Thomas J. Carew

Born:

Los Angeles, California
July 25, 1944

Education:

Loyola University, Los Angeles, CA, Psychology BS (1966)
University of California at Riverside, Riverside, CA, PhD (1970)

Appointments:

Postdoctoral Fellow, Department of Psychiatry, NYU School of Medicine (1970–1972)
Instructor, Department of Psychiatry, NYU School of Medicine (1972–1974)
Assistant Professor, College of Physicians and Surgeons, Columbia University (1974–1981)
Associate Professor, College of Physicians and Surgeons, Columbia University (1981–1983)
Professor of Psychology and Biology, Yale University (1983–1999)
Professor, Neurobiology and Behavior, University of California, Irvine (2000–2011)
Professor, Center for Neural Science, New York University (2011–present)
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Honors and Awards (Selected):

National Institute of Mental Health, Career Development Award (1975–1987)
National Institute of Mental Health, MERIT Award (1990)
Yale College Dylan Hixon Prize for Excellence in Teaching in the Natural Sciences (1991)
Endowed Chair, Yale University: John M. Musser Professor of Psychology (1991)
Guest Professor, Free University, Berlin, Germany (1993–1998)
Elected Fellow, American Psychological Association (1991)
Endowed Chair, University of California, Irvine: Donald Bren Professor (2000)
Elected Fellow, American Academy of Arts and Sciences (2004)
Endowed Chair, New York University: Anne and Joel Ehrenkranz Dean of the Faculty of Arts and Science (2011)

Thomas Carew has explored the cellular and molecular mechanisms of memory in the model system Aplysia californica. *Early in his career, he was a member of the team in Eric Kandel's laboratory that provided the first evidence for both long-term memory and associative learning in* Aplysia. *These discoveries gave rise to independent behavioral, synaptic, and molecular studies in the Carew laboratory first at Yale, then at the University of California–Irvine, and finally at New York University. In this work, he developed several lines of research that provide mechanistic insights into learning and memory. For example, with his research team, he was able to dissociate several different types of memory on both behavioral and synaptic levels, showing, for example, in contrast to a prevailing theoretical view, that it is possible to induce both intermediate-term and long-term synaptic changes underlying memory in the absence of any short-term changes. He also identified three mechanistically distinct phases of synaptic facilitation in the central nervous system, each of which predicted the existence and molecular features of distinct temporal phases of behavioral memory. The predominant focus of his work for the last several years has centered on two basic themes: first, the critical importance of pattern and timing in memory formation; and, second, the essential roles that growth factors play in the induction of long-term memories. In exploring both of these themes, he has been able to identify the contribution of a number of specific molecular cascades, as well as their interactions, in the induction and consolidation of different forms of memory. The overarching goal of his research has been the forging of direct connections between specific synaptic and molecular events to bona fide instances of memory expressed behaviorally.*

Thomas J. Carew

Prologue

The singular event that most affected my life was a radiator boiling over; specifically, the radiator of my 1954 Ford station wagon. This stately vehicle had no backseat to make room for my surfboard. It also had no muffler, which had two consequences: first, it made the car sound like a beast, and, second, it caused the floorboard in the back to get really hot, prompting the occasional hitchhiker to ask to get out before his intended destination. I will return to the radiator in a bit.

The Early Years

I was born in Los Angeles in 1944. I grew up in a lower middle-class neighborhood called La Puente and had a rather unremarkable youth. My brother and I went to Catholic elementary school where our classmates were predominantly of Mexican American heritage. I still have fond memories of negotiating lunch deals on the playground where I would trade my typical lunch of tuna fish sandwiches for my classmates' lunch, usually cold refried beans wrapped in flour tortillas: gourmet meals all around.

High school consisted of a Catholic boarding school in the San Fernando Valley. At the time, I felt that being in boarding school was a little like being in jail, but when I look back, the education I received was certainly of high quality. Following high school, I attended Loyola University in Los Angeles. I was a first-generation kid. My dad was a salesman and my mother was the secretary at a local Catholic school. So, for my parents, my going to college was very important, because it offered me a chance to have a shot at a better life than they had.

Loyola University was a terrific experience for me. Being trained by the Jesuits was extremely important for my early intellectual development. The key elements of this training were an emphasis on logical thinking, on rigorous argument, and on intellectual discipline. It was at Loyola where I first fell in love with learning in general, and with science in particular.

