting Professorship in Psychiatry, Johns Hopkins University Med. Ctr., 1994
Co-recipient (with H. Akil) of the Robert J. and Clair Pasarow Foundation Award for Neuropsychiatric Research, 1994
Member, Institute of Medicine of the National Academy of Sciences, 1994
Fellow, American Association for the Advancement of Science, 2000
Named "Highly Cited, Influential Researcher in Neuroscience" by ISI, 2000
Ralph Waldo Gerard Professor of Neurosciences in Psychiatry, 2006
New York Academy of Medicine, Thomas W. Salmon Award (with H. Akil), 2010
Rhoda and Bernard Sarnat International Prize in Mental Health (with H. Akil), 2012

Huda Akil and Stanley Watson have made seminal contributions to our understanding of the neurobiology of emotions and affect, including pain, stress, depression, and addictive behavior. They began their research work at the time of the discovery of endorphins and participated in the development of that field through a combination of behavioral, anatomical, molecular, and translational studies. Throughout their careers, they have developed new technologies for studying the function and regulation of neural circuits, as well as new paradigms and animal models of affective behavior. This includes the study of the neurobiology of temperament and its relevance to differences in mood and addictive disorders. They are currently engaged in large-scale studies to discover new genes and proteins that cause vulnerability to major depression and bipolar illness. The hallmark of their work is its multidimensional, collaborative, and integrative nature.

Huda Akil and Stanley J. Watson, Jr.

Tales of Endorphins and Other Adventures

> In science, unlike the usual situation in human affairs, reality frequently exceeds expectations.
>
> *Daniel Klepner, 1998*

In this chapter, we describe what led us to become neuroscientists, and then we focus our recollections on the birth and early years of the field of endorphins—the brain's naturally occurring opiates. We were privileged to witness the dawn of that field and to contribute to its development. We each came at it not only from different scientific angles but literally from different worlds—Huda having grown up in Damascus, Syria, and Stan in New Orleans, Louisiana. Our collaboration has been a journey that has defined our scientific and our personal lives.

We tell this tale from our unique perspective while trying to acknowledge the huge influence that many great scientists have had on our lives—first and foremost, our mentors who have shaped our thinking and given us unimaginable opportunities. But also, scientists we met along the way who influenced us through their ideas, their example, their generosity, their collaboration, their advice, and even their doubt. We also touch on some lessons learned—the importance of considering contrary opinions but also the power of having a strong hunch about the nature of a system or an approach; the value of using the right technology at the right moment, or if need be, inventing new technologies to advance the field; and mostly, the remarkable power of teamwork.

Our only regret is that by limiting these recollections to the early days, we have not recounted some equally exciting and more recent scientific adventures, nor have we given proper credit to many of our trainees and colleagues who came along since then. Our trainees have a chimeric name, the Wakils—for Watsons and Akils. Had we been writing about the *future* of neuroscience, it would have been all about them. Instead, we dedicate this chapter to all the Wakils, the scientific branch of the family—our students and postdocs—and to the biological branch of the family—our children and grandchildren. May your lives be filled with adventure!

Huda

I was standing on a table looking out of a large window watching an airplane take off. I must have been about three years old, and the airplane carried my mother away to be with my dad. I knew she was going to be gone for a long time, and something felt heavy inside me. But I was mostly wondering how that plane was able to fly. Standing beside me, my uncle and grandmother congratulated me on being a big girl and not crying about my mother's departure. My cousin, who was my age and very attached to my mother, was screaming and asking to go with her.

This is my first fully fleshed memory, and it is very vivid. We were in Damascus, Syria, in the late 1940s and I was around two to three years old. I had been allowed to go up on the airplane to see the inside—in retrospect, quite an unusual opportunity. I retain the impression of a very narrow space, the presence of people dressed in military uniforms, and a vague sense of worry about my mother being trapped in there. I knew she was flying to England to be with my father while he completed his doctorate in London, and I could tell she was sad to be leaving me.

Looking back on that moment, I recognize many elements that were to define me. That education was of paramount importance, enough to warrant long separations from those I love, proved to be one the consistent themes of my young life. But equally strong was the abiding sense that my parents loved me. Separations never felt like abandonment but rather like a sacrifice we all made together toward a shared and important goal. Now that I study the neurobiology of stress and resilience, I can recognize the elements in my life that were stressful without being overwhelming, and I can see how they combined with my emotional makeup and family structure to give me the resilience to emigrate from Syria to the United States to become a scientist. But chance and good luck played an equal role in this improbable journey.

Damascus—Early Life

My early childhood was spent in the context of an extended family revolving around my maternal grandmother and her five sons, two daughters, and one stepdaughter, headquartered in a building owned by my grandparents. The bottom floor of my grandparents' residence was a large and comfortable home. It had a courtyard with a fountain in the back, and the building was surrounded by a garden with a kumquat and a lemon tree, blooming forsythias, and the scent of jasmine on summer evenings. This is where my grandmother lived together with the unmarried members of the family. There were smaller apartments above, and my nuclear family occupied one of them. So, when my parents went away, I simply moved downstairs to be next door to my aunt, whose big room was filled with magical knickknacks such as a vessel that holds kohl for eye makeup, which I now own. Even my

paternal uncle called my grandmother's place home. He was much younger than my dad and had a sweet tooth, so he took me out regularly on special outings to eat *chocolat mou*, the local equivalent of a chocolate milkshake. I had a special plan for each of my relatives—something sugary that I loved to buy when I was out with them, and together they made me feel special and cared for. But there was no denying the hole in my heart until my parents and I were reunited.

In later years, the separations were due to my father's work. He was a professor at the University of Damascus, quickly becoming very well known within Syria and throughout the Arab world, in part due to several lay books and articles he wrote introducing psychological concepts to the Middle East, including coining Arabic terms for concepts such as "reinforcement" and "feedback." He was also in demand by the United Nations Educational Scientific and Cultural Organization (UNESCO) and would take positions in remote places that needed his expertise to create new educational communities. Sometimes we all moved with him, and at other times he went alone for several months at a time.

One such UNESCO assignment took us to Egypt in 1953–1954, when I was in third grade. Because my father was stationed in a remote rural place that did not have a decent school, I was sent to a boarding school for girls run by French nuns, called Le Collège de la Mère de Dieu, in Garden City, Cairo. Looking it up on the Internet, I can see that it is an impressive place that offers a great education, but it is fair to say that I was utterly miserable there. Once again, I missed my mother, father, and little brother (who was two), and this time I did not have an extended family to turn to. I also could not understand the Egyptian accent, and the other kids made fun of mine. The nuns seemed remote and spoke only in French, which I understood but barely. The only highlight of my day was the 3 p.m. break when they distributed chocolate and French bread as a snack. I spent weekends with my parents, sometimes in Cairo where they kept an apartment, and sometimes in Sers El-Layan, the village that was the site of the UNESCO project. I remember holding onto my composure tightly as my parents came to pick me up for the weekend, trying to remain strong and to prepare for my eventual return to school. I have to believe that these separations, stressful as they were, gave me the ability in my early twenties to pick myself up and move away from my family, friends, and country—once again for the sake of education.

That year in Egypt was memorable in many ways. They had a great zoo, and I begged to go there on the weekends. I spent hours watching the monkeys interact, groom each other, take care of their young, eat, and play. Little did I know then that I would spend much of my adult life studying animal behavior. But it was also an education in local politics. Then, as today, Egypt was in turmoil. Its first president, Muhammed Naguib, resigned shortly after we arrived, and I recall seeing pictures of Gamal Abdul-Nasser

plastered all over Cairo. My dad, who was passionately interested in Middle Eastern politics and who was missing his Damascene friends, discussed the developments with my mother at every opportunity. The issues of military rule versus democracy, regional tensions with Iraq, the fate of Sudan as well as the Suez Canal dispute were common conversations at the dinner table. At one such dinner, my parents talked about the escalating tension between Cairo, Israel, and the West and the fact that the U.S. Sixth Fleet was moving in the Mediterranean. Consequently, all the UNESCO experts had to leave Egypt immediately. It was probably the start of the tension that led to the Suez Canal crisis a few years later, but I had no idea what any of it meant. Still, these developments made "the Americans" sound as though they had the power to change our lives.

The rest of my schooling through grade, middle, and high school took place in Damascus in a single school also run by French nuns—L'Ecole des Franciscaines, Missionnaires de Marie. My parents must have known that my boarding school experience had been painful, so they resolved to keep me in the same school and let me live with relatives whenever they needed to leave on UNESCO assignments—this happened over four years between fifth grade and the end of high school. I missed my parents, my younger brother Bisher, and my much younger sister Mayada. But I loved school and was at the top of my class, which was very important to my family. It was in that school that I consciously started dreaming of becoming a scientist.

Thinking Science Thoughts

Like most scientists, I was a curious child. I asked a lot of questions about how things worked, how they were made, or why they happened as they did. I am grateful that my parents never dismissed these questions as annoying. Rather, they took them seriously and answered me thoroughly. An example that has stayed with me is my query about grapes. I must have been six or seven years old, and I had inquired about how plants grow. My father explained about seeds and pits and how they contain all the information needed to grow the plant. One day, I was eating a seedless grape and asked how such a plant could grow. He said he was not exactly sure but would try to find out. A few days later, a friend of his who was an agricultural engineer came to our house and explained to me all about propagation and grafting of seedless fruits. The meta-message from that one episode has stayed with me since—the integrity to admit one's ignorance and the importance of searching for knowledge wherever one can find it. More importantly, it told me that I was asking reasonable questions that were worth a real answer. It is only in retrospect that I have come to realize how amazing a gift my parents gave me, and how unusual it was for a little Syrian girl to receive it.

But it was one of the nuns that helped me go from being an inquisitive child to dreaming of becoming a scientist. I remember the one-room library

in my Damascus school with the desk of the librarian nun near the door. I must have gone there often because the librarian seemed to know me well. I was around 10–11 years old when, one day, as I was leaving the otherwise empty library, she handed me a thin volume in French and told me that she thought I would be interested in it. It was an abbreviated biography of Marie Curie, and it transformed the way I thought about the world. That a young woman from Poland could pick herself up and move to Paris to study science was a revelation. I thought that science, even though it had flourished in the Arab world in the Middle Ages, was now the domain of the lucky few—the French, the British, the Americans. But here was someone who was none of these nationalities and yet was able to be a scientist! I also had never heard of a woman scientist. The fact that she went on to win two Nobel Prizes and that she worked with her husband and raised a daughter who herself won a Nobel Prize was breathtaking. Going to Paris to become a scientist became my secret dream.

From that point on, I tried to get a hold of French or Arabic books about the lives of scientists, but they were not easy to come by in Damascus. By the time I was in high school, I could read English well enough to a have a few more options. I vividly recall reading the life of Sir Alexander Fleming, discoverer of penicillin for which he received the Nobel Prize. I was struck by the fact that his discovery was accidental—he was culturing staphylococcus, left his cultures on a laboratory bench during a holiday, and returned to find that a fungus had grown in one of the dishes, and it had inhibited the growth of the bacteria around it. Rather than being disappointed by the contamination, he pursued it, grew the fungus systematically, tested it against a range of bacteria, and discovered its actions. He also struggled to extract it and to get it accepted as an important breakthrough. This awakened me to the power of observation, the importance of turning an apparent setback into an opportunity for discovery. I could tell that for Alexander Fleming and Marie Curie, the glamour came after much obscurity, frustration, and disappointment. But the discovery of penicillin held a personal meaning for me. My mother had suffered from tuberculosis soon after I was born and had gone to a sanatorium in Lebanon for more than a year. I had no memory of that particular separation, but she often spoke of it with great sadness and bemoaned the fact that she had had no access to antibiotics, even though they had already been discovered. The juxtaposition of my mother's illness with reading the story of the drug gave me a personal sense of how science can impact people's lives.

Stan

Early Life

One of my earlier memories is lying in the tray of our family sedan, the area between the top of the back seat and the rear window, as my dad drove down

very dark roads, looking at the night skies and searching for patterns in the stars. In fact, most of my very early recollections are visual. A few years ago, when our family was exchanging tales of first memories, I described a specific ceiling pattern that I used to watch as I was falling asleep. It was distinctive enough that my mother could pinpoint it to a particular apartment where we lived for a short period of time. Apparently, I was still in my crib when this image was imprinted in my mind! So, maybe it is not too surprising that I grew up to be a neuroanatomist, with astronomy as my other serious endeavor.

The long nighttime trips that began my star fascination took place because my father, Stanley J. Watson, Sr., took us along on trips for his work as a minister, as he would "substitute" all over Oklahoma in small churches that needed a pastor. When he was younger, my dad would hitchhike to the church, often halfway across the state, preach and counsel, then go back to college. It did not pay much but kept him in school. It was on one such trip that he met my mother, Johnie Lee Butler, and they married shortly thereafter. Both families were prototypical European-American families from that era. My father's family came to the United States in the late 1700s as carpenters and farmers, and some became sheriffs and ministers, with one U.S. marshall in the Oklahoma Territory. The Butlers, remotely related to President Andrew Jackson, were farmers and small businessmen, and my grandfather, John Butler, was relatively successful as a farmer, proprietor of a small coalmine, general storekeeper, and as an owner of the local "movie house." They lived through the Dust Bowl of the 1930s and the Depression in a comfortable way. Both families were oriented toward physical work and survived through intelligent and careful management.

I grew up in Oklahoma playing outside, running hard, and walking alone to my grandmother's house, even though I was four years old and she lived more than a mile away on a dirt road. I remember listening to my dad preach and loving my mom's smile. My mother was fairly ill for several years, during which time I took care of my younger brothers, Mark and Dave, and cleaned house (sort of).

We moved from Oklahoma to New Orleans for my father's seminary education, back to Oklahoma for more education, and finally settled into an apartment at the New Orleans Baptist Theological Seminary, where my father had a faculty position. The years from five to twelve were both positive and challenging. I changed schools four times, but I always did well and learned how to fit in. My favorite subjects were math, science, and oddly, history.

Throughout my childhood and adolescence, I worked any job I could find in order to buy bicycles, telescopes, movie tickets, radios, and eventually a motorcycle and a car. When I was 10, I had earned enough to buy my first bike and a year later my first telescope (a three-inch Newtonian). It was that telescope that provided me with my first taste of science. As a child, I was a tinkerer and was often testing some idea or another. If I did not understand

something, I wondered if it was really true—it bothered me until I figured it out for myself. My first such effort with thinking about astronomy was during an event called the "opposition of Mars" when the Earth passes between the sun and Mars, and the planet can be seen in the "opposite" direction from the sun. Through my telescope, I could see the white polar caps (largely CO2) and a hint of other features in the more equatorial areas. But I had placed my telescope in a position where I could be blinded by a street lamp—so I moved it. I then began to worry that, by moving, I was actually looking at a different part of Mars! It took me a couple of weeks and some effort with my astronomy to realize what parallax was and how silly my worry was. I finally began to understand the small angle I was working with, and I came to realize that Mars from any angle on Earth would look the same.

Growing up, I did everything from cleaning rain gutters to selling shoes and tools, to working on the docks on the Mississippi and cutting trees to make paper—work was a point of pride as well as money, and my parents were intent on raising "thoughtful, skilled, and independent children." For example, when my brother Mark was 14, he worked on the wheat harvest at our grandmother's home in western Oklahoma. My parents let him leave to follow the harvest all the way to mid-Canada. He was a superb mechanic and was in charge of the repairs for all the wheat harvesting machinery (combines) during that trip. At the end of the summer, he bought a 50cc Honda motorbike and rode the 2,000 miles home, looking very tanned and happy. I too was very interested in understanding how machinery works and how to repair it when it malfunctioned, and eventually I made a living in college partnering with my friend at repairing old cars and selling them.

My junior high school in New Orleans was a very tough place—knives, 18-year-old seventh-graders, and a principal who tried to jump off the building twice! Being a 70-pound 12-year-old, I was at a significant disadvantage! Within a month of starting school, a large 18-year-old with an alcohol problem demanded my math homework. So, I made a deal with him: I would let him copy my math homework only if he would let me explain the problem and its solution. It worked! He became a great ally for that entire year, and the experience taught me the importance of forming strategic alliances! During my eighth and ninth grade years we had a new principal—a large, ex-Marine who was tough but fair to students and faculty alike. He restored some order and school was fun again, especially math and science. In fact, during my eighth-grade year, I had a superb teacher who taught us general science and encouraged more independent thinking. I selected as my project the mapping of sunspots during the solar maximum (an 11-year cycle). I was proud to confirm the work of real astronomers, which showed that sunspots moved faster at the equator than at the poles. I am sure they were relieved!

During my last year of junior high, I was approached by the school counselor to see if I was interested in attending Benjamin Franklin Senior High

School in New Orleans. It was, and still is, a very selective public school that draws students from the entire area with a tough evaluation process for admission. I was lucky enough to be selected as one of the 50 students admitted in 1958. That education was the hardest I have ever experienced—including college, graduate school, medical school, and residency training. My peers were exceptionally bright, the teachers and the courses very strong and challenging. To my love of math, science, and history, I now added Latin and German. I was encouraged to prepare a science project for the tenth-grade competition, and I chose to explain the Kant-La Place theory of the origin of the solar system. It basically hypothesized that a dust cloud condensed into the sun and the smaller eddies turned into planets. I chose to demonstrate the theory with a fluorescent painting of the proto-planetary disk placed in a darkened box illuminated by black light. The project did not win but was very popular. It was very visual, and it strikes me that I have not gone very far from that work even now. In general, Franklin High School taught me a great deal of specific academic information but also enhanced my social skills and gave me a clear perspective on my academic strengths and weaknesses. I felt ready for college!

Huda

Beirut—College Years (1963–1968)

I had always expected to go to college at the University of Damascus, where my father was a professor. Typically, Syrian girls who were lucky enough to go to a university were not sent away for their education. But one day, my father sat me down in his study and told me that I had the opportunity to receive a scholarship to attend the American University of Beirut (AUB), his alma mater. He had discussed my options with the chair of psychology at AUB, had sent him a copy of my transcript, and his colleague had encouraged my application. Miraculously, I received a Rockefeller Scholarship to AUB that covered my tuition, room, and board. I was thrilled and petrified. One big hurdle was that the curriculum was American, a big change away from my French education. I had taken English as a requirement for the French *baccalaureat*, but it was a distant third language for me and I could not imagine managing my entire education in it. I was also accepted directly as a sophomore because the French baccalaureat was considered the equivalent of completing the freshman year. It seemed like a recipe for failure. Both my parents encouraged me to take a chance, but it was my mother's regret at never having had such an opportunity herself that made me realize how lucky I was.

I worked very hard that first year. A requirement for keeping my scholarship was maintaining an A average, and the combination of skipping a year and studying everything in English was a challenge. But at one point,

I realized I had done it before—that year in Egypt. I had started near the bottom of my class and ended up near the top. That early experience helped me regain confidence in myself, and soon it felt easy and then downright fun.

My years at AUB transformed my life. I met people from all over the world and established close friendships that have lasted until today. It also gave me a taste of what it must be like to be American. During my first year, I had an American roommate, Patty, whose father was a diplomat in the Middle East. Pretty, perky, and flirtatious, she was very popular among the male contingent at AUB and went on fabulous dates but still missed her all-American high school boyfriend. She explained concepts to me such as going steady and being pinned, and I was shocked to hear that her parents wanted her to date around rather than focus on a single person. I, being a nice Syrian girl, had never had a date in my life.

During my first term in college, President Kennedy was assassinated, and Patty and her older sister, who lived down the hall, were devastated because they had a direct personal connection to the Kennedy family. This made the United States seem less remote, more human, and accessible. It was not just a superpower that influenced Middle Eastern politics, it was made of people I was meeting, and their president was connected to someone I lived with! That moment may have been the seed of my notion that I could actually live there someday and realize my educational dreams.

Early Research and Scientific Interests

In spite of my desire to differentiate myself from my father, I became a psychology major because I found the subject fascinating. I wanted to understand thinking and felt that language was a window into that. But I was also very interested in social psychology, in belief systems, and in the role of culture in thought. As an undergraduate, I conducted a research project on the social beliefs of Syrian women across generations and socioeconomic levels. I visited several families and spoke to grandmothers, mothers, and daughters and systematically asked them a set of questions about their lives using a questionnaire I had devised. But I also collected stories they wanted to share with me—tales of being married before reaching puberty, of being disciplined by stepchildren who were older than them, of never having a normal cycle because of being either pregnant or nursing continuously, and of secret attempts at abortions that were mostly unsuccessful. The voices of these women still reverberate within me—the combination of resignation and courage even among those who considered themselves more lucky than not. Even though some of them had never known another life, they seemed to glimpse other possibilities, a sense that the world could be different. My mere presence, without a hijab and coming from a university abroad to conduct this research, was enough to confirm that women could

have different lives. But they did not seem to resent me; they wanted to be heard, to share their stories, and to impress upon me their uniqueness. Just as Marie Curie had shown me one way that life could unfold, these women showed me its polar opposite. Coupled with the voice of my own mother, who seemed to have yearned for a greater measure of self-determination, these women's voices shaped my desire for self-definition, rather than being tossed around by various rules and customs.

Late into my undergraduate work, I decided to focus on psycholinguistics as a means to probe the process of thinking. I had a British mentor, Professor Ernest Darlymple-Alford, who taught cognition, learning, and memory as well as psycholinguistics. Here again, my first research project was focused on cultural aspects of language and thought. I wondered whether bilingual individuals think differently when they are using different languages. To test this notion, we relied on the pool of bilingual (Arabic–English) students at the AUB and administered a personality scale that tests the degree of authoritarianism. This scale (the so-called F-scale) was devised after World War II to understand the propensity to be authoritarian and follow figures of authority—presumably the behavior that contributed to the spread of fascism. The scale was known to be very culturally sensitive, with Western cultures scoring lower than Middle Eastern or Asian cultures. So, we asked: Would native Arabic speakers be equally high on this scale regardless of which language they were operating in? Because the scale had split-half reliability, we administered to each subject one-half in Arabic and the other half in English (the two halves were counterbalanced across subjects). Remarkably, every person tested had a higher F-score when functioning in Arabic than when functioning in English! I never published this work, but it was my first foray in personality testing, something to which I have returned more recently in the context of understanding the neurobiology of temperament.

Early Exposure to Behavioral Neuroscience

I decided to pursue a master's degree in psycholinguistics at AUB, under Professor Darlymple-Alford, while also working as a teaching assistant. My master's thesis had a more classical "verbal learning" topic that did I did not find as exciting. But it was during that graduate year that I took a course in physiological psychology from Professor Conrad Consalvi that I found fascinating. I still recall being in the stacks of the AUB library and discovering a paper by James Olds about self-stimulation in rats. I knew next to nothing about the brain, its organization, or its function—back then psychology was quite separate from biology and neuroscience as a field was barely in existence. After reading a *Science* paper by Olds on using self-stimulation to test psychoactive drugs, I remained in the library for more than eight hours and tracked down every paper I could find written by him

and others on intracranial stimulation. Feeling dazed, my heart hammering, I thought: "I want to do that! Get right in the brain and figure out how it controls behavior!" The fact that it was possible was stunning. But I also felt that this was clearly beyond my reach—I had no access to animal research, electrical stimulators, or any means of looking directly at the brain. I could not have imagined that I would wind up doing exactly that sort of work, in some of the very institutions where Jim Olds had worked (UCLA and University of Michigan), working under someone who had been his student (John Liebeskind), and even meeting Olds in person.

Coming to America (1968)

As I was completing my bachelor's and master's degrees, it was becoming evident that my parents were increasingly worried—had they created a geeky monster who would never fit within the expected framework of Damascus society? Arranged marriages are still the norm in Syria, and during my junior year at AUB, I had become engaged under such an arrangement, only to discover that the groom-to-be was much more religiously conservative than my parents had estimated and would not abide my dress style and independent habits, much less my long-term professional goals. As importantly to me, he seemed to resent the company of my younger siblings, Bisher and Mayada, and they were wary of him. My father helped me dissolve that relationship. After a year hiatus, another engagement was arranged. I was more enthusiastic about this second one because the fiancé was a physician completing his residency in the United States. Here, suddenly, was my ticket to go to America! Better yet, he seemed open-minded and willing to see me apply to graduate schools. I was in heaven. My fiancé, whom I had barely gotten to know during his vacation away from his residency, returned to the United States. But something was obviously wrong because his letters were progressively less frequent and more cryptic. And then one summer day, in June 1967, while I was home in Damascus because of the Six-Day War, I received an uncharacteristically thick envelope from him. He explained to me that, before we met, he had been in love with an American nurse. His parents were opposed to the relationship and had made him promise to break it off and marry someone from Damascus. And although he had done his best to honor his promise to his parents and to me, he was still in love with his previous girlfriend and was feeling very guilty toward me. I wrote him back immediately absolving him from the arrangement and encouraging him to stick to his guns with his parents and marry the woman he loved. I was finishing the letter when my father walked in and found me in tears. I recounted what happened, and my father asked me whether I had fallen in love with the guy. I had to laugh and explain that my disappointment was entirely because I had lost my opportunity to go to the United States and pursue my studies!

I believe it was my mother who convinced my father to still allow me to apply to graduate schools in the United States. I marvel, once again, at my parents' remarkable behavior for that time and place. Maybe they decided I was not cut out for arranged marriages or that they had somehow failed to arrange the perfect one because they left me to my own devices during that last year at AUB. I applied to and was accepted in a number of programs (although my current academic home, the University of Michigan rejected me summarily!). But the big hurdle was money. Even if my parents could afford it, there was no way to move money out of Syria and get it to the United States. The only positions available in these graduate programs were teaching assistantships, and they were not being offered to some foreign candidate, sight unseen. So, I resigned myself to staying in Beirut, working for a year to save enough money to go to the United States, and to convincing one of the programs to give me an assistantship.

It was July 1968, toward the end of my master's thesis, when I opened my mailbox at AUB and found a letter from the University of Iowa, where I had been accepted with no financial support. It told me that one of the graduate students slated for a teaching assistantship had been drafted to Vietnam, and they were willing to offer me that position. The surge of joy I felt quickly died when I realized that the letter had been dated weeks before but sent with insufficient postage to be sent first class. I immediately placed a call to Iowa and almost fainted with relief when they told me the position was still available!

Suddenly, it was a mad scramble. Rather than job hunting, I needed to complete my thesis, pack my bags, and get myself to the United States! But a passport with an "exit visa" from Syria allowing me to go to the United States presented yet another hurdle. Syria had cut off its relationship with the United States, and if I ever wanted to return home, I needed legitimate papers. Once again, I found myself in Damascus, despairing from ever achieving my dream. And once again, my parents came through. My father used his connections and secured a visa for me at a time when no one else was getting one. Armed with a thesis in need of revisions, a suitcase of clothes that were woefully inadequate for life in Iowa, and $200 in cash, I tearfully said goodbye to my family and began my journey to the United States. My friends were taking bets on how long I would last—I believe six months was the longest period anyone bet on.

Stan

University and Graduate School (1961–1969)

My undergraduate college years were generally unremarkable with the exception of an academic "vacation" when I drifted for a while and two courses that revived my interest and led me to my fascination with the brain.

Early in my college years, I felt bored and uninterested in academic life. But I became significantly more engaged after a course on basic psychology by Professor Irving Tucker (I later learned that he subsequently moved to Beirut and taught Huda, half a world away!). The second course was one on behavioral analysis by a PhD student of B. F. Skinner's. Both courses deeply interested me but felt, in the final analysis, incomplete. Neither presented much about the brain itself, except for one slide that Tucker showed from the Swedish histochemical group demonstrating catecholamine pathways in the brain—now that fascinated me! In retrospect, my interest in machines and fundamental mechanics was the key to my desire to migrate from pure psychology to neurobiology. It took some years for me to recognize that my fascination was not just in behavior or psychopathology but in the underlying physical nature of the brain and how it functions.

During my senior year in college, I applied to several graduate schools, based on Tucker's suggestions. I was admitted to the psychology department at the University of Iowa. It was almost as "tough" a place as my high school. Iowa was a bastion of behaviorism and stimulus-response theory, very much in competition with the more cognitive/social modeling style on the West Coast. The focus at Iowa was on behavior with the brain as an unknowable "black box." It was a driven, tense department, although many of the faculty members were pleasant. My personal interest turned toward clinical-experimental pathology, mainly schizophrenia, which I viewed as the most severe of the major mental illnesses. My perspective was shaped by my faculty mentor, Dr. Robert Callahan—a bright and charismatic psychologist who had served as a World War II navigator and bombardier. Somehow, I hoped to learn more about the brain by focusing on this illness.

During the third year of graduate school, clinical psychology students typically took a full-year clinical internship. Mine was at the Palo Alto Veterans Administration Hospital. The residents and faculty were from the department of psychiatry at Stanford's school of medicine. It was arguably the most prized internship in the nation, and I was overjoyed. I loved the San Francisco Bay area. That internship was spectacular and represented a major influence on my life and career. The ward had about 30 inpatients, an excellent staff, and superb Stanford psychiatry residents. I worked with one resident (Warren Crick) who countersigned my orders—resulting in a "Watson-Crick" duo, much to the amusement of everyone. This unit was for acute and subchronic cases. Outside of severely disturbed patients on a locked ward, we took the worst cases. The range of illness was huge, covering the full spectrum of psychopathology. We saw overt psychoses, posttraumatic stress disorder (PTSD), psychiatric depression, panic disorders, and sociopathic behavior. The patients ranged from World War II vets to Vietnam vets, Special Forces, bikers, mathematicians, and doctors. All in all, it was a very rich, yearlong experience, one that stood me in good stead for all my subsequent clinical work.

By the end of my psychology internship, I realized that I wanted to focus on the biology of severe mental illness. I considered postdoctoral training as a psychologist but decided that, with a bit more time, I could become a psychiatrist. Upon returning to the University of Iowa and informing the members of the department of my decision, many seemed upset and angry about my choice. I explained that I was not abandoning my PhD but continuing and expanding it. It was a fascinating view of how closely related fields viewed each other with suspicion.

A few days after my return to Iowa City, I met a delightful young woman. She was sitting at the desk of my former girlfriend, and somehow I immediately knew that Huda and I were a good match. She was bright, humorous, and could compete with the best. That last year of graduate school was complex for me—beyond my relationship with Huda, I was completing my dissertation work, making plans for the following year, and helping my parents recover from the strongest Gulf hurricane ever seen. My PhD thesis on sensory processing in severe schizophrenic patients (Watson, 1969; 1974) proved interesting. My studies focused on the response of acute versus chronic schizophrenia under quite demanding and stressful stimuli, and the impact on the acutely ill subjects was clearly overwhelming. It convinced me that acutely psychotic patients were quite vulnerable to stress, but it clearly lacked a true biological approach. I was ready to move on.

Huda

A Year in Iowa (1968–1969)—Meeting Stan

As Ozark Airlines landed in Cedar Rapids, Iowa, I was revising my expectations about seeing skyscrapers. In all my American dreams, I had imagined New York, or the beaches of southern California, not the American Midwest. In the few days before school started, I felt completely alone in the world. Telephone calls to my family were very difficult, and I knew no one. The differences in culture were so much greater than I had expected. But as the term unfolded, I began to meet other graduate students. One of them was Stan Watson, who would become my husband and lifelong collaborator.