Now back to the radiator. Loyola at that time comprised all male students. The companion university up the road a bit, Mount St. Mary's, consisted of all women. Occasionally on a Friday night, there would be a dance, called "a mixer," in which the Loyola men and the Mount St. Mary's women would get together. In my sophomore year at such a mixer, I met a girl whose name was Mary Jo Sick (that's right, Sick). I also met another girl that evening: her name was Julie and she was Mary Jo's best friend

from high school. On a Saturday morning a week or two later, I thought it a good idea to drive up the road and drop in to say hi to Julie (unannounced of course; I was not a terribly sophisticated fellow). An important element of the story is that Mount St. Mary's is on a steep hill. On the way up that hill to visit Julie, my trusty station wagon overheated and the radiator boiled over. So I turned around and coasted down the hill, wondering who else I might know that would want an unannounced visitor? Oh yeah, that interesting gal named Mary Jo. Importantly, she lived at the bottom of the hill, in a fancy neighborhood where she worked as an *au pair*, taking care of the children of a wealthy family.

So, I dropped by to say hi to Mary Jo. She took my unannounced arrival in stride. We sat out on the front lawn of the wealthy folk's home and chatted . . . for a long time. That was in October 1963. We were married the next August (yes, I'm leaving out the part where virtually everyone thought that we were crazy to be married as juniors in college). That was 53 years ago. It's gotten better every day.

The Formative Years

Two critical events happened while I attended Loyola. First, as I mentioned, I fell in love with science. I always have truly enjoyed mystery stories (I do to this day. Truth be told, I have a serious addiction to Irish crime fiction), and doing science was like being engaged in a real-world mystery story. At Loyola, I was a psychology major and became extremely interested in a field that was then called "physiological psychology" (one of the forerunners of modern neuroscience). I was especially interested in the broad field of learning and memory. Little did I know at the time that this deep interest would profoundly shape my career for the rest of my professional life.

The second critical event that happened at Loyola was the birth of our first daughter, Maura Beth. I have to admit that, on graduation day, carrying Maura on my hip when I went up to get my diploma was quite special.

Immediately after graduating from Loyola, I enrolled in a master's program in Physiological Psychology at California State College at Los Angeles, hoping to strengthen my chances of attending graduate school for my PhD. I earned my master's degree in 10 months and, in the following year, enrolled in the graduate program in the Psychology Department at the State University of New York at Stony Brook, where several faculty were interested in studying learning and memory.

While at Stony Brook our second daughter, Amy, was born. So now our family was complete. Soon thereafter, my PhD advisor (Lewis Petrinovich) was recruited to become chair of psychology at the University of California, Riverside (UCR). So with kids in tow, we packed up everything and headed back west.

As a graduate student at UCR, I had little to no mentoring or guidance in the laboratory. I worked pretty much on my own. But that was just fine; the upside was that I could study virtually anything I wanted. I just had to figure out how to do it, from designing the experiments, to building the equipment, to analyzing the data. I have no doubt that problems that took me a week to solve would've taken a well-mentored student an hour or two. But the trade-off was well worth it, as I learned a critical life skill: not to be afraid of trying something . . . anything. The worst that could happen is that it wouldn't work. And when it did (occasionally) work, wow, what a feeling.

For my thesis project, I studied retrograde amnesia in rats. I recorded from cortex (extracellularly) in freely moving animals, and identified some interesting correlates of memory impairment (Carew 1970). In addition, working with a fellow graduate student and great friend, Terry Crow, we also found some interesting (and surprising) dissociations between cortical and behavioral reflections of a phenomenon called "cortical spreading depression." That study gave rise to my first paper in *Science* (Carew et al. 1970).

Are You Finished Yet?

A brief aside is warranted here. Recall that my mom didn't go to college and had little to no idea of what a "scientist" did. Throughout my graduate years, she would repeatedly ask me a single question: "Have you finished your experiment yet?" This was not an innocent question; it was a less-than-subtle Irish-Catholic guilt trip. And whenever I answered "No, mom, not yet," there was a moment of silence before she went on to ask about the grandkids.

The New York University Years

I completed my studies for a PhD in 1970. Reflecting on those graduate years, I am quite proud of what I accomplished. I learned not to be afraid to try anything on my own. I also learned that any decent scientific experiment will take at least five times longer to complete than anticipated. But most important, I came to love the feeling of being on the hunt intellectually for important clues as to how the brain accomplishes this trick called "memory," the effortless and yet magical feat of acquiring information about the world, somehow storing it, and then later retrieving it.