Stan walked into the office that I shared with two other female graduate students, greeted them, and asked me who I was. I was already leery of answering that question—giving my foreign name only to be asked where I came from, with Syria typically eliciting a blank stare. So, I decided to get it out of the way all at once and said: "I'm Huda Akil, and I'm from Syria. But I don't imagine you know where that is." "Sure I know," he said, "You've been winning a few wars lately!" He was sarcastically referring to the Syrian losses during the Six-Day War, and I was incensed. He chuckled, told me I was sitting at the desk of his ex-girlfriend, asked me if I had any idea how cold it would get in the winter, explained that he had

spent the previous year doing a clinical psychology internship at Stanford and had plans to go to Lake Tahoe on a ski vacation over Christmas break (he already had the ticket in early September), and sauntered out. I was fuming at his attitude—every other American I had met seemed so gentle and friendly! One of my office mates told me not to mind him—after all, he was from New Orleans and rode Harleys. She also warned me not to go out with him, and I explained that I had no interest, and was already dating a Lebanese physician, a friend of a friend, who was doing his residency at the university hospital. And yet, within a few weeks, Stan and I had progressed to a serious relationship that would survive three years of separation leading to a long-lived marriage.

A Year in Iowa (1968–1969): Lab Work and Local Politics

At the University of Iowa, I worked with a young professor and recent PhD from the University of Michigan, Dr. Stephen Fox, who was interested in studying electrophysiological mechanisms of learning in cats and humans. Steve was extremely creative. Instead of searching for neural correlates of learning, he was using operant conditioning to directly alter the late components of sensory evoked potentials and to determine the neurophysiological correlates of this learning within the area and in other components of the circuit. During my stay, the lab published two papers in *Science* demonstrating that such conditioning at the electrophysiological level was possible in cats and in humans (Fox and Rudell, 1968; Rosenfeld et al., 1969). So, in my first exposure to animal research, I began to learn electrophysiology, and I perfected the intricate surgery that introduced multiple recording electrodes aimed at various cortical and subcortical sites, along with a large cannula lodged into the cat's palate to allow instantaneous delivery of the milk reward. The evoked responses were being computed online (using the then state-of-the-art PDP8), and the late components of the response were selected for shaping. When the criterion for a learned change was met, the system triggered delivery of the milk—a remarkable feat considering the computational capacity available at the time. Alan Rudell was the programming genius behind the operation, but there was a group of people (e.g., Chris Kaneko, Bob Norman) who loved to fiddle with computers and electrophysiology rigs and discuss technicalities for hours. I was in awe.

My Iowa year also introduced me to more biochemical and pharmacological aspects of neuroscience—particularly through an excellent course taught by the late John Harvey (who later became chair of pharmacology at Drexel University). John was the first person to ask me what I hoped to do with my graduate training. He suggested that I might wish to incorporate pharmacological approaches into my research because they would be more "portable" and less technically demanding should I wish to return to Syria and establish a research operation there.

By the spring term of that year, several faculty members, including the department chair, were strongly suggesting that I should reconsider my choice of laboratory. For reasons that I did not fully grasp, matters had become quite tense between Steve and several of the senior faculty. But I liked Steve and my colleagues in the lab, and I felt that I was being pressured not necessarily for my own best interest but as a pawn in a battle that I did not fully understand. Stan, who was completing his PhD that year, encouraged me to get out of the situation and leave Iowa, a possibility I broached with Steve. When Steve discovered that I had been accepted to UCLA the year before, but without financial support, he told me he had a plan. He explained to me that one of his students at the University of Michigan, John Liebeskind, was a junior faculty member at UCLA and he would contact him and vouch for me as a scientist and as a teacher. He made the call immediately, and John asked to speak with me—a sort of mini-interview. A few days later, John called back to say that my previous acceptance had been re-activated and I was being offered a teaching assistantship at UCLA! Although Steve was losing one of his new students, he had the satisfaction that the department would also lose that student. The pawn had landed on her feet!

A New Phase: The UCLA Years (1969–1972)

Long before my move to UCLA had been arranged, Stan and I had decided to break up at the end of the academic year because there was no future in a relationship between an American guy and a girl from Damascus. But he needed to complete some premed courses and had chosen to return to Stanford to accomplish that, and we decided to make the cross-country drive together. Stan helped me locate a miniscule studio apartment in Los Angeles, bought me a tiny little TV to go with it, and we said our goodbyes as he headed north to the Bay Area. Once again, I was alone in a new place, and this time, I had broken up with someone I loved.

Los Angeles was yet another culture shock, but John Liebeskind was the nicest man in the world and one of the best mentors anyone could dream of—warm, supportive, modest, generous, funny, and yet erudite and extremely smart. His lab in the C floor (i.e., three floors below ground) of Franz Hall became my new home and the true foundation of my scientific career. John had completed his PhD at the University of Michigan, working first in the laboratory of James Olds before switching to work with Steve Fox. He had completed a sabbatical in Paris with Mme. Denise Albe-Fessard, and his research was focused on central mechanisms of pain control (see Terman, 1999). He had a small group consisting exclusively of graduate students. But we were surrounded by the laboratories of several newly minted faculty members whose work spanned the emerging field of neuroscience—Frank Krasne's focus on neural plasticity using invertebrate

models, Donald Novin's work on mechanisms of motivation, Larry Butcher's interest in creating animal models of Parkinson's disease, and others. The atmosphere was both exciting and collegial, and several graduate students from across labs became my friends.

The UCLA Years (1969–1972): The Discovery of Stimulation-Produced Analgesia

It was at UCLA that I had my first taste of scientific discovery. As soon as I arrived and as part of my training, I was recruited to help Tom Wolfle, a veterinarian who was completing his PhD research under John. Tom was trying to model the central pain syndrome, a phenomenon that typically results from injury to the nervous system. The site of perception and processing of central pain was unknown, but Liebeskind had hypothesized that activation of some specific brainstem structures in the dorsal tegmentum would mimic this phenomenon. Tom was attempting to show that electrical stimulation of these brain sites was in fact noxious, and that rats would learn to escape it or even to actively avoid it. For comparison, he was alternating between the central stimulation and a peripheral pain stimulus (a shock to the paw) and testing escape behavior.

Tom had an easy manner, friendly ways, and a southern drawl. He was very patient with me and taught me a great deal about gently handling rats, programming electronics, and experimental design. But one afternoon, as we were holed up in the animal testing room, Tom seemed uncharacteristically frustrated. He pointed out to me that animals receiving stimulation in the brainstem periaqueductal gray matter (PAG) did not seem to learn to avoid the central stimulus as expected; moreover, they actually did not escape it for quite a while. Even stranger, they appeared to show greater tolerance of the peripheral pain after having been stimulated in the PAG. These anomalies were not helping Tom complete his thesis work as fast as he had hoped because he had a deadline for returning to his position in the armed forces. We decided that we needed to discuss the problem with John—this is when I learned one of my biggest lessons in science.

Instead of looking at it as a problem, John seemed fascinated. He wanted to see the phenomenon and to learn more about it. And he brought into the discussion David Mayer, a senior and very talented graduate student in the lab. We were all intrigued: Could stimulation of the PAG be doing the opposite of what was expected? The PAG was known to be a site that encoded distress and fear, and a very recent study had shown strong negative responses to stimulation of the brainstem in humans (Nashold et al., 1969). But our observations seemed to hold, and given Tom's tight timeline a division of labor ensued. Tom would test whether the PAG site might be rewarding and could support self-stimulation, whereas David Mayer would take the lead in testing the effect on pain responsiveness. Tom completed his work

promptly and published a paper (Wolfle et al., 1971) showing that although most areas of dorsal tegmentum supported escape and avoidance behavior, there were sites in the ventral PAG that did in fact support self-stimulation.

Meanwhile, Dave Mayer set out to ascertain whether stimulation could indeed block pain and if so, what other brain regions participated in that phenomenon. I was still in the "flunky" mode and was helping with various aspects of the study. We tried multiple pain tests, some classical (such as the tail flick test) and some improvised. We experimented with a wide range of current parameters to optimize the analgesia. We invented ways to prove that the animals were indeed analgesic but not otherwise impaired. For example, we would set the rats in a pan containing ice water. Within a few seconds, a control animal would escape, presumably due to the uncomfortable temperature. But if we turned on the PAG stimulation, the animal remained standing in the ice water, unperturbed. We could hand him a pellet of chow, and he would happily munch on it. As soon as we would turn off the current, he would escape. These and other demonstrations were completely convincing—we were producing a complete blockade of pain sensation while leaving other sensory and motor modalities intact. David, though only a few years older than me, was a great teacher as well as an imaginative and self-assured experimentalist. I learned from him the importance of extensive observation, of truly getting to know the animal's behavior with some granularity rather than simply performing pre-designed tests.

It was around that time that we learned that Reynolds had observed a similar phenomenon and reported that he could perform abdominal surgery with no anesthesia in three rats that were receiving stimulation near the lateral edge of the central gray (Reynolds, 1969). Consequently, my next lesson was to learn how one moves past being "scooped." John felt that the Reynolds study, although convincing, was rather limited in scope, and he thought that we would more fully characterize the phenomenon, map its extent and brain distribution, and ascertain whether or not it was associated with reward pathways (shades of James Olds!). So, David went on to do just that as part of his dissertation work. This body of work demonstrated that there is an extensive circuit that supports pain inhibition across the mesencephalon and diencephalon, particularly in periacqueductal and periventricular gray matter. It also showed partial overlap with reward pathways. Thus, some sites supported self-stimulation with no analgesia, others supported analgesia but not self-stimulation, and yet others supported both. We termed the phenomenon stimulation-produced analgesia (SPA). This was my first neuroscience publication (as third author after David Mayer and Tom Wolfle) and it was in *Science* (Mayer et al., 1971)!

Getting the paper accepted by *Science* was no mean feat, and once again John demonstrated his remarkable flexibility and scientific acumen. The paper was returned with positive reviews but insufficient enthusiasm for appearing in *Science* (presumably because of the Reynolds paper). I recall

sitting with David in John's office trying to decide how to better make our case. We were convinced that the depth of our characterization of the SPA phenomenon, the evidence that it was distinct from generalized sensory inhibition, the anatomical extent, and the relationship to reward pathways represented compelling evidence of a significant discovery. We wondered whether it would be useful to make the analogy to opiates, the only known class of drugs that could produce that degree of analgesia without being anesthetic. It was soon thereafter that John discussed the issue with Brooks Carder, a faculty member in the psychology department who was interested in substance abuse research. Brookes pointed John to an article in *Scientia Sinica* where the sites of action of morphine had been mapped in the rabbit, using microinjection (Tsou and Jang, 1964). Here was a new, key thought to add to the discussion—that the system we were describing was at least partially overlapping with the site of actions of morphine in the brain. This allowed us to make the case, albeit circumstantial, that brain stimulation and morphine activate a common system whose primary function was pain inhibition as opposed to pain appreciation: "We propose that brain stimulation attenuates pain by activating a neural substrate that functions normally in the blockage of pain" (Mayer et al., 1971). In the next phase, David and I went on to test this notion more directly.

The UCLA Years (1969–1972): James Olds' Influence, Once Again

While I was working with David on the demonstration of SPA, I had the chance to meet James Olds and his wife and scientific colleague, Marianne, and once again, unbeknownst to him, he reshaped my scientific path! Stan had had a roommate at Stanford who was now a graduate student at Caltech working with the Olds. He was inviting Jim and Marianne over for dinner at his house and asked me whether I would like to meet them. I, of course, eagerly accepted and told John Liebeskind about it. Because John had briefly worked with the Olds at Michigan before joining Steve Fox's lab, he wanted to make sure that I would get their input on our recent (yet unpublished) observations about electrical stimulation in the PAG and its effects on pain responses. I had the feeling that this went deeper than a desire for a scientific perspective, that John was hoping that they would be impressed by these recent discoveries.

The dinner consisted of only our host, the Olds, and myself, so I had plenty of chance to interact with them—and it felt like a parallel universe to be in the same room with the person who was the subject of my Beirut epiphany. They were both very friendly, with Marianne telling me about the challenges of being a female scientist and Jim seeming pleased by the depth of my knowledge about every paper he had published. When he asked me what I was working on and with whom, I excitedly told him about John and our analgesia story. He said that I should be careful to not over-interpret

the observations—that sometimes it is easy to create a functional lesion through brain stimulation. In other words, the animals may be analgesic because we were functionally lesioning the pain circuitry during stimulation! I explained that pain sensation returned within minutes of terminating the current, and he pointed out that functional lesions are not permanent.

The next day in John's office, as I relayed the conversation, I watched his initial disappointment turn into determination. He was sure the functional lesion hypothesis was wrong, but we needed to argue against it and maybe even prove the opposite. One can see the resulting argument being developed in the discussion of the (Mayer et al., 1971) *Science* paper—that this was an active mechanism not simply an interruption of pain perception. We were talking to Jim Olds in that paragraph! The analogy to morphine could have been problematic—after all morphine could work by creating a functional lesion in the processing of pain perception. However, as we state in the paper, "the integrity of certain neurotransmitter systems appears necessary for morphine to exert its analgesic effect" (Way and Shen, 1971).

The UCLA Years (1969–1972): Dissertation Work

The process of writing the discussion of the Mayer et al. paper, in order to get it accepted into *Science* and to implicitly respond to Olds' alternative interpretation, framed the rest of David's dissertation and defined mine. Before that dinner, I was supposed to identify brain sites that caused self-stimulation only when the animal was in pain but not under non-noxious conditions. However, following the discussion with Olds, it was more urgent to prove the true presence and active nature of the pain inhibitory circuit.

Thus, my thesis work had two goals: (a) to test the hypothesis that *brain stimulation was indeed an active mechanism* by demonstrating that it required intact neurotransmission in order to be effective—that is, that blockade of certain neurotransmitter systems would prevent SPA, their replenishment would restore SPA, and their basal activation would enhance it. We reasoned that if central gray stimulation were merely producing a functional lesion, then adding to the lesion by blocking various neurotransmitters would not prevent the analgesia; (b) to test the hypothesis that *SPA activates the same circuit that morphine activates*, by asking whether the same neurotransmitters that were implicated in morphine analgesia (e.g., serotonin) were also implicated in SPA. We also decided to use direct blockers of the action of morphine, in particular a drug that had been used by Tsou and Jang (1964), nalorphine. Meanwhile, David would look at the possibility that tolerance to morphine would alter the efficacy of SPA, in order to show that the two means of activating the system would exhibit cross-tolerance.

At that point, we were not specifically thinking about the molecular mechanism of morphine action or the existence of opiate receptors. Although opiate pharmacologists had suggested the existence of such

receptors (Goldstein, Lowney, and Pal, 1971), and a race for demonstrating their presence in the brain was underway, this was beyond our ken. To us, morphine and other opiates were somehow able to activate a neural circuit that can block pain by releasing known neurotransmitters along its neural components, and we were accomplishing the same effect with direct electrical stimulation of that circuit. But which neurotransmitters should we focus on?

We focused on monaminergic neurotransmitters for several reasons. We were stimulating close to the dorsal raphe, a major cell group in the brainstem that synthesizes serotonin, so serotonin was clearly one of our targets especially because it had been implicated in morphine analgesia (Samanin et al., 1970; Samanin and Valzelli, 1971). We were very aware of the work of the Swedish histochemists, especially because our lab neighbor, Dr. Larry Butcher, had learned monoamine histofluorescence in Sweden and was establishing the Falck-Hillarp technique at UCLA. Others had implicated noradrenergic mechanisms in reward and because some of our sites supported self-stimulation that was a reasonable target. Finally, dopamine was gaining increasing attention not only for motor control but in reward pathways (Ungerstedt, 1971).

Indeed, this work demonstrated convincingly the importance of monoaminergic neurotransmitters in SPA (Akil and Mayer, 1972). The main paper that resulted from this work concluded: "Dopamine and serotonin appear to facilitate SPA, whereas noradrenaline appears to inhibit it" (Akil and Liebeskind, 1975). We discussed the results in relation to studies by others on the site and mechanism of morphine's analgesic action and noted striking parallels between SPA and morphine analgesia. This body of work was used to support "the existence of a common pain-inhibitory system in the brain activated by morphine and by focal electrical stimulation" (Akil, Mayer, and Liebeskind, 1972, 1975; Akil and Mayer, 1972).

The UCLA Years (1969–1972): The First Physiological Evidence for Endogenous Opiates (Endorphins)

One of the pharmacological tools I had planned to use to address the analogy between SPA and morphine was nalorphine, a morphine analogue used by Tsou and Jang (1964) to block morphine analgesia. However, the drug proved problematic to use in our paradigm. When I gave nalorphine, it produced some analgesia in its own right, in the absence of brain stimulation. However, this effect would wear off, and I was able to find a time window where nalorphine no longer had an effect on the baseline pain responsiveness but was able to partially block stimulation analgesia. However, the timing was tricky and the results variable. This was rather baffling, until a chance meeting with an opiate pharmacologist helped me immensely!

John Liebeskind had planned to attend the 1971 Winter Conference on Brain Research in Colorado but changed his mind and sent me in his stead.

Walking around the meeting wearing John's nametag was an immediate icebreaker. One evening, at the bar where everyone gathered after meeting and skiing, a gentleman walked up to me and asked me with an impish smile if my name was really John. His name was E. Leong Way (Eddie Way). I had no idea that I was talking to the chair of pharmacology at University of California, San Francisco (UCSF), a world expert in opiate pharmacology! Around us, it was loud and people were drinking and dancing, but somehow I managed to tell Eddie about my work and especially my bewilderment over the results with nalorphine. He said: "My dear, you're using the wrong drug! Nalorphine is a bear—it's a mixed agonist-antagonist at the opiate receptor. You should use naloxone, it's a pure antagonist." Then he laughed and said, "You'll probably get nothing though—giving naloxone is like giving water if the animal doesn't have an opiate on board! But if they're addicted, then watch out!" I was still processing all that when he added: "Get yourself an opiate pharmacologist on that dissertation committee" and then he asked me to dance! So I did as he suggested: I danced, I got a pharmacologist on my committee, and I used naloxone in my study.

In fact, I returned to Los Angeles and immersed myself in reading about opiates and opiate antagonists. I learned that, indeed, a pure opiate antagonist is expected to be inactive and works primarily by blocking the actions of opiate agonists. This did not augur well for my study. By then, it was early 1972, John was on sabbatical in Paris, and David was planning to join him there. I was on my own. But I had seen signs of blockade of SPA by nalorphine, albeit ephemeral. So, in spite of the literature and Eddie Way's warning, I decided to trust my instincts and give the study a try. I obtained some naloxone and started playing around with doses and timing of administration to learn how it works. Indeed, it had no effect on the baseline pain response in several tests but was an excellent blocker of morphine analgesia. I was finally ready to test its effect against stimulation-produced analgesia.

I implanted my animals and tested them for SPA. By then, I was adept at recognizing a "good animal," and I selected those with pure pain inhibition with no side effects of the stimulation (which meant hitting the ventral, rather than dorsal central gray). It was late in the evening, and I was alone in the basement of the psychology department, so I thought I would sneak a peek and test one animal. I gingerly tried my first dose of naloxone. And it worked! There was no effect of the drug on baseline pain responsiveness but a significant reduction in the analgetic impact of the stimulation. I decided to run another animal, and then another. I ran the controls with saline injections. I let the effects of naloxone wear off (because it is short-acting) and could see that the analgesia would return in the same animals that had previously lost it under naloxone's influence. I stayed in the lab all night and through the early morning hours and completed the entire study! I felt a huge sense of excitement. I somehow knew this was important, even though I could not have explained why.

I immediately shared the results with David and sent them to John in Paris. He too seemed very excited. Shortly thereafter, he told me that he wanted to communicate the results as soon as possible through Dr. Fessard to the *Comptes Rendus de l'Académie des Sciences*, the French equivalent of the *Proceedings of the National Academy of Sciences* in the United States. The paper appeared (Akil et al., 1972) and included the study from that frenzied night of work. However, it is fair to say the opiate field did not take particular note of it.

By June 1972, I had wrapped up my experimental work for my dissertation and was getting ready for my final defense. I was getting increasingly interested in learning about pharmacology and the mechanism of actions of drugs on the brain, and because the Fifth International Congress of Pharmacology was taking place in San Francisco, I asked John to allow me to present our work there. This was to be my first public talk and although my submitted title was "Serotonergic Mechanisms Underlying Stimulation-Produced Analgesia," I decided to use my allotted 10 minutes to summarize all my work—the SPA phenomenon, the hypothesis that it was a distinct system in the brain, the evidence for its active nature based on the monoamine studies, and its analogy to morphine based on the naloxone study. The session, focused on pain and opiates, was packed and I was beyond nervous.

At the end of my talk, a scruffy looking man walked up to the microphone in the middle of the aisle and asked belligerently: "Did I hear you right? Did you say you could stop pain?" I was baffled by the tone but decided he was objecting to my terminology so quickly apologized for using the word "pain" when I should have said "nociception" because the animals cannot report on their pain and it is inferred. My response did not have the expected effect of quieting the objection. Instead, my questioner seemed more agitated and started shaking his fist at me, yelling: "Only God can stop our pain! Do you think you are God?! You're going to burn in hell!" I stood there stunned as two security guards pulled the man out of the room while he was still yelling and shaking his fist at me. I later learned that it was a mentally ill man who had wandered in from the street, and he had asked the first scientific question I ever received in a public talk!

I was still on the podium in a state of shock when a short, elderly gentleman with thick round glasses and a heavy German accent stood right in the front to ask the next question. He congratulated me on the work and said that the most remarkable finding was the naloxone effect—were we surprised by it? I said we realized that it was supposed to be an inert antagonist, but naloxone seemed to be blocking *something that we were releasing through the electrical stimulation*. I had never quite phrased it that way, but he nodded his head and seemed to agree with that notion. His next question was even more surprising: "So, are you currently purifying the chemical that you are releasing with your brain stimulation?" I said no, we were not (and wondered silently how one would go about doing such a thing!). He thanked

me politely, and I went back to my seat next to Stan and felt everyone staring at me. The session ended shortly thereafter, and a man in front of me turned around, introduced himself as Ed Domino from the University of Michigan (again!) and said: "Good for you for confounding everyone!"

After the session many people walked up to me to discuss my talk—equal part interest in the results I had presented and in the homeless man episode. I gathered from these interactions that it was Hans Kosterlitz, a giant in the field, who had asked the second question about naloxone. I also came to understand that he, along with Avram Goldstein and others, had concluded (based on the body of pharmacological evidence) that opiates must work via their own unique receptors. But why would the mammalian brain have receptors for opiates unless it produced an opiate of its own? They reasoned that an endogenous opiate system must exist. But if it did, then one should detect physiological or behavioral consequences of preventing its actions (e.g., when blocking it with naloxone). Yet all previous attempts at showing an effect of naloxone in the absence of an exogenous opiate had failed. I had just handed them the first physiological evidence of such an effect of naloxone, and by implication, of an endogenous opiate system! I had even provided an explanation for why it worked for us and not in other studies: You had to first activate the system, as we did with electrical stimulation.

It was terrifying!

Stan

At Stanford—A Transition Year (1969–1970)

I was very fortunate to be accepted in the laboratory of Ernest (Jack) Hilgard at Stanford. This was not a formal postdoctoral position but a kindness on his part, to allow me to work with him in the morning and retake some required courses in the afternoon. Many states insist that the premed science requirements be taken within five years of applying to medical school, so I retook undergraduate chemistry and biology at Stanford. I then worked from 4 p.m. to midnight at the Psychiatric Emergency Room (ER) in the San Mateo County Hospital, located between San Francisco and Palo Alto. My job was to see ER patients and work with the psychiatrists moonlighting there—all psychoanalysts from Mt. Zion in San Francisco. Having been trained at Iowa, I had a limited perspective on the psychoanalytic tradition. However, after a few months of working with these psychoanalysts, I came away really impressed with their very sharp skills in reading people, sizing up the situation no matter how complex, and in dealing most effectively with a wide range of patients on an emergency basis.

The patient population was even more varied and complex than the one I had seen on the inpatient Veterans Administration (VA) ward. On an average night, I would see about 10 to 15 patients often with their families,

friends, or the San Mateo police and occasionally with federal agents. The range of cases typified the San Francisco Bay Area at the peak of the Haight-Ashbury boom. Beyond the wide range of severe mental illnesses, we saw a huge number of drug patients including those on LSD, and numerous suicide attempts including by a couple of 10-year-old children. Patients included organized crime members, outlaw bikers, and children living in the woods that had been exposed to any and all the crude drugs that the drug camps could make. The stories were tragic and incredible, and the experience memorable and unique. This period, coupled with my earlier VA internship, taught me a great deal about the political, social, governmental, and practical aspects of the mental health system in California. When I returned as a psychiatry resident, I had little to fear in terms of learning the ropes of the system.

At Tulane Medical School (1969–1974)

After that year in California, I was accepted into medical school at Tulane University, in my hometown of New Orleans. I had forgotten how complex and socially inbred that city was in those days. If you were a doctor or medical student, you received amazing support and forgiveness from everyone from the mayor to the cop on the beat. Louisiana's legal system is based on the Napoleonic Code, not British Common Law, and under this codified legal system there were four seats of power: the mayor, the district attorney, the chief of police, and the coroner. The latter was a very powerful representative of "the crown" and had many responsibilities across civil society. If you were in medicine, it meant that he was your ally and a very powerful one at that. All of this was laid out to us on the first day of medical school. "If you are in trouble, just call us" and they meant it. I was amazed, baffled, and yet felt right at home!

The academic side of medical school was straightforward, with five to six hours a day of lectures. But the clinical aspect was another matter altogether. Charity Hospital was a free medical system put in place by the infamous Huey Long, former governor of Louisiana, for the poor population of the state. It had 2,800 beds, few nurses or attending doctors, and served 80 percent of the New Orleans population. It was the closest thing the United States had to the medical system seen in undeveloped nations. All medical matters were run by residents and fellows, and to some extent, by the medical students. It was a colorful, dynamic, primitive hospital. No medical experience since then has come close to Charity in complexity and in demonstrating the raw face of human illness.

During my first year of medical school, I had little time for anything but the standard curriculum, except for one elective—a three-hour per week course. For that, I chose a class on human anatomy, with a special focus on neural circuits, taught by a British surgeon/anatomist by the name of

Jeff Ellison. I can see in retrospect that Jeff was decades ahead of the field. His area of emphasis was understanding the cholinergic and monoaminergic systems, and especially developing better methods for studying them. After that course, I asked to work with him, and spent all of my available time—nights, weekends, and holidays—setting up the monoamine fluorescent method, known as the Falck-Hillarp method (Falck, 1962; Falck et al., 1962). It was a tedious yet beautiful method. It was amazing to watch the fluorescent green signal of the catecholaminergic system and the yellow signal of the serotonergic system as they came to life. I mapped the cells and fibers of these monoaminergic systems throughout the brain, replicating the work of the Swedish histochemists—Fuxe, Hokfelt, Dahlstrom, and more than 20 other colleagues who pioneered that area of neuroscience (Agnati et al., 2010; Dahlstrom, 2010; Fuxe et al., 2010; Granholm et al., 2010; Hökfelt, 2010; Perez de la Mora et al., 2010). It was a thrill to finally have a glimpse of the brain's operational systems.

But the Falck-Hillarp method was too unreliable for a humid environment like New Orleans. The freeze-dried blocks of rat brain rapidly absorbed water and the monoamine fluors were lost. Anders Bjorklund had proposed the use of glyoxylic acid as a substitute for formaldehyde in the freeze-dried tissue. But I felt that the need for freeze-drying the tissue to prevent monoamines from diffusing was a dogma of the histofluorescence method that was worth testing. Maybe there were alternative strategies to prevent diffusion. So, I decided to modify the technique and devised a perfusion technique using glyoxylic acid, rather than freeze-drying it (Watson and Barchas, 1975; Watson and Ellison, 1976; Watson et al., 1977a). It was a frustrating period, wrestling with a very complex method while being a medical student and working part time and knowing that I was trying to compete with world-class scientists. It took two years of playing with the conditions, but it finally worked! Somehow, maybe due to ignorance on my part, I never really doubted that it would. And in spite of the difficulties along the way, I enjoyed the technical challenge and the process of making it succeed. I was able to obtain a much brighter signal, and it was a bit longer-lived. It is important to recall that these were endogenous fluors that resulted from a ring closure with one or two carbon donors (Fuxe et al., 2010). The catecholamine fluors lasted about 1.5 minutes and the serotonin signal was visible for only 10 seconds under a fluorescent scope before it disappeared. Once it faded it was gone forever! Worse, I only had ASA 200 speed color film. Once I found the cells or fibers and focused, I only had one shot at producing an image! Thus, extending the time with glyoxylic acid felt like an important advantage. When I presented this new method at the 1975 International Union of Pharmacology (IUPHAR) meeting in Helsinki, Tomas Hökfelt and Kjell Fuxe came to my poster. They were pleasant to me, but I was very intimidated because of their fame and because they spoke to each other for at least a half an hour in Swedish! I was left wondering what they thought about my method.

Years later, when I mentioned this to Tomas, he was surprised and amused. He had forgotten the entire episode.

In spite of these challenges, this experience crystallized my sense of excitement about the importance of understanding neural circuitry and of linking neuroanatomy to the function of specific neurotransmitter molecules—in this case, the monoamines. It also gave me a direct sense of the importance of advancing technology if we are to increase our understanding of brain structure and function.

The rest of my "free" time, while in medical school, was spent seeing patients as a licensed clinical psychologist and in helping my father set up a mental health system in rural Alabama. I also spent holidays and one summer at UCLA with Huda and worked at the Neuropsychiatric Institute at UCLA as a "fill-in" psychologist.

The summer between my first and second years of medical school was especially memorable. I spent it working for Dr. David Hamburg, then the chair of the psychiatry department at Stanford. David was a major influence on my career in psychiatry, and to this day, he remains a great colleague and mentor to Huda and to me. During that summer, he had me develop protocols for UNESCO for studying adolescent gang violence. But he also taught me a great deal about psychiatry in general as a field and as a social structure. And he would later facilitate my admission to the residency program at Stanford.

In the fall of 1972, Huda moved to New Orleans, and I finally convinced her to marry me. During 1972–1973, she worked as an instructor in neurosurgery studying the endogenous opioid systems, leading up to a series of amazing papers on humans. Near the end of medical school, I was admitted to Stanford for my residency in psychiatry and was allowed to take my last medical school rotation in Boston in the laboratory of Dr. Seymour Kety, the leading figure in the biology of severe mental illness at the time. The medical part of my internship (six months) took place in the Pacific Presbyterian Medical Center in San Francisco, which offered a striking contrast to Charity Hospital in terms of its setting and the condition of the patients.

Huda

A Year in New Orleans: More Naloxone Studies (1972–1973)

I defended my doctoral thesis in the fall of 1972 and moved to New Orleans, where Stan was a medical student at Tulane University. Stan and I had known each other for four years by then but had been in different cities for three of those years. We planned our wedding for December 1972. I needed to find a lab to work in for that one year, while Stan would apply for a residency in psychiatry and I would seek a longer-term postdoctoral position at the same university. So, Stan investigated options for me at Tulane and

spoke to a number of researchers on my behalf. He eventually linked me up with a neurosurgeon, Dr. Donald Richardson, who was interested in pain research, had in fact read some of our brain stimulation papers, and seemed very willing to invite me for an interview.