But as much as I loved the *big questions* that I had studied surrounding the broad area of learning and memory in the brain, I realized that I had, in fact, gained little insight into the *actual mechanisms* utilized by the brain to achieve this feat. Said another way, I came to appreciate that, although I had acquired a rich vocabulary with which to describe the processes of learning and memory in the brain, I had achieved no true

mechanistic insights into how those processes were actually implemented. It was this insight that prompted me to search for a simple model system in which it might be possible to achieve such a mechanistic understanding. Enter *Aplysia*!

I had read extensively about different invertebrate model systems, and came to appreciate that a premier system was provided by a simple marine mollusk, *Aplysia californica*. And when I read these papers, far and away the most exciting work was being done by an investigator named Eric Kandel at New York University (NYU). So I wrote to Eric asking him whether it might be possible to join his laboratory as a postdoctoral fellow and, to my delight, he said yes.

On my flight from California to New York City to visit Eric for the first time, I read three extraordinarily exciting new papers from his lab that had just appeared in *Science*. The coauthors of these papers were Harry Pinsker, Irving Kupfermann, and Vincent Castellucci (Pinsker et al. 1970; Kupfermann et al. 1970; Castellucci et al. 1970). Little did I know at the time, that these three members of the Kandel group would become lifelong friends. These back-to-back *Science* papers described the analysis of a simple form of learning, habituation. What was incredibly exciting to me was the fact that this elementary form of learning could be simultaneously studied at three levels of analysis: a behavioral level, a circuit level, and a synaptic level. I couldn't wait to get to NYU!

Joining Eric's laboratory was a truly transformative experience, for several reasons. First, I was exposed to incredibly smart, energetic, and highly motivated colleagues. These included the primary members of Eric's lab: as I mentioned above, Vincent Castellucci and Harry Pinsker, as well as Earl Mayeri. Another extremely talented graduate student in the lab, Jack Byrne, had a strong background in engineering. Jack became, and to this day remains, my closest friend.

In addition to Eric's lab, there were three other laboratories in the overall group. One belonged to a terrific colleague and friend, Irving Kupfermann, who had just formed his own independent group studying the feeding system in *Aplysia.* There were also two other primary laboratories: one studying sensory processing in the mammalian cortex, led by Alden Spencer, and the other studying fundamental principles of neurochemistry, led by Jimmy Schwartz. Collectively, this consortium of laboratories was highly collegial and extremely interactive.

A truly positive aspect of joining the Kandel lab was the feeling that I had arrived in the major leagues. I felt that the scientific questions about learning and memory that we were trying to solve were fundamentally important, and the analytic techniques with which we attacked these questions were state of the art. Finally, and perhaps most important, was the fact that it was just plain fun to work with these colleagues, each and every day.

Lunchtime Was a Classroom

Another key feature of life in the Kandel lab was lunchtime. In his college years at Harvard, Eric was a student of history. And that background deeply informed every conversation we had at lunchtime. It was routine for Eric to tell us stories about the first person to discover a particular scientific phenomenon, the system and methods that were used, and why the particular finding was important in the field of neuroscience. I cannot overstate the importance of these lunchtime conversations. As a complete tyro in this exciting new field of neuroscience, I had a daily tutorial that made a deep and lasting impact.

Long-term habituation: One of the first major projects that I worked on in the Kandel lab was with my colleague Harry Pinsker. Harry and I were continuing the analysis of habituation that he and other members of the group had carried out the previous year. We developed a method for studying habituation of a simple reflex response (the gill and siphon withdrawal reflex) in freely moving animals. One day Harry and I were examining the magnitude of response (in the siphon-withdrawal reflex) of an animal that we had trained the previous day. To our surprise, the animal still showed evidence of habituation training 24 hours after its original learning experience. This immediately suggested to us that *Aplysia* might be capable of remembering the habituation training for several days. We set out to examine this phenomenon in detail and found that, when animals received behavioral training for four consecutive days (with only 10 trials per day), they showed significant retention of the memory for habituation for far longer than 24 hours later, even as long as three weeks later (Carew et al. 1972). This was the first demonstration of long-term memory in *Aplysia*, and opened the door to a wide range of future studies. Next, Harry, Eric, and I carried out a series of studies examining the acquisition and retention of long-term habituation, not only at the behavior level but also at the cellular level. We found that we could identify specific synaptic signatures of long-term memory for up to three weeks after initial training (Carew and Kandel 1973). This was an exciting time for us, as it was the first instance of long-term memory that could be studied in detailed synaptic and mechanistic terms.

Long-term sensitization: Soon after our analysis of long-term habituation, Harry, Eric, and I turned our attention to another form of nonassociative learning called sensitization, an increase in behavioral responsiveness following the presentation of a strong stimulus. We were joined in this study by a graduate student, Wayne Hening. In parallel to the long-term habituation studies that we had carried out the year before, we found that *Aplysia* also could exhibit long-term sensitization of its withdrawal reflexes. Specifically, when we delivered a noxious stimulus to the tail of the animal repeatedly

over a few days, the animal would show memory for sensitization that lasted for several weeks (Pinsker et al. 1973).

Thus, in a period of only a couple of years in the Kandel lab, my colleagues and I discovered that *Aplysia* was fully capable of at least two forms of long-term memory, for habituation and for sensitization, opening the door to the possibility of detailed mechanistic studies in the coming years. But first, a change of address!

The Columbia Medical School Years

In 1974, our entire scientific group, composed of the Kandel, Kupfermann, Spencer, and Schwartz labs, was recruited to the Columbia College of Physicians and Surgeons located a few miles uptown in New York City. This was a major move that took some serious coordination. But move we did. And by the end of the year, we were all back in business in a wonderful new space up at Columbia Medical School.

Synaptic Studies of Long-Term Memory

Soon after our move to Columbia, Vince, Eric, and I began a series of studies examining long-term habituation and sensitization at a mechanistic level. Informed by synaptic studies that we had carried out previously (Carew and Kandel 1973), Vince and I examined the synaptic connections between identified sensory neurons and motor neurons making up an elementary component of the reflex circuit for the withdrawal reflex that we studied. We discovered that we could in fact identify clear changes at the synapses of intact animals that had received the behavioral training. Specifically, in one set of studies, we found that, in animals that had received long-term habituation training, the synapses were significantly depressed for more than a week (Castellucci et al. 1978). In striking contrast, in a separate set of studies we found that, in animals that had received long-term sensitization training, the same synapses were enhanced, also for more than a week (Carew et al. 1979).

The Quest for Associative Learning

A major feature of learning and memory in higher animals (including humans) is the ability to associate stimuli with either a positive or negative consequence. In our laboratory, we were extremely interested in examining whether *Aplysia* could exhibit this type of associative learning.

Our first step in this direction was carried out with a talented new graduate student named Terry Walters. Terry, Eric, and I examined a form of associative learning in *Aplysia* that commonly was known as "fear conditioning." We found that *Aplysia* could learn to associate the taste

and smell of a chemical in seawater with a negative consequence, such as tail shock. Specifically, after pairing the chemical stimuli with tail shock, subsequent delivery of this chemical stimulus alone would enhance the reflex responsiveness in the animal. We went on to study this interesting form of associative learning at both a behavioral level and at the level of neural correlates of the learning (Walters et al. 1979, 1981; Carew et al. 1981). This was the first evidence of associative learning that we identified in *Aplysia*.

Differential Classical Conditioning

Perhaps the most familiar form of associative learning is called "classical" or "Pavlovian" conditioning. A major goal of the lab for many years was to examine whether *Aplysia* were indeed capable of classical conditioning. A wonderful new postdoctoral colleague, Bob Hawkins, recently had joined the laboratory, as had a very bright young behavioral technician, Emily Marcus, who had just graduated from college. So the team was ready to go. We set out to examine the question of whether *Aplysia* were capable of exhibiting bona fide classical conditioning. We struggled with this problem for months and finally, after several frustrating attempts, we were successful. Specifically, we discovered that *Aplysia* were fully capable of a rather sophisticated form of associative learning called "differential classical conditioning" (Carew et al. 1983). We went on to study this phenomenon in behavioral detail, and subsequently Bob, Eric, and I, together with a new postdoctoral colleague, Tom Abrams, were able to identify a powerful candidate synaptic mechanism for classical conditioning called "activity-dependent facilitation" (Hawkins et al. 1983; see also Walters and Byrne 1983). These were indeed exciting times in the Kandel laboratory.

My First Grant Award!

During these years in Eric's lab I was privileged to be free from the stress of having to get independent grant funding. That said, it was truly important for me to jump into this arena and learn the ropes of National Institute of Health (NIH) applications. My first serious foray into the grant world was my submission for a Career Development Award from the National Institute of Mental Health (NIMH). And what do you know, I got the award the first time out. Moreover, I was truly fortunate to renew that award twice more. Thus, I had continuous funding by the Career Development Award from 1975 to 1987. I remain deeply appreciative of this early support of my work by the NIMH. I have been privileged to enjoy continuous funding from NIMH throughout my entire career (to the present day, more than 42 years).