Don and I hit it off immediately. A dashing, Southern gentleman who was talented and fearless, he explained to me that he was currently performing experimental surgery to relieve human patients from intractable pain by lesioning certain areas of the thalamus. But he thought SPA might represent an alternate approach because lesions were far from ideal and often produced irreversible side effects. Don did not have a research grant or a lab group, but he did have a laboratory that he supported out of his clinical practice, and where he took time from his clinical work to perform electrophysiological studies on cats—a gentleman scientist! He obtained a title for me as an instructor in neurosurgery at Tulane, used his personal funds to pay me—with the expectation that I would continue to conduct animal work but also bring my knowledge of SPA to his human patients. Here was my opportunity for "translational research"—a notion the importance of which would emerge decades later, but which I immediately found fascinating.

The first order of business was to set up the laboratory for testing SPA in rodents as well as cats. I had several follow-up ideas relating to my dissertation work that I needed to complete before publishing it. Moreover, the meeting in San Francisco had made me realize the importance of the naloxone study. Equally compelling was a telephone call from Dr. Avram Goldstein, who had somehow traced me to the lab at Tulane. He told me that he had missed the session in San Francisco but had heard about my presentation and wanted to ask me some questions. He proceeded to quiz me for almost an hour about every detail of the naloxone experiment—the dosages of the drug I had tried, the timing of administration and recovery, and possible alternative explanations. I knew him to be a major figure in the field of pharmacology, especially in the area of opiates, and I was quaking as I was answering his queries. I decided I needed to replicate the effects of the opiate antagonist on SPA in a different setting and across additional conditions. I was now operating solo, although I would occasionally chat with John Liebeskind on the phone about my progress. I was able to replicate my observations from the UCLA study, but once again, the reversal was incomplete—in other words naloxone diminished the magnitude of the analgesia induced by electrical stimulation but did not abolish it completely. I attempted higher doses of naloxone, but they did not seem any more effective. I also identified current parameters that produced partial analgesia, to ensure that the stimulation was not supramaximal. But even then, the naloxone reversal was partial. Therefore, in our eventual publication of this work (Akil et al., 1976), we argued for two important ideas: (a) The brain stimulation was releasing a "morphine-like substance" from the central gray that the naloxone was blocking; and (b) because the blockade was partial,

we were *also* either bypassing the morphine-like synapse and activating the downstream mechanisms, or there were other endogenous mechanisms of analgesia that were non-opiate in nature.

A Year in New Orleans: Stimulation-Produced Analgesia in Humans (1972–1973)

Don Richardson was gracious enough to let me complete my own animal work, but we were both eager to test the approach in humans. After all, as I had politely explained to my unusual questioner in San Francisco, we can never be certain about the disappearance of pain without some sort of explicit corroboration.

Our first approach was to test SPA in five patients who were suffering from intractable pain and were slated for a thalamic lesion (Richardson and Akil, 1977a). We would first introduce the electrode, aim it at the brainstem periaqueductal gray matter, and then ask whether we could find current parameters to produce pain relief. The first surgery was memorable. It was my first time in the operating room, and I was truly curious to see whether what had worked in rats and cats had a chance of working in humans. The patient was a gentleman who had lost his foot to diabetes and suffered from phantom limb pain. He was not only distressed by the pain itself but deeply embarrassed for having this sensation in a nonexistent limb. The patient was awake during the surgery, and we were able to question him about his pain in the operating room. Several sites and current parameters proved ineffective. But at one point, as we stimulated the central gray matter, he suddenly reported that the pain had floated away! Even his sensitivity to pinprick was altered, but the pain relief was also accompanied by a number of other sensations, some of them unpleasant. This was in fact the pattern with each of the patients we tested acutely: Pain relief was evident but autonomic symptoms accompanied the brain stimulation including nystagmus, nausea, vertigo, and a feeling of a "rising vapor." My task in the operating room was to manipulate the current parameters and adjust them to obtain maximal, side effect–free analgesia, but this proved almost impossible to achieve reliably in the periaqueductal gray.

However, my knowledge of David Mayer's mapping study led me to suggest testing more rostral, periventricular sites. Don designed the electrode path in such a way that we could stop the penetration at several points in the medial thalamic region and test for pain sensations and pain relief. We were excited to find that a site in the periventricular gray area between the nucleus parafascicularis and the third ventricle, at the level of the posterior commissure, produced significant, bilateral pain relief of acute and chronic pain, with minimal side effects. The patients reported a feeling of relaxation, and remarkably, one minute of stimulation could result in pain relief lasting up to an hour. Therefore, we chose this region as the target of

permanent implants to allow self-administration of brain stimulation for chronic pain control.

We carefully selected the next set of eight pain patients for chronic electrode implant with the hope that brain stimulation would suffice as the sole method for pain control (Richardson and Akil, 1977b). The surgery was conducted in two stages, on two separate days. On the first day, the electrode was introduced under local anesthesia and aimed at the previously identified periventricular site, and the patient was tested in the operating room for pain relief. The electrodes were connected to a temporary percutaneous lead to allow testing for pain relief in the hospital over a period of seven to 14 days. During the second stage of surgery, carried out under general anesthesia, the electrode was permanently connected to a Medtronics induction receiver and rectifying unit that was implanted over the pectoralis muscle. The patient could then use a pocket-sized Medtronics stimulator, connected to an inductance antenna, to transmit the pulse current when placed over the subcutaneous receiver.

We worked with each of the patients to ensure the best combination of stimulation parameters to ensure analgesia. We also remained in contact with them and tested them in the lab at regular intervals to track the effectiveness of the treatment on their chronic pain and on some acute lab tests of pain responsiveness (Richardson and Akil, 1977b). Seven out of the eight patients achieved good to excellent results and could obtain pain relief without classical pain medications. In some cases (n = 4), we relied on the antidepressant amitriptyline to augment the magnitude and/or duration of analgesia. This was a direct translation of my dissertation work on the role of monoamines in the pain inhibitory circuit, and we showed that the effect was immediate rather than the delayed timing of the antidepressant effect. A follow-up study of these and other patients (n = 30) showed that the effectiveness of deep brain stimulation as a primary means of achieving pain control was sustainable (Richardson and Akil, 1977c). Although deep brain stimulation is not a routine procedure for pain control, undoubtedly because of its invasiveness, its efficacy has been replicated across several medical centers. A relatively recent meta-analysis described results from 424 cases studied over a period of 20 years after we published our work and reported a success rate of approximately 80 percent for stimulation in the periventricular/periaqueductal gray areas (Bittar et al., 2005).

The experience of seeing our work in animals helping people with intractable pain was profoundly moving for me. My initial interest in neuroscience was entirely intellectual—a commitment to science for its own sake. But within a few years, I had witnessed personally the power of basic scientific discoveries to make a difference in the lives of people. Our patients would call to thank me because they could now pick up their kids for the first time in years or spend time with their family or have a good night's sleep. One patient was terminally ill with cancer and was grateful to be able to

spend his last few months of life with the ability to alleviate his own pain. Many continued to call me for several years after I left Tulane and moved to California. Science had gotten much more personal.

A Transition Year and a Memorable Meeting in Massachusetts, 1973–1974

During 1973–1974, we were in Boston. Stan was completing his senior year medical school rotation at Mass General, and I was writing up the work I had completed during my time at Tulane. I was also submitting proposals for awards (e.g., the Sloan Fellowship) that would be "portable" in order to increase my options at a postdoctoral position. We were once again facing the challenge of coordinating our careers, and because Stan was applying to residency programs, he had little control over the outcome.

It was David Hamburg who, once again, gave us wonderful advice when he pointed us to Jack Barchas as a potential mentor at Stanford. During our visit for Stan's residency interview, I met with numerous distinguished scientists, but it was immediately evident that the Barchas lab would be my first choice. Jack was warm and thoughtful, had a great perspective on basic and clinical neuroscience, and ran a very exciting, energetic, and broad research operation. So, we felt extremely fortunate when Stan was accepted into the Stanford residency and Jack offered me a position in his laboratory starting in the summer of 1974.

While in Boston, I met Dr. Steven Matthysse, a young faculty member at the McLean Hospital under Dr. Seymour Kety. One day, Steve informed me that he and Solomon (Sol) Snyder were involved in organizing a special meeting in the spring focused on opiate receptors. This would be sponsored by the Neuroscience Research Program (NRP) series that was headed by Dr. Francis O. Schmidt of MIT. Steve indicated that the program was very exclusive because only a small number of leaders in the field would be invited to attend. Small books resulted from these NRP meetings, but because the participants were not asked to write chapters, NRP used "scribes"—young scientists would be asked to take notes, go over the taped lectures, and come up with the first draft of the book. The speakers would then be able to edit the summaries written by the scribes. As one of the organizers, Steve invited me to serve as one of the four scribes. I was thrilled! Two of the other scribes were from Snyder's group and were to become major players in the field of neuropharmacology—Ian Creese and Gavril Pasternak.

The NRP meeting took place May 19–21 at a beautiful site in Brookline, Mass., called the Brandegee Estate. The wisteria bushes were blooming, all my heroes were in attendance, and I was in heaven. I met Solomon Snyder, Avram Goldstein, Floyd Bloom, Eric Simon, Lars Terenius, Leslie Iversen, Arnold Mandel, and several others, and most would become lifelong colleagues and some of them very good friends. I also met Candace Pert, the graduate student from Sol's lab who was the first author of the opiate

receptor paper. I reconnected with Eddie Leong Way and told him how much his input had influenced my research. My UCLA lab mate, David Mayer, now at the Medical College of Virginia, was representing our collective research at the meeting. Importantly, Hans Kosterlitz and his young colleague, John Hughes, were in attendance.

The atmosphere of the meeting was electric. The title was "Opiate Receptor Mechanisms" (Snyder and Matthysse, 1975), and the primary focus was intended to be on the recent exciting demonstration of opiate receptor binding in the brain—work independently published in 1973 by three separate groups led by Lars Terenius, Solomon Snyder, and Eric Simon (Terenius, 1973; Pert and Snyder, 1973; Simon et al., 1973). Indeed, there had been some very interesting developments about the nature of receptor binding and distinctive responses to ionic conditions that were beginning to hint at multiple conformations and types of opiate receptors (cf. Snyder and Matthysse, 1975). Moreover, from the outset, there were rumors that there would be revelations about an "endogenous ligand" for the opiate receptor.

I have a very strong visual memory of the presentation by John Hughes on May 20, 1974. But I am also in possession of the audiotapes from this NRC meeting and have since listened to his talk. Against the scraping of chairs, Hughes began with the statement: "There are noradrenergic, cholinergic serotonergic, and GABAergic neurotransmitters. I shall now complicate the picture by suggesting the existence of morphinergic transmission." He then proceeded to describe the use of the guinea pig ileum (a tool optimized by Hans Kosterlitz to characterize opiate receptor function) for purifying an endogenous "morphinergic" substance. He reported the extraction of material from the brainstem and the use of naloxone reversibility as a criterion for identifying the fractions that contain opiate-like material. The enriched fractions were successively purified and "sent to the chemist" for characterization. Hughes stated that they were proposing the name "enkephalin"—from *kephalē* (Greek) or cephalon, meaning from the "head." This is in analogy to adrenaline, which was purified from the adrenal gland. At the end of John's talk, there was a flurry of questions, especially about whether the material resembled an opiate alkaloid. Lars Terenius, who shortly thereafter spoke of early evidence for the existence of an endogenous opiate factor, said he believed it was a peptide (Terenius and Wahlstrom, 1974). I remember making note of his statement even though I was not completely sure what a peptide was. Leslie Iversen, who was the meeting discussant, stood up at the end and said in his summation that what we had heard "boggles the mind"—and proceeded to imagine the repercussions of such a discovery. It was indeed mind-boggling to me! Here was the material we were releasing with our electrical stimulation, our own natural opium! I literally was unable to sleep all night from the excitement. As I worked with the other scribes, the sense of anticipation was palpable—this was the start of a new era.

Huda and Stan: Stanford (1974–1978)

Thanks to the generosity of David Hamburg and Jack Barchas at Stanford, we had finally aligned our careers with positions that allowed us to continue our training and pursue our research interests—Stan as a research resident in the psychiatry department and Huda as a postdoctoral fellow in the Barchas lab. Nowadays, our son, Brendon, refers to Stanford as the "mothership" because of the number of times our family has gone back to it at critical points—including our daughter, Katie, who currently works there. Back in 1974, we made the trip from Boston to the West Coast in our trusty Volkswagen van, amid the gas crisis and with news of Patty Hearst on the radio. The first few months, we lived in San Francisco so that Stan could complete his internship at Pacific Presbyterian and Huda could start her training in neurochemistry—an entirely new area of research for her. We then moved to Palo Alto in June of 1974 and remained there until September 1978.

This was the start of a relationship with Jack Barchas that has proven to be one of the most important in our lives, scientifically and personally. Jack's mentorship, collaboration, and support have continued unabated for four decades. The Barchas lab was unlike any other we had encountered. Jack was still young, in his late thirties, but ran a large and diverse research operation with a sense of excitement and with state-of-the-art technology. His interests spanned the entire range of neuroscientific analysis, from *in vitro* studies of synaptic function to the pathophysiology of psychiatric illness. This intellectual and technical breadth was based on his implicit and explicit view that these different levels of discourse can and should be integrated to achieve a real understanding of brain function and dysfunction. The lab felt mature—dominated by postdoctoral fellows and senior technicians. It also included MD–PhDs such as the late Roland Ciaranello, who was starting his child psychiatry residency after returning from a fellowship with Julius Axelrod. Even then, Jack was very aware of psychiatry's need for a new brand of physician–scientists, and he consistently went out of his way to nurture their careers and to ensure that they received the strongest basic research training while honing their clinical and translational skills. But possibly the most remarkable aspect of Jack's research enterprise was the freedom he gave lab members to come up with their own research projects. Rather than assigning each person to a specific sub-project of an existing grant proposal, Jack challenged us to express our original scientific ideas, enunciate our questions, and to elaborate our plans. Once he was convinced about the value of the project, he would go out of his way to ensure that we had the tools, equipment, and support needed to make it a success. He encouraged our attendance at meetings to represent the lab and our involvement in grant writing and site visits. His fundamental respect for different perspectives and ideas and his unwavering commitment to developing

the careers of young scientists are values we have tried to emulate in our own research. And Jack seemed as excited as we were about the challenges offered by the new field of endogenous opiates!

A New Function of Endogenous Opiates—Coping During Stress

Our work in Jack's lab began shortly after that famous 1974 NRP conference. The book was not yet published, but news of the endogenous "morphine-like" factor was spreading. An obvious study for us was to prove that this system was indeed activated during stimulation-produced analgesia and to determine how it might mediate that analgesic function. That meant we needed to be able to measure it in specific brain regions of individual animals—no mean feat because we did not know its structure at the time, only its activity. During the NRP conference, it had become clear that one way to measure the morphine-like factor of Hughes was by using the opiate receptor binding assay. Indeed, the Snyder group was detecting and characterizing the endogenous opiate(s) by using brain extracts to compete with radioactive ligands at the opiate receptor (Pasternak and Snyder, 1975; Simantov and Snyder, 1976). So, it was critical to set up opiate receptor binding as a technique in the lab and to adapt the extraction method of Snyder's group to be able to analyze changes in levels of endogenous ligands before or after analgetic brain stimulation. Each one of these steps proved challenging, especially in the hands of a behaviorist (Huda) who had never held a pipette, much less set up new assays and biochemical extraction methods. The rest of the lab had expertise in monoamine assays and other neurochemical methods, and a fellow postdoc biochemist, Robert Patrick, was extremely helpful in getting us over these initial challenges.