Moving On

In the fall of 1982, Eric and I were both invited to speak at a conference on learning and memory held at Princeton University. At that conference, I was approached by a senior member of the Psychology Department at Yale, Allan Wagner, a renowned learning theorist whose work I deeply respected and admired. After a brief introductory chat, he asked me a straightforward question: Would I ever consider joining the Psychology Department at Yale? I was both truly surprised and deeply honored by the question. The answer was simple: of course, I would.

Soon thereafter, I visited Yale and gave a talk to the students and faculty of the Psychology Department, as well as to the broader neuroscience community. They were an intellectually stimulating group of people who were great fun to meet and get to know. Following this visit, I was fortunate enough to receive an offer from Yale. After a few weeks of discussions with Yale (and a good bottle of wine with Mary Jo to consider what this opportunity would mean for the family), we were on our way to New Haven.

The Yale Years

As soon as I arrived at Yale, it was very important to me to set up an active, independent laboratory. I was extremely fortunate to have an early key partner in this endeavor, as Emily Marcus, that very talented behavioral technician who had joined the Kandel lab at Columbia a few years earlier, agreed to come with me to Yale to help establish the new laboratory.

Setting Up the Lab

Emily and I worked tirelessly for months setting up the lab. One notable memory deserves mention. In the lab we had several large (400 gallon) seawater tanks in which to study the behavior of *Aplysia*. To keep each animal separately housed, we developed a system of individual "cages" that were actually plastic colanders, individually fitted with small Styrofoam cubes to keep them afloat in the tank. To set up this behavioral system, one day Emily and I set out to a local large grocery store to buy the colanders. We found about three dozen of them on the shelf and put each and every one of them in our shopping cart. When we went to the checkout stand, the cashier looked at us strangely and remarked: "Man, you folks must really like spaghetti!" Now I had two choices: One was to tell her exactly what we were going to do; namely, individually house marine mollusks in a very large aquarium to study their learning and memory. The other option was to simply say, "Yeah, we really can't get enough of the stuff." Prudence was the better part of valor: I opted for the latter explanation, leaving the cashier with a good story to tell her coworkers later that day.

Now What Do I Do?

In setting up my lab at Yale, a major goal was to chart out new experimental territory to establish my scientific independence. I knew that I wanted to continue to examine learning and memory at multiple levels of analysis, and that meant continuing to use the powerful system of *Aplysia* that I was so fortunate to learn in the Kandel lab. There were two novel areas that I wished to establish right away, both using *Aplysia*. One program focused on the development of learning and memory, and the other on neural mechanisms underlying operant conditioning. In both of these endeavors, I was extremely fortunate to be joined by very talented colleagues.

Development of Learning and Memory in Aplysia

The overall rationale for the developmental program in the lab was this: Development can serve as a powerful dissection tool with which to examine the relationship between different forms of learning. Here is a simple example. Two forms of nonassociative learning that were commonly studied at behavioral and cellular levels in *Aplysia* were sensitization (the facilitation of a nondepressed reflex response) and dishabituation (the facilitation of a previously habituated response). Both can be induced by exactly the same stimulus (e.g., a mild shock to the tail), and phenotypically they look identical. But are they? One way to answer this question is to examine when these forms of learning emerge developmentally. It turns out they have very different developmental timetables (see below), showing that they are indeed, at least in part, different processes. The ability to launch this major research program at Yale was founded on previous groundbreaking work of Arnold Kreigstein, Steven Rayport, and colleagues in the Kandel lab who were responsible for several technical and conceptual breakthroughs in the analysis of the development of *Aplysia* from their early larval stages all the way into adulthood (Kriegstein et al. 1974; Kandel et al. 1980; Rayport and Kandel 1986).

The development of habituation and sensitization: In the examination of the development of learning and memory, I hit pay dirt right away, as two gifted postdoctoral fellows, Cathy Rankin and Tom Nolen, joined the lab. Cathy assumed primary responsibility for the behavioral analysis in the project, while Tom took on the cellular component. Also at this time, the lab began to grow further. A talented young undergraduate at Yale, Mark Stopfer, joined the group as a graduate student, and to my delight, Emily Marcus decided to apply to the Department of Biology at Yale and begin her graduate career in our lab. Thus, the lab began to gel into a small but well-organized community.

As we began our behavioral studies, I clearly remember chatting with Cathy about her project. Using a high-powered inverted microscope, she

was going to study the development of habituation of the siphon withdrawal reflex in individual larval animals, each of which looked just like a speck of dust in a test tube of seawater. Cathy held up the tube and asked me straight away: "Can we really do this??" And with great confidence I answered, "Absolutely!" And then (as Cathy learned later), I went across the hall, closed my office door, and asked myself the question: "Can we do this?" Luckily, the "we" was Cathy, who had magic hands and plenty of resolve.

So Cathy and Tom, together with Emily and Mark, tackled the developmental project with equal amounts of creativity and high energy (with a healthy dose of skepticism thrown in, appropriately so, given the extremely challenging technical nature of the experiments on both behavioral and cellular levels). But thanks to their hard work and perseverance, the overall project proved quite successful and offered novel insights into the relationship among three important forms of nonassociative learning: habituation, dishabituation, and sensitization, each of which we found to emerge behaviorally according to different developmental timetables (Carew 1989).

In the ensuing years, I was extremely fortunate to have a number of extraordinary colleagues join the lab both as graduate students and as postdoctoral fellows. Among the postdocs were Tom Fischer, Nigel Emptage, Carolyn Sherff, Bill Wright, Diana Blazis, Julianne Mauelshagen, Sharen McKay, and Lore Gruenbaum. These young scientists were highly motivated and soon identified projects that would allow them to pursue their interests in the laboratory. I'll expand on this theme shortly.

At different times, we also were joined by three extraordinary senior scientists: Alison Mercer from Otago University in New Zealand, Uli Mueller from the Free University in Berlin, and Yadin Dudai from the Weissman Institute in Israel.

In addition to these postdoctoral fellows and senior scientists, several graduate students came on board. These bright young folks included David Cook, Fred Kinsey, Kent Fitzgerald, Laura Stark, René Marois, Teresa Nick, Stephen Fisher, Adam Bristol, Angela Purcell, and Michael Sutton.

So those were the Yale teammates who collectively, over a span of 15 years, made this phase of my career a true joy, both scientifically and personally. Let me now give you a sense of some of their accomplishments.

Developmental timetables for behavioral plasticity: The deep theme of the developmental project informed many of our studies in those years. Kathy, Tom, Mark Stopfer, Emily Marcus, and I published several papers examining different forms of learning in terms of their developmental timetables and underlying mechanisms (Rankin et al. 1987; Rankin and Carew 1987, 1988, 1989; Nolen et al. 1987; Nolen and Carew 1988; Stopfer and Carew 1988; Marcus and Carew 1998; Stark and Carew 1999). This team was also able to differentiate and analyze a wide range of behavioral plasticity in the adult animal. For example Emily, Tom, Kathy, and I found that, just like in

the developing animals, we could behaviorally dissociate habituation, dishabituation, and inhibition in adult animals (Marcus et al. 1988a). This finding paved the way for important future mechanistic studies.

Structural development of a modulatory serotonergic circuit: Another extremely talented graduate student in the laboratory, René Marois, tackled a very difficult problem. René wished to study the development of the modulatory circuit in *Aplysia* at the ultrastructural level. René was fearless in his project. Because none of us in the laboratory had ever done electron microscopy before, René got the training he needed from another extremely helpful colleague at Yale, Susan Hockfield (who, a few years later, went on to be the president of MIT). Thanks in large measure to Susan's critical guidance, René produced a seminal series of papers describing in detail the ultrastructural development of serotonergic modulation in the larval stages of *Aplysia* (Marois and Carew 1997a, 1997b, 1997c).

Operant Conditioning in Aplysia

The second novel project that we tackled was the examination of a form of associative learning called operant (or instrumental) conditioning. The rationale for this project was this: Because *Aplysia* had proven to be such a powerful system for studying diverse forms of nonassociative learning (habituation, dishabituation, and sensitization), and a major form of associative learning (classical conditioning) at a mechanistic level, it might also prove to be a good system for studying another major form of associative learning, operant conditioning.