A fundamental question in the field was: Why should the brain have its own endogenous opiate system, with a morphine-like substance and a receptor? Given his interest in addiction, Avram Goldstein, who was now our Stanford neighbor, suggested a role of endogenous opiates in eliciting the thrills of pleasure—for example, while listening to music (Goldstein, 1980). To us, an obvious physiological function of our own opiate was the control of pain—as demonstrated by the body of work with stimulation-produced analgesia. But aside from direct brain stimulation, what would trigger this anti-pain protective mechanism? Discussions with Jack and a graduate student in the lab, John Madden, led to the hypothesis that during stress, pain needs to be inhibited in order to allow the organism to fight or flee. We therefore set up the behavioral model and demonstrated that, indeed, stress was accompanied by significant pain inhibition, a phenomenon we termed "stress-induced analgesia." Similar to SPA, stress-induced analgesia is naloxone reversible. Using our newly minted assays, we were able to demonstrate that it was accompanied by significant changes in the

levels of the "opiate-like factor" in several brain regions (Madden et al., 1977). This work provided early evidence of one of the adaptive functions of the endogenous opiate system—pain inhibition to allow coping with other environmental demands. We should note that there is also a non-opioid form of stress-induced analgesia that was described by Liebeskind's group, who went on to contrast the opioid and non-opioid forms of pain inhibition during stress (Lewis et al., 1980).

The Endorphins—Endogenous Opiates Galore (1974–1976)

Through the remainder of 1974 and throughout 1975, an intense race was underway to identify the endogenous morphine-like factor (see North and Hughes, 2013). The Goldstein group was relying on the bioassay used by Hughes and Kosterlitz—the guinea pig ileum—to identify the nature of the ligand and its naloxone reversibility (Teschemacher et al., 1975), while Snyder's group was relying on the opiate receptor binding assay (Pasternak and Snyder, 1975). By late 1975, however, we heard that Hughes and his colleagues had indeed identified the chemical nature of their "enkephalin." We saw a preprint of the *Nature* paper (Hughes et al., 1975), which was published in December, and the big surprise was that there were two pentapeptides: tyrosine-glycine-gycine-phenylalanine-methionine, which was termed met-enkephalin, and tyrosine-glycine-gycine-phenylalanine-leucine, which was termed leu-enkephalin. We immediately swung into action—we finally had a chemical structure and needed to have these peptides in hand to test their impact on behavior and to raise antibodies for radioimmunoassays and for anatomical studies. We promptly located a local peptide synthesis company (Peninsula Labs) and ordered the synthesis of one gram of each of these peptides. Before the end-of-year holidays, when we called to inquire about the status of the synthesis, the company founder asked: "What is this that you ordered? Yours was the first request, but since then everyone in the world wants this stuff!" We were off to the races!

In their seminal paper, Hughes et al. (1975) pointed out that the sequence of met-enkephalin could be found within the sequence of a previously identified pituitary hormone called beta-lipotropin (β-LPH). It was known that smaller peptides were derived from the processing of larger peptides or proteins via enzymes that cleaved at specified sites. Given that all of met-enkephalin was embedded in β-LPH, it was reasonable for them to propose that β-LPH could be the precursor of met-enkephalin. That sequence identity triggered a series of questions and threw the field into a state of confusion that was only resolved through the use of anatomical approaches.

β-LPH had been isolated from the pituitary gland by C. H. Li at the University of California in San Francisco. Li, a prominent biochemist, who had purified and sequenced many of the major pituitary hormones, was searching for a molecule that might control obesity and had identified β-LPH

as having "fat mobilizing properties," hence, the name. But the enkephalins had been isolated from the brain and β-LPH was in the pituitary—what was their relationship? Some scientists suggested that the primary source of endogenous opioids was indeed the pituitary and that the smaller peptide products represented retrograde flow back to the brain. Another issue was the precursor for leu-enkephalin. Was there a β-LPH equivalent with leucine in position five that would serve that function? And were both forms of β-LPH present in the brain?

Meanwhile, a new opiate-like peptide was born. The laboratory of C. H. Li isolated a 31 amino acid peptide from camel pituitary extracts and determined its structure (Li and Chung, 1976). Remarkably, the structure was identical to the sequence of carboxyl-terminal 31 amino acids of ovine β-LPH. The authors noted that this "peptide possessed very low lipotropic activity but significant opiate activity." Indeed, Li, Loh, and their colleagues showed that it has remarkable analgesic activity that seemed to far exceed that of the enkephalins (Loh et al., 1976). Meanwhile, Roger Guillemin at the Salk Institute (who had received the Nobel Prize for his work on neuropeptides) was also identifying fragments of β-LPH and showing that they had opiate activity (Ling and Guillemin, 1976). At a meeting in 1974, Eric Simon had proposed the use of the term "endorphin" to indicate all the endogenous opiate molecules being discovered. This terminology was rapidly embraced, and C. H. Li termed his peptide β-endorphin because it was a fragment of β-LPH. Guillemin named the shorter fragment he had isolated α-endorphin. Another term for endogenous morphine-like or opiate-like molecules was adopted: opioids.

Once again, the discovery of β-endorphin threw the field into confusion, evident especially in discussions at scientific meetings. Because β-END appeared more potent and longer lasting in its behavioral effects relative to met-enkephalin, showing analgesia, cross-tolerance to morphine, as well as "catatonic" effects (Bloom et al., 1976; Loh et al., 1976: Tseng et al., 1976), some argued that it was the true endogenous opioid, while met-enkephalin was merely a breakdown product. Kosterlitz countered that long-lasting activity is not typical of classical neurotransmitters because they are typically rapidly degraded and suggested that β-endorphin might be a hormone (given its pituitary origin), whereas the enkephalins might be neurotransmitters (especially considering their neural origins).

Neuroanatomy to the Rescue!

A major thrust of our efforts in the Barchas lab was for Stan (who was carrying a full load as a psychiatry resident) to establish techniques for functional neuroanatomy, in order to allow us to study the neural circuitry implicated in pain control, including the monoamines and the endorphins. Jack Barchas secured the funds for the purchase of a cryostat and microscope

and provided Stan with a darkened room in order to set up this technology in the lab. Indeed, the first joint Watson-Akil publication married our two research experiences up to that point by demonstrating an overlap between the brain sites that support stimulation-produced analgesia and the dorsal periventricular catecholamine bundle (Watson et al., 1977b).

In the early 1970s, Boyd Hartman and colleagues published their method for immunocytochemical visualization of dopamine-β-hydroxylase, the final enzyme in the biosynthesis of nor-epinephrine (Hartman et al., 1972), and the Swedish histochemist Thomas Hökfelt and his colleagues pioneered the use of immunocytochemistry to describe the distribution of the monoamine synthetic enzymes and of neuropeptides including somatostatin and substance P (Goldstein et al., 1971; Hökfelt et al., 1974, and cf. Hökfelt, 2010). These and similar papers opened up the entire world of protein and peptide visualization in the nervous tissue. And remarkably, although biochemistry and peptide biology had been critical in the discovery of the endorphins, it was immunohistochemistry that provided the answers to key questions that were plaguing the field.

Jack Barchas was one of the organizers of a meeting that was held at Asilomar, Calif., in January 1976, soon after the description of the enkephalins by Hughes and colleagues. John Hughes was invited at the last moment and stayed at our home in Los Altos on the way to the meeting site. This was the start of many years of friendship with John. Tomas Hökfelt was also at the meeting and asked us to introduce him to John. Hökfelt approached Hughes about a possible collaboration centered on mapping the distribution of the enkephalins in the brain, but John had already made other collaborative arrangements. Instead, each of their labs set out to develop their own antibodies against the enkephalins. By early summer of 1976, while at a meeting in Aberdeen, Scotland, we learned that Hökfelt's group, in collaboration with Lars Terenius, had mapped leu-enkephalin in the brain (Elde et al., 1976). This was particularly important because it put to rest the idea that enkephalin was merely a breakdown artifact. Meanwhile, we raised our own antibodies to met-enkephalin (Sullivan et al., 1977) and used them in immunohistochemical studies to characterize its distribution in the brain—a pattern that appeared to resemble that of leu-enkephalin (Watson et al., 1977c).

But a key remaining question was the relationship of the enkephalins to β-LPH and β-endorphin. To this end, we requested a meeting with Dr. C. H. Li at UCSF, and he agreed to see the two of us. Being young scientists—a newly minted postdoc and a first-year resident—negotiating with Dr. Li was quite intimidating. A tall, regal, and reserved man, he clearly had his own agenda for us—for example, he wanted us to use behavioral techniques to show that β-END was important in memory and to develop a plasma radioimmunoassay that could be used in humans. But he also was interested in the mapping studies and finally agreed to provide us with the necessary antisera.

These antisera were great and terrible. Their working titers were quite high and thus gave a strong signal, but the antigens were purified from pituitary, which meant they were far from pristine. Unfortunately, protein purification in the mid-1970s was rather flawed in that many molecules of roughly the same size and charge were co-purified. For example, the β-LPH antibody also detected growth hormone, neurophysin, and prolactin. To handle this problem, it was necessary to pre-absorb the β-LPH antiserum with all of the aforementioned pituitary hormones in order to visualize the single population in the pituitary that expressed β-LPH (Watson et al., 1977d). Eventually, we raised our own antibodies against β-endorphin using synthetic peptide coupled to a carrier antigen.

A central question was whether β-LPH/β-END existed in brain. The idea that retrograde flow from the pituitary into the brain was responsible for the brain analgesia needed to be addressed, especially because it was espoused by some very distinguished scientists, including Nobel Prize winner Rosalyn Yalow. So, it was very exciting to visualize for the first time the presence of β-END in brain! Using immunohistochemistry, we identified a major cell group that expressed these peptides—the arcuate nucleus of the hypothalmus (Watson et al., 1978b). It was also quite revealing because the anatomy was so distinctly different from that of leu- and met-enkephalin and contrasting them became critical.

Two Distinct Opioid Circuitries in the Central Nervous System—or When Science Meets Everyday Life

By the fall of 1977, we had compelling evidence that there were two separate opioid systems in the brain—the two enkephalins that were widespread in their distributions and that appeared to overlap in their location and β-END, which had a single major cell group in the hypothalamus with long projections both rostral and caudal. (Our later studies showed a smaller β-END cell group in the brainstem nucleus of the tractus solitarius [Khachaturian et al., 1984]). Indeed, our *Nature* paper (Watson et al., 1978b) was entitled: "Evidence for two separate opiate peptide neuronal systems." This was remarkable news because it laid to rest many questions in the field while raising new ones. Yes, the enkephalins and β-END appeared to be bona fide neurotransmitters—not just breakdown products and a pituitary hormone; and no, it did not seem as if met-enkephalin derived from β-END. So, the synthetic origin of the enkephalins remained in question. We were aware at the time that Floyd Bloom at the Salk Institute was working on this same neuroanatomical question in collaboration with Roger Guillemin, and we were hoping that our findings were consistent with his—and indeed they were (Bloom et al., 1978).

Meanwhile, we were expecting our first child in early December. But an important meeting was looming large—that of the American College of

Neuropsychopharmacology (ACNP) taking place December 14–16, 1977, in San Juan, Puerto Rico, where a whole symposium was devoted to the latest developments in the opioid field. This was before our anatomical studies or Floyd's were published, so we decided that the meeting was important enough that Stan should try to attend it even if it was a few days after Huda's delivery. But that baby was not following our schedule and would not arrive for another two weeks, so Stan had to cancel his trip to the meeting. With Jack's encouragement, we decided to contact Floyd Bloom, whom we knew would be talking at the meeting and ask him whether he would be willing to show some of our slides on the anatomy of the two opioid systems. He generously agreed. In the midst of false labor cramps, we stayed up late preparing a set of slides for him to choose from, along with a cassette tape describing the experimental details for each of them. We remained in Palo Alto awaiting the baby's arrival and news from the meeting with baited breath. To our utter amazement, we heard that Floyd spent most of his talk presenting our slides, saying that he had similar data but that our images were more clear-cut. He explained the reason for Stan's absence and apparently the baby's delivery became a topic of frequent inquiry at the meeting! We were awed that, at a time of such heated competition, Floyd would be so incredibly generous and rise above the fray! In so doing, he set an example of scientific values that we have never forgotten. And his generative style appeared contagious. Not only did many people contact us with warm personal and scientific messages, but Avram Goldstein took extensive notes at the meeting, had them typed up, and sent them as a "baby present." Meanwhile, C. H. Li had wished us the best of luck on the delivery, urged us to have a son, and encouraged us to collect blood from mother, child, and umbilical cord to ascertain changes in levels of β-END during delivery! We followed his advice, which meant we had to first establish a plasma radioimmunassay for β-END. It also meant that Stan had to be collecting blood during the delivery! We subsequently published a paper showing that β-END was in fact increased during pregnancy and labor, although its functions in the circulation remain unknown (Akil et al., 1979).

Endorphins and Stimulation-Produced Analgesia—Direct Evidence

With radioimmunoassays in hand, we finally had the opportunity to directly test the hypothesis that was put forward at UCLA—that analgetic brain stimulation activates an endogenous pain inhibitory system and releases opiate-like factors. We collaborated with Don Richardson at Tulane University who was continuing to use the deep brain stimulation approach in human subjects, and he collected cerebrospinal fluid from the third ventricles of these patients during the course of the surgery.

We obtained evidence of enhanced levels of both enkephalin and β-END following electrical stimulation (Akil et al., 1978a, 1978b). The elevation of β-END was more sustained, and given its highly analgesic effects when exogenously administered and the reversal of the analgesia by naloxone including in humans, it was reasonable to conclude that it was a critical player in SPA. This was supported by the anatomy of β-END, whereby the descending projection from the arcuate cell group runs along the medial periventricular sites coincident with those that elicit analgesia (and in the opposite direction to the ascending periventricular catecholamine bundle). However, we also showed that enkephalin could be rapidly degraded (Sullivan et al., 1978, 1980), which suggested that it might produce local modulation of pain responses but might not survive long in the cerebrospinal fluid. The findings on release of endogenous opioids in humans upon deep brain stimulation represented the integration of animal and human studies culminating in a remarkable "translational" outcome.

But Wait—There's More! A Molecule of Pain, Addiction, Stress, and Skin Pigmentation

As if the endorphin tale were not exciting enough, there arose a whole new dimension associated with β-END/β-LPH. As noted earlier, β-LPH was isolated from the pituitary gland, and anatomical studies showed that it was localized exclusively to the corticotrophs—that is, the cells of the pituitary that synthesize and release adreno-corticotropin hormone (ACTH), the stress hormone. It should be noted that the N-terminal region of ACTH (ACTH1–13) can be cleaved and modified by N-acetylation and C-terminal amidation to give rise to alpha-melanocyte stimulation hormone (α-MSH). This hormone was known to be important in skin pigmentation as well as playing a role in stress responses in certain animals (e.g., frogs). Remarkably, β-LPH contained a homologous sequence in its N-terminal domain termed β-MSH, which also possessed the ability to alter pigmentation. Recall that β-LPH also contains the entire sequence of β-END at its extreme carboxy-terminus. Thus, beyond the presence of β-LPH in the corticotrophs, there was evidence of some structural and functional relationships between β-LPH/β-MSH/β-END on the one hand and ACTH/α-MSH on the other. But what exactly was that relationship?