At about this time, David Cook asked to join my laboratory as a graduate student. David arrived with lots of ideas and full of energy. He approached this difficult problem with great creativity and came up with a way to establish that *Aplysia* could indeed exhibit operant conditioning. And he did so in a novel way: David took advantage of a natural occurring behavior of *Aplysia* called head-waving. When the animal is suspended above a substrate underwater, it naturally waves its head back-and-forth searching for food or for a place to gain a foothold. David found that *Aplysia* do not like bright light. So he combined these two facts and asked whether he could operantly condition *Aplysia* to change their head-waving response in response to the presentation of bright lights (this would be called negative reinforcement in the learning trade). David found that he could indeed teach *Aplysia* in this fashion. Animals readily learned to change their head-waving response to avert the bright lights (Cook and Carew 1986). This constituted a basic but effective form of operant conditioning in this simple model system. For his thesis, David went on to examine operant conditioning at three levels: (a) at a behavioral level, (b) at the level of identified electrical activity in the muscles that produced the operant response (head waving), and (c) at the

cellular level of identifying possible reinforcement pathways in the central nervous system that mediated this form of learning (Cook and Carew 1989a, 1989b, 1989c; Cook et al. 1991).

Temporal Domains of Memory

Another theme that emerged during the Yale years was the examination of the mechanistic differences between various temporal phases of memory. We examined three phases in depth over the ensuing years (and we continue to do so to this day in my current lab at NYU): short-term memory (lasting minutes), intermediate-term memory (lasting hours), and long-term memory (lasting days to weeks, and even longer). As I will illustrate with several examples, we found that the best way to differentiate these three temporal domains was not on the basis of time, but rather on the basis of what we call their "molecular signatures." This overall thread of research that began at Yale has remained an active focus of our research efforts for more than 20 years.

Long term in the absence of short term: Allison Mercer, Nigel Emptage, and I combined pharmacological and synaptic physiological approaches to examine different forms of synaptic plasticity at the sensory neuron to motor synapse in the reflex circuit. In one of our studies, Allison made use of a specific pharmacological blocker that differentiates different kinds of serotonergic receptors and, with Nigel, found that we could completely block short-term synaptic plasticity, while leaving long-term plasticity completely intact (Mercer et al. 1991). In another study, Nigel and I went on to conclusively show that long-term synaptic facilitation could be observed in the complete absence of short-term facilitation (Emptage and Carew 1993). These studies were a major breakthrough for us, because they addressed a fundamental question in the neurobiology of learning: Is short-term memory required for long-term memory? Allison and Nigel's data showed quite clearly that it was possible to generate clear long-term synaptic facilitation (the cellular correlate of long-term memory in *Aplysia*) in the complete absence of short-term facilitation.

Synaptic analyses of dynamic gain control in a well-defined neural circuit: I first met my postdoctoral fellow Tom Fischer when he was a student at the Neural Systems and Behavior course at the Marine Biological Laboratory in Woods Hole, MA, in which I participated for several years (more about this later). Tom was a gifted synaptic physiologist. He became very interested in an inhibitory circuit for the withdrawal reflex in *Aplysia* and carried out several seminal studies in this well-defined circuit. With several colleagues, including Diana Blasis, a new postdoctoral fellow, Tom showed that this inhibitory synapse could provide a form of "dynamic gain control"

in the reflex circuit (Fischer et al. 1997). In a particularly interesting study, Tom further expanded our horizons by elucidating a phenomenon called "meta-plasticity," which essentially means that the plasticity exhibited in this dynamic gain control process could *itself* be shaped by experience. This exemplified the power of combining synaptic physiology with a conceptual analysis of neural processing in this simple reflex circuit (Fischer and Carew 1995a, 1995b; Fischer et al. 1997; Fischer et al. 2000).

A final word about Tom is relevant: In addition to being a terrific scientist and a die-hard hockey player (who often came back from a "lunch break" at the Yale hockey rink with a few bruises to his credit), he was also a deep thinker. For example, in a lab meeting one day, he posed the question: "24 hours in a day; 24 beers in a case; coincidence?" As I said, Tom was a truly thoughtful colleague.

Synapses and cell bodies learn to get along: Another creative postdoctoral colleague, Carolyn Sherff, significantly advanced our understanding of the induction of long-term synaptic facilitation. In a series of clever experiments, Carolyn developed a chamber in which the cell bodies of the sensory neurons could be functionally separated from their synapses onto motor neurons by means of a watertight barrier. This allowed Carolyn to independently manipulate the molecular environment of the synapses, or the cell bodies. In one experiment, Carolyn asked whether local application of the neuromodulator serotonin at the sensory neuron *synapse* could contribute to the induction of long-term synaptic facilitation produced by serotonin applied at a later time to the *cell body*. And, indeed, she proved that was the case. She found that long-term synaptic facilitation could be induced, with a striking form of "cooperativity" between the cell bodies of the sensory neurons and their remote synapses (Sherff and Carew 1999).