It was the elegant work of Edward Herbert and his students, James Roberts, Richard Mains, and Betty Eipper, that moved the field dramatically (Mains and Eipper, 1977; Roberts and Herbert, 1977a,b; Eipper and Mains, 1978). They showed that all of these active peptides derive from a common precursor protein that came to be known as pro-opiomelanocortin (POMC). The name is descriptive because this common precursor encodes β-LPH (and therefore β-END and β-MSH) at its carboxy-terminal end and encodes ACTH (and therefore α-MSH) in its middle region. It was clear that there

was a large N-terminal region of this precursor of unknown sequence and function. Painstaking work showed that the active peptide hormones derived from the systematic cleavage of this precursor via specific enzymes that recognize appropriate dibasic cleavage sites. Different lobes of the pituitary gland process these peptides to different extents, with the anterior lobe making ACTH and a mix of β-LPH and β-END, whereas the intermediate lobe processes POMC more fully to α-MSH, β-MSH, and β-END.

Given that we had seen β-LPH and β-END in the brain, could we provide evidence of the existence of other POMC related peptides in the central nervous system? To this end, we demonstrated the existence of ACTH immunoreactivity in the arcuate nucleus and the co-existence of ACTH with β-LPH/β-END in the same area (Watson et al., 1978a).

In a landmark paper showing the first cloning of a mammalian gene, Nakanishi et al. (1979) provided the full nucleotide sequence (1,091-base pair) of a cDNA encoding bovine POMC. The amino acid sequence revealed that the heretofore-undescribed N-terminal domain of the precursor encodes a third melanotropin sequence that they termed gamma-MSH. Thus, the cloning of POMC revealed that a single gene could encode multiple active products (ACTH, MSH, β-END) as disparate in their function as skin pigmentation, stress control, and pain and affect control. It also demonstrated that a given gene could have repeated motifs (i.e., the MSH core sequence). But equally notable was the fact that there was no evidence of a sequence that could give rise to leu-enkephalin, further supporting the anatomical evidence that suggested that the enkephalins were distinct from β-LPH/β-END and did not derive from the POMC precursor. Interestingly, a collaborator on the Nakanishi et al. (1979) paper was the pioneer in molecular engineering, Stanley Cohen, who worked one hallway away from the Barchas lab. Even though both our groups were studying POMC and had some key reagents (e.g., antibodies) that might have been helpful, the two labs were unaware of these parallel efforts.

Moving to Michigan

We began our search for faculty positions while still expecting our first child. We faced the issue of coordinating two careers at a time when there was less awareness of such issues. But we were also very lucky to wind up with several options. Gardner Quarton, the director of the Mental Health Research Institute (MHRI) at the University of Michigan, was a patrician, thoughtful, and sophisticated man who clearly understood our individual and combined career needs. Bernard Agranoff and Bernard Carroll were also members of the MHRI, and Bernie and Barney (!) were very critical in our recruitment. As importantly, the institute (now renamed the Molecular and Behavioral Neuroscience Institute–MBNI) represented an ideal setting for us. We were both interested in fundamental neuroscience

research, but we were also interested in moving between animal and human work—translational science, before it was so designated. Our interests were moving toward not only understanding the basic biology of opioids in brain and pituitary but also exploring their functions in drug abuse and in the biology of stress—especially considering our work on stress-induced analgesia. In many ways, the sequence of POMC was our research program. Moreover, while at Stanford, we had also pursued work on the potential role of endorphins in schizophrenia (Watson et al., 1978, 1979), and the institute's close ties to the psychiatry department were a major draw. But so was the fact that this was, in fact, a neuroscience institute. Indeed, Ralph Waldo Gerard, one of the founders of the Society for Neuroscience who had coined the term "neuroscience," was also one of the original members of the institute. The director, Gardner Quarton, had worked at the Neuroscience Research Program (NRP)—the same entity that had sponsored the opiate receptors meeting where Hughes had revealed the existence of enkephalins. And Bernard Agranoff had carried out his landmark work on the biochemical nature of memory. By December 1977, at about the time that the ACNP meeting took place and our first child was born, we decided to make the University of Michigan our next academic destination. We, our son Brendon, and our daughter Kathleen (Katie), who was born in Ann Arbor, have called it home ever since!

For Huda, in particular, there were several ironies in this choice—for example, an early rejection of her graduate school application from the University of Michigan Psychology Department. But more positively, the fact that three important people in her life had worked not only in this same university but in this very institute: James Olds, Steve Fox, and John Liebeskind! In fact, while awaiting the arrival of new furniture for our labs, we dug out some lab stools from storage and on the bottom of several of them was written boldly: "Olds Lab." It seemed fated.

Indeed, the MBNI, the department of psychiatry, and the University of Michigan more broadly have proven to be a wonderful academic home to both of us, and Bernie Agranoff is yet another remarkable mentor who taught us how to remain passionate about science while navigating the administrative world thoughtfully, strategically, and with humor.

The Regulation and Function of Pro-Opiomelanocortin and Its Products

Moving to Michigan, we began to define a research program that would integrate our combined interests and skills. We had two separate labs, but we knew we would be collaborating actively. Although many issues were still swirling in the field, it seemed to us that a central one was how to think about the function of these opioid peptides in the central nervous system—not just as neurohormones or pituitary releasing factors but as

bona fide neurotransmitters. We were used to thinking about monoamines, but the biosynthesis, release, regulation, and receptor actions of neuropeptides remained a mystery. Moreover, POMC presented a unique challenge. It contains so many different active peptides, and we knew that it was processed to different extents in the anterior versus intermediate lobes of the pituitary. How was it handled in the brain? And how do we conceive of an integrated function of these POMC peptides, when one could regulate pain and pleasure and the other could encode stress or modify skin color? Finally, although we understood some mechanisms of cellular regulation of classical neurotransmitters, we had no insights regarding these neuropeptides. Pharmacological tools that interact with the opioid receptors abounded, but no tools were available to manipulate the availability or release of the opioid peptides. Finally, the role of the endorphins in opiate tolerance, dependence, and addiction remained unexplored. There was clearly no shortage of questions for us to tackle.

Our early work clearly showed that the processing of POMC was distinctive in the brain—more complete than what is seen in the anterior pituitary corticotrophs but with fewer post-translational modifications than what is seen in the intermediate lobe, where β-END is N-acetylated, thereby losing its ability to activate opiate receptors (Akil et al., 1981). Further, ACTH was processed in the arcuate to α-MSH (Watson and Akil, 1979), although it would be many years before its function in controlling feeding would become evident. But our work suggested that the different POMC products, which are presumably released simultaneously at the synapse, could work in a coordinate fashion; thus, when we microinjected α-MSH in the periaqueductal gray, we observed analgesia similar to what is seen with β-END, although the α-MSH induced analgesia was non-opioid in nature (Walker et al., 1980). This suggested that peptide products deriving from the same precursor could work coordinately on the same target function, albeit via different receptor mechanisms.

We also pursued the question of interplay between stress and opioids (which we had initiated at Stanford) in much greater depth, acquiring a mechanistic understanding of how stress triggered changes in the biosynthesis, processing, and release of POMC in the pituitary and in the brain (Akil et al., 1985; Young and Akil, 1985a,b). Finally, we examined the impact of chronic morphine on the regulation of POMC and especially on the synthesis of various forms of β-END that differed in their opiate potencies (Bronstein et al., 1988). This was a way of addressing the dynamic regulation of this system. Thus, we stated in a review (Akil et al., 1984): "It is conceivable that the modification of β-END 1-31 into β-END 1-27, which is ten-fold less active as an opioid, may serve a physiological function. Furthermore, such a modification appears to require time, and may not take place if β-END 1-31 is freshly synthesized. Thus, a measurement of the ratio of these two forms may yield an index of activity in the system and may correlate with various

functional states of the animal." These types of questions required that we establish methods such as pulse-chase studies, high-pressure liquid chromatography (HPLC) coupled to radioimmunoassays, as well as receptor-binding assays to study the dynamics of function and regulation of POMC in the brain and pituitary, under physiological conditions.

These and other regulatory studies revealed a pattern of POMC communication and regulation that greatly broadened our views of synaptic transmission—it no longer seemed to be a simple on/off system but rather relied on a host of molecules of different lengths, post-translational modifications, receptor selectivities, and durations of action. Moreover, the exact mix changed under different physiological conditions such as pain, stress, or addiction. In other words, although we began by thinking that synapses were monosyllabic, neuropeptides taught us that they actually spoke in sentences.

One More Opioid Family

In 1979, Avram Goldstein's lab identified a new 13 amino acid opioid peptide termed "dynorphin 1–13," which they characterized as "an extraordinarily potent opioid peptide" because it was 700 times more potent than enkephalin in the guinea pig ileum. Remarkably, the first five amino terminal residues of dynorphin were identical to the full sequence of leu-enk. Could this be the missing precursor for leu-enk, or was it a situation similar to β-END, where the sequences were overlapping but the anatomy was distinct?

Soon after our arrival at Michigan, Avram approached us about mapping this new peptide in the brain. And once again, the anatomy proved to be highly revealing. Dynorphin distribution was distinctly different from that of either the enkephalins or POMC. Thus, dynorphin immunoreactivity was most prominent in the paraventricular nucleus of the hypothalamus and overlapped with the expression of arginine vasopressin, with projection to the posterior pituitary (Watson et al., 1981, 1982a). We carried out a systematic analysis, using improved antibodies against a host of opioid peptides to compare the distribution of dynorphin versus the enkephalins in brain. This paper concluded, "It thus appears that the brain contains at least three separate opioid neuronal networks: an enkephalin family . . . , a beta-endorphin family, and a dynorphin family" (Watson et al., 1982b). These anatomical observations proved to be completely consistent with biochemical studies showing the existence of three separate precursors for these peptides (see following).

However, at the behavioral level, dynorphin appeared to be an unusual sort of opioid. In spite of its potency in the guinea pig ileum, it did not produce very good analgesia. Moreover, we showed that intracerebroventricular administration of dynorphin produced potent and long-lasting effects on motor function and on the electroencephalogram in rats, and

local iontophoretic or pressure ejection of dynorphin consistently inhibited hippocampal unit activity. None of these effects were significantly affected by naloxone even at high doses. Moreover, a fragment of dynorphin that failed to displace any number of tritiated narcotics from rat brain homogenates produced similar effects on these physiological measures *in vivo*. We suggested that dynorphin was an unusual opiate and that its sequence also contained a second biologically active site that is capable of quite potent but non-opiate effects (Walker et al., 1982). These behavioral observations added to a host of findings (e.g., differential profiles in the guinea pig ileum and mouse vas deferens) that suggested the existence of multiple opiate receptors (see following), as well as activities mediated via non-opioid peptide products.

Everyone Finds a Home

In 1984, we wrote a review of the opioid field (Akil et al., 1984) that began with the following summary statement: "While most would agree that the mid-seventies were vintage years for endorphin research, 1982 is certain to be 'a very good year.' Less obvious to the public eye, it is nevertheless a turning point in endorphin research because it is the year in which all the brain opioids found a home." Indeed, after several years of uncertainty about the relationships between the various opioid peptides, a combination of biochemical studies and molecular cloning in peripheral tissues such as the pituitary and the adrenal clarified the major structural issues. By the early 1980s, more than a dozen opioid peptides had been isolated from the brain, pituitary, and adrenal medulla. They all shared the Tyr-Gly-Gly-Phe sequence followed by either met or leu at their N-termini. And then they either stopped at five residues (enkephalins) or had various extensions ranging from two to 26 amino acids. Molecular cloning showed three distinct families: *POMC* (as described earlier) containing β-END that could be processed to various forms; *pro-enkephalin*, which contained seven copies of the Tyr-Gly-Gly-Phe core—these included leu-enkephalin, four repeats of met-enkephalin, and two extended forms of met-enkephalin (with two and three additional amino acids following methionine); and *pro-dynorphin*, which included three copies of the Tyr-Gly-Gly-Phe core, each followed by leucine and with various extensions yielding the peptides dynorphin A (the first one cloned by Goldstein), dynorphin B, and neo-endorphin. Many of these peptide domains could be further modified post-translationally with additional cleavage, N-acetylation, or carboxy-terminal amidation to alter their stability, opioid selectivity, and potency at the receptor and *in vivo* (Nakanishi et al., 1979; Comb et al., 1982; Kakidani et al., 1982; Noda et al., 1982).

These cloning results corroborated the body of work carried out on the brain distributions of these peptidergic systems across several laboratories. In turn, the co-expression of opioid peptides from the same precursor within

the same neurons was confirmed by immunohistochemical studies (e.g., Khachaturian et al., 1983a, 1983b, 1985; Watson et al., 1983) and by extensive characterization in particular brain loci (Dores et al., 1985).

Multiple Opioid Receptors

The original receptor binding studies in the brain characterized a receptor that was "classical" in its pharmacology in that it recognized opiate drugs with affinities that were consistent with their analgesic potencies and with their effect in the guinea pig ileum. In effect, this was the classical morphine receptor. However, in the mid 1970s, Martin and his colleagues (1976) suggested that there were multiple opiate receptors based on studies of analgesia, tolerance, and cross-tolerance in the dog. In particular, they proposed the existence of a *mu* (for morphine preferring) and a *kappa* (ketazocine preferring) opiate receptor. A third receptor they suggested, sigma, eventually proved to be non-opioid in nature. However, Kosterlitz and his colleagues had suggested that the mouse vas deferens contained an opiate receptor that had properties distinct from those of the guinea pig ileum, which they termed the "delta opiate receptor." The enkephalins appeared to be particularly good ligands at that delta site, whereas dynorphin was not (Goldstein et al., 1979). Thus, by the early 1980s, the field had identified three types of opiate binding sites: *mu*, *delta*, and *kappa* (Kosterlitz et al., 1980; Gillan and Kosterlitz, 1982).

In the same review (Akil et al., 1984) in which we summarized the existence of three precursors that encode all the known opioid ligands, we addressed the issue of multiple opioid receptors by stating: "Less clear, but equally critical, is the issue of multiple opioid receptors. Unquestionably, the heterogeneity exists. What remains to be established is whether each of the three families of opioids has its own receptor, or whether a given family can interact with more than one subtype, and each receptor subtype with more than one family. More critical to physiology is whether these unique combinations result in different biological events and are involved in different functions."

Of course, the answer turned out to be the more complicated one. The most straightforward relationship between the opioid ligands and their receptors is the one between pro-dynorphin and the *kappa* opioid receptor, in that *kappa* has high affinity only to the products of the pro-dynorphin family. However, there is no symmetry in that members of the pro-dynorphin family also interact with other opioid receptors, especially *mu*. The delta opioid receptor recognizes with high affinity the members of the pro-enkephalin and of the POMC family. Finally, the classical *mu* opioid receptor can interact with high affinity with members of each of the three precursors. Each of these receptors also has a unique profile vis-à-vis synthetic peptides and opiate alkaloids (see Gutstein and Akil, 2006, for review). It should be noted

that whether based on their signaling properties or their anatomy, activation of each of the three types of opioid receptors yielded a different pharmacological and behavioral profile. For example, activation of the *kappa* receptor can be analgetic but can cause dysphoric responses, unlike the combination of analgesia and positive responses elicited by activation of the *mu* receptor.

The complexity of peptidergic neurotransmission was evident once again—a given precursor gives rise to multiple opioid (and non-opioid) peptides that are capable of interacting with multiple opioid receptors. The signaling in any given location would depend on the exact juxtaposition of neuropeptides and specific receptors determining the eventual functional signal. It was therefore critical to map the distribution of the three types of opioid receptors, an extensive effort that we undertook in the brain of multiple species and across development (Mansour et al., 1987, 1988, 1995 a,b). Maps of the three opioid peptide families and the three opioid receptors produced by our group appeared very popular and hung on many lab walls.

However, the purification and the cloning of the opioid receptors proved remarkably stubborn. It was not until 1992 that two groups, using functional cloning strategies, succeeded in cloning the *delta* opioid receptor (Evans et al., 1992; Kieffer et al., 1992). This allowed other groups including ours to clone the other two opioid receptors, *mu* and *kappa* (Meng et al., 1993; Thompson et al., 1993; Xie et al., 1994). This opened the door to extensive studies on the anatomy, regulation, function, and structure activity relationships of these receptors. For example, we generated numerous mutants and chimeras of these receptors to identify the domains that are critical to selectivity and discrimination among the various opioid peptides (Meng et al., 1996; Watson et al., 1996).

Developing Technology to Study Gene Expression and Regulation in Brain—*In Situ* Hybridization

The history of opioids brought home the importance of developing technologies that are both powerful and aimed at understanding neurobiology at the level of neural circuits. Techniques that married neuroanatomy with the ability to examine specific functional molecules had transformed neuroscience in the 1960s and 1970s. These techniques included monoamine fluorescence and immunocytochemistry. Yet both had significant limitations that made the study of regulation in a neuroanatomical context challenging.

Monoamine fluorescence was not only limited to a small set of neurotransmitters, but the fluors decayed rapidly, rendering quantitative studies very difficult. Immunohistochemisty (ICC) had clear advantages because the fluorescent signal lasted up to a few hours, and subsequent staining methods (horseradish peroxidase or alkaline phosphatase) produced color precipitates that were essentially permanent. Yet ICC had several pitfalls, especially when dealing with a novel target molecule. The size of the average

epitope for most antibodies is in the range of four to six amino acids, and yet this short sequence is the key determinant of the specificity of antibody binding. This results in numerous issues of cross reactivity with other proteins, such that any one serum can provide misleading information. There are some reasonable solutions to this issue—the use of multiple antisera aimed at the same peptide/protein, affinity purification of the antibody to isolate a specific population, or the use of monoclonal IgGs. However, polyclonal antibodies offer certain advantages because they can contain subpopulations that prefer different forms of a given molecule—for example, the full length POMC precursor, or an intermediate such as β-LPH, or a final product such as β-END. This flexibility is particularly helpful when the exact nature of the product in the brain is still unclear.

But arguably the biggest drawback of ICC is that it is poor for quantitation, and therefore not readily conducive to regulatory studies. The variation in structure of the target protein coupled with the variation in IgG pools combine to make a quantitative analysis less than optimal. Moreover, the methods of preparing an epitope for immunization need to be adapted to the nature of the sequence and the three-dimensional structure of the target protein. Essentially, each antibody is custom-prepared and requires specialized methods for effective visualization—rendering comparisons between proteins extremely difficult.

So, how could one circumvent these issues and study gene expression and regulation in a neuroanatomical context? Given the revolution in molecular biology at the time, it became evident (to us and to others) that adapting the tools of that field to neuroscience would be extremely powerful. In the 1980s, developing a tool to visualize messenger RNA (mRNA) in a neuronal context became a major thrust of the Watson lab in a close collaboration with the lab of James Roberts. Jim had been in the Herbert lab in Oregon and had played a critical role in the early identification of the common ACTH/LPH precursor (Roberts and Herbert, 1977a,b). He had moved by then to Columbia University, and he brought to the collaboration his great knowledge of molecular biology, his warm and generous style, and a wonderful sense of humor and adventure. Other key members of the team were Connie Gee, in Jim's lab, and Robert Thompson in the Watson lab (now a friend and faculty colleague at the Michigan). Together, this team developed a technique that came to be known as *in situ* hybridization (ISH), and they published the first paper using this tool in the brain, aptly showing the presence of POMC in the arcuate nucleus of the hypothalamus (Gee et al., 1983). The power of ISH was immediately recognized by the neuroscience community—our poster at the Society for Neuroscience annual meeting in 1984 was mobbed, suggesting that this was a tool whose time had come.

At the time of our first efforts at *in situ* hybridization, the world of molecular biology typically quantified mRNAs by Northern gel analyses—that is, by extracting the mRNA from tissue, running it on a gel, and

identifying it by using a P32 labeled, double-stranded DNA probe. The method was reliable and quantitative but sacrificed the entire tissue block, leading to only very crude anatomical resolution.

There had been very few efforts toward a more anatomical approach. A notable one was a brief, two-page report by John Shine using ^{32}P double-stranded DNA probes to visualize POMC in a rat pituitary. The image was rather blurry with no cellular resolution because ^{32}P scatter is huge. Still, it was a proof-of-concept. But how do we ensure cellular resolution, quantitate the signal, and obtain high sensitivity? We also had to struggle with tissue fixation and probe penetration into the tissue section.

Our initial experiments with ^{32}P-labeled, double-stranded DNA led to the same blurry signal seen in Shine's paper. We considered ^{3}H-labeled probes, given that they have approximately 200 times less scatter, but they were weak emitters. In the end, we found ^{35}S to be a good compromise. It has a scatter of only one to three microns and is a considerably stronger emitter. Indeed, the next set of experiments using ^{35}S-labeled, double-stranded DNA yielded much better anatomy. Single cells were visible, even in the brain! But the method was rather variable in the intensity of the signals it produced. Quantitation was not reliable, and sensitivity to low copy number mRNAs (which are common for signaling molecules) was suboptimal.

We determined that the next hurdle was the biochemical nature of the probe (i.e., being double-stranded DNA). It defeated itself. After labeling, it was necessary to raise the temperature to melt the strands apart and to allow the antisense DNA strand to hybridize with the target mRNA; unfortunately, it could also re-hybridize with the sense strand DNA, interfering with quantitation and sensitivity. Ideally, we needed a single-stranded probe of a defined and consistent length. This would allow us to control hybridization kinetics and therefore accurately estimate the number of mRNA molecules of interest. But there was nothing available to resolve this problem at the time.

Even so, we made substantial progress at the technical, anatomical, and biochemical levels during this phase. For example, we were able to show that POMC neurons not only contained all the POMC products (Watson et al., 1977d, 1978a,b) but also the mRNA that coded for POMC (Kelsey et al., 1986; Lewis et al., 1986). Although such an observation is obvious now, it was critical in responding to the stubbornly lingering notion that POMC peptides flowed from the pituitary into the blood stream and then back into the brain where they were taken up and concentrated.

We soon began to consider the use of oligonucleotides (15–50 bases) as probes because they are single stranded and produce a reliable and semi-quantitative signal. However, we had to wrestle with their lack of sensitivity because we could only add one or two radioactive nucleotides to the ends of the probe. Then a postdoctoral fellow in the lab, Michael Lewis, came

up with an ingenious solution (Lewis, et al., 1986). He suggested using 3' deoxynucleotidal transferase to add a random radioactive tail to the oligo probes. This worked much better, and we could make tails of up to 50 nucleotides without much loss of stability.

The next breakthrough was the availability of tools for *in vitro* transcription of single-stranded, labeled mRNA. The transcription was high fidelity and produced a highly radioactive, antisense strand. Even with that first gel: a single band! We had achieved the clean, highly radioactive ^{35}S-labeled antisense probe we needed. It produced a lovely signal with greatly improved background at the single-cell level of resolution. Beyond the nature of the probe, the key was to treat the tissue with RNase after the label hybridized. This post-treatment would not digest RNA:RNA double strands but would digest unhybridized labeled RNA, thereby dramatically reducing the background noise.

Just as the Swedish histochemists at the Karolinska Institute explored any and all variables associated with their methods, we felt it was important to follow suit in optimizing this technique. We used more than 10,000 slides in this process, defining the conditions and strategies to ensure specificity of the signal and reliability of the quantitation. We were also committed to being explicit about these conditions and to training others in the use of this technique. It is gratifying that since our first paper appeared in 1983, thousands of researchers have relied on this tool as a means of studying gene expression and regulation in the nervous system.

In Situ Hybridization in Service of Understanding the Biology of Opioids and Stress Systems

Once a practicable version of *in situ* hybridization was developed, there was an obvious shift in the nature of neuroanatomical studies. Neuroscientists could see not only the anatomical value of ISH but its power to quantitate mRNA levels in their paradigms involving behavior, lesions, electrical stimulation, electrophysiology, pharmacological and physiological manipulations, and developmental studies. In a sense, neural circuits became measurable, in terms of their basal activity and their response to stimuli, and the connection between genes and function could now be more easily envisioned.

Of course, for us, one of the earliest applications of ISH was the study of the opioid system at the gene-expression level. This included the original demonstration of POMC mRNA in the arcuate nucleus (Gee et al., 1983), as well as the discovery of a small POMC cell group in the nucleus tractus solitarius (Bronstein et al., 1992). It also included the full mapping of the gene expression of two other opioid precursors as well as the opioid receptors (Mansour et al., 1995a,b)

But we also put ISH to a range of other uses all in service of understanding regulatory biology of specific molecules in the context of neural circuits.

For example, we showed that in hypothalamic magnocellular neurons, dynorphin gene expression is coregulated with vasopressin and oxytocin genes by osmotic challenge (Sherman et al., 1988). Additional modifications of the technique allowed us to study the co-expression of multiple genes in a single neuron via dual ISH, thereby defining functionally distinct subpopulation of neurons in brain areas relevant to reward (e.g., Curran and Watson, 1995) and stress and negative affect (Day et al., 1999). We were also able to use ISH to assess c-fos gene expression and to couple it to track tracing in order to define specific circuits involved in the neural integration of the stress response (e.g., Herman et al., 1989; Cullinan et al., 1993). An underappreciated but extremely helpful tool is the use of intronic *in situ* hybridization. In this approach, the hybridization probe is aimed at specific introns within the gene of interest, allowing the detection of heteronuclear RNA (hnRNA). This is the primary transcript that exists briefly immediately following transcription and before the deletion of introns that results in mature mRNA. Because the half-life of a given intron is so short, the levels of introns directly reflect changes in the rate of hnRNA synthesis and can therefore be used to detect changes in transcription (Herman et al., 1991; Itoi et al., 1999). Thus, intronic ISH allows the study of dynamic changes in gene activity in the context of specific neurons and neural circuits.

But ISH also became the "go-to" approach for assessing the expressing of newly cloned genes of relevance to neural functions. Thus, our group relied on it to provide the initial mapping of gene expression upon the cloning of the dopamine receptors (e.g., Meador-Woodruff et al., 1989), the corticosteroid receptors (Herman et al., 1989), or of new peptide families such as orphanin/nociceptin (Neal et al., 1999). Coupled with other anatomical, functional, and molecular tools, it continues to be a cornerstone of studies of regulatory biology in the nervous system.

Telescoping to the Present Day

The study of opioids was the jumping off point for our broader interest in the study of regulation of emotions. The endogenous opioids exemplify the integrated way in which the brain and neuroendocrine system orchestrate a range of affective and motivated responses. Endorphins control pain, pleasure, stress responses, appetite, and sleep as well as autonomic, neuroendocrine, and digestive functions. Indeed a single gene, POMC, encodes peptides classically associated with the control of stress (ACTH), pain, pleasure (β-END), and appetite control (α-MSH). Our laboratories initially focused on the neurobiology of substance abuse on the one hand and of stress on the other as model systems of regulation of affective pathways. But it soon became clear that these two domains are closely intertwined—for example, in the way that stress contributes to various aspects of addictive behavior,

and addiction, in turn, alters coping and stress responsiveness. Moreover, the interplay with stress applies not only to opiates but to many drugs, such as cocaine and alcohol.

Although the reductionist approach had clearly paid off in the opioid arena, a true understanding of affective behavior requires a more integrative approach. Given our combined interests in genetics, molecular biology, neuroanatomy, pharmacology, and behavior, we felt ready to face the broader challenge of understanding affect regulation under "normal" conditions and in pathological states. Our translational perspective, including our various studies in humans, had underscored the variability in affective responses among individuals and the fact that the same event is perceived as a stressor by one person and as a positive challenge by another. Yet, differences in vulnerability or resilience to environmental and psychosocial challenge, although hugely important in psychiatry, were rarely studied in basic neuroscience.

Indeed, epidemiological and genetic studies have consistently suggested that there are stable personality and temperamental styles that could predict the types of psychopathology that a person might develop. Thus, a more inhibited "internalizing" personality style is associated with vulnerability to anxiety and depression, whereas a more impulsive "externalizing" personality style is associated with a propensity to conduct disorders and substance abuse. Moreover, these behavioral traits were very stable characteristics of humans, evident during early childhood and greatly predictive of future behavior (Kagan et al., 1989; Mischel et al., 1989). Here was a fundamental dimension of behavior that defined not only affect but its relationship to cognition and psychopathology. Yet, at the time, the neurobiology of "temperament" was not a serious topic of neuroscientific analysis. We decided to tackle this issue, bringing to bear on it a combination of genetic, neurochemical, anatomical, behavioral, and translational perspectives. We have been successful in creating animal models of "internalizers" versus "externalizers," and remarkably, our selective breeding of these behavioral phenotypes maps beautifully onto the human characteristics for predicting propensity to anxiety, depression, aggression, and substance abuse (Flagel et al., 2013). We have identified a number of molecular mechanisms, genetic and epigenetic, that lead to these stable differences (e.g., Turner et al., 2011) and shown that these animals respond differently to the world around them, from affective challenges to associative learning (Flagel et al., 2011, 2013). Importantly, this animal model of "temperamental" differences provides us with an excellent vehicle for studying the function of candidate genes that are emerging from human genetic and postmortem studies of psychiatric illness, especially in relation to mood disorders.

Arguably one of the most ephemeral targets of neurobiology has been to understand the basic biology of mood and the pathophysiology of mood disorders. The concept of mood is itself ephemeral in nature,

and yet dysregulation of mood and affect represents one of the largest burdens of disease worldwide. Our work in opioids and stress biology, and the well-established role of stress as a trigger of mood disorders, sparked our initial interest in the study of brain mechanisms of depression (e.g., Young et al., 1994). But this work has mostly flourished in the context of an unusual, broad-scale and long-lasting collaboration called the Pritzker Neuropsychiatric Research Consortium. Beyond our group and colleagues at the University of Michigan, the consortium involves our mentor, Jack Barchas, who is now the chair of psychiatry at Cornell University, along with Alan Schatzberg at Stanford, William (Biff) Bunney at University of California at Irvine, Rick Myers at the HudsonAlpha Institute, and the late Edward (Ted) Jones at the University of California at Davis. This group of scientists and their colleagues bring together a range of scientific fields—genetics and genomics, informatics, neuroscience, and psychiatry, along with the rich and ever-evolving toolset of modern biology in order to investigate the brain biology of severe psychiatric illness. We rely on a world-class brain bank (headed by Biff Bunney) for postmortem analyses, along with the study of human subjects, animal models, and even *in vitro* systems. This collaboration has led to numerous insights regarding the neurobiology of psychiatric disorders (e.g., Evans et al., 2004; Scott et al., 2009; Bernard et al., 2011; Turner et al., 2012; Li et al., 2013) and deserves its own recounting. But it underscores the need for new scientific models to address the major challenges that the field of neuroscience continues to face because of the complexity of the problems that it tries to unravel.

Final Thoughts

The Danish scientist and mathematician Piet Hein said: "Problems worthy of attack prove their worth by attacking back." We feel lucky that, in more than four decades of neuroscience research, we have been attacked back on a regular basis by the problems we have chosen. This has kept us energized, excited, and ready to pick a new fight. As we solve one little mystery about the functioning of the brain, dozens of new ones emerge. Our simplistic hypotheses have been crushed by biological reality on a regular basis, and the process is truly humbling. This is exactly why we treasure any contributions we have made that have stood the test of time.

During this journey, we have learned the importance of relying on each other—not just each other as a couple, but on our research group, our students, our mentors, our collaborators and colleagues, and our scientific community, including those who have come before us. We have come to understand the critical need for different kinds of minds working together to try to solve the ultimate mystery: how the mind works.

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