The Molecular Architecture of Learning and Memory

A deep theme of our work for many years (and to the present day) has been the examination of the molecular mechanisms that underlie the diverse forms of learning exhibited by *Aplysia*. This research program really flourished at University of California Irvine (UCI, as I will describe). But without doubt, it had its inception at Yale, with the help of two friends and colleagues, Uli Mueller and Yadin Dudai.

Protein kinase A: I mentioned before that Uli Mueller, old friend and colleague from Berlin, spent a year in my laboratory at Yale. Uli had a deep understanding of second messenger signaling in neurons. His scientific work was primarily in honeybees, so he was no stranger to invertebrate neurobiology. When he came to the lab at Yale, we decided to examine different phases of activation of a key second messenger in neurons, the cyclic

AMP-dependent protein kinase, or protein kinase A (PKA). PKA already has been well established as an important second messenger system in *Aplysia* through the groundbreaking work done by Jimmy Schwartz and his colleagues back in the NYU days. What Uli brought to our laboratory was a novel technique that would allow us to examine in great detail the temporal choreography of different phases of PKA activation in the sensory neurons. In one series of experiments, Uli and I found that serotonin produces temporally and mechanistically distinct phases of persistent activation of PKA in the sensory neurons (Mueller and Carew 1998). This finding significantly enhanced our understanding of the role of this critical second messenger system in the production of long-term memory in *Aplysia*.

Another important step in our molecular studies was provided by Yadin Dudai, who spent several months in the lab at Yale and introduced us to additional molecular techniques for studying cAMP in *Aplysia*. This greatly aided our work at the time and would prove to be especially valuable for our later work. He was also a real mensch.

Molecular pathways and memory formation: An additional series of experiments began with the arrival of a new graduate student, Michael Sutton. In his first series of studies, Mike examined two distinct forms of synaptic facilitation at the sensory-motor synapse. Complementing the findings that Uli provided a year earlier, Mike found that parallel molecular pathways mediate the expression of two distinct forms of intermediate-term facilitation at the sensory-motor synapses (Sutton and Carew 2000). Through these experiments, Mike opened up an entirely new direction of experimental inquiry in the laboratory, one that greatly advanced our collective understanding of the temporal domains of memory formation.

Early Studies of Growth Factors in Aplysia*: Foreshadowing the Future*

Soon after a talented developmental biologist, Sharon McKay, joined the lab as a postdoctoral fellow, we began to examine the role of specific molecules, "growth factors," that are critical in wiring up the brains of developing animals. Growth factors were first identified by Rita Levi-Montalcini and Stanley Cohen, who showed that these molecules are critically important during the development of the nervous system. For this groundbreaking work, Levi-Montalcini and Cohen received the Nobel Prize in 1986.

Sharon and I were interested in the question of whether growth factors might also have significant effects in adult animals, specifically in the induction of synaptic and behavioral plasticity (McKay et al. 1999). About that time another postdoctoral fellow, Laurie Gruenbaum, also joined our group. I was extremely fortunate to have Laurie come to the shop because she had been a graduate student with Uli Mueller back in Berlin, and thus was truly well trained. A final critical player in this new growth factor team was Angela

Purcell, a new graduate student in the lab. Angela was highly motivated and tenacious in her experimental pursuits. This small team of colleagues in the lab was very energetic and productive. The collective insights provided by the team's work showed that growth factors in *Aplysia* could significantly influence the following: (a) the pattern of growth of identified neurons, (b) the specific kind of facilitation that is induced at the synapse, and (c) specific molecular cascades during long-term synaptic facilitation (Gruenbaum and Carew 1999; McKay et al. 1999), These early findings helped to launch an experimental program that, to this day, remains a central focus of my laboratory.

A tragic postscript: One of the most gifted and committed young scientists I have ever met was a young postdoctoral fellow in my lab, Julianne Mauleshagen. Julianne had been a graduate student with Randolf Menzel in Berlin. She came to my laboratory to study synaptic mechanisms of learning and memory (Mauleshagen et al. 1996, 1998; Bunge et al. 1997). Julianne had two great passions: science, and mountain climbing. When she wasn't in the laboratory, she was scaling a mountain somewhere around Connecticut. One evening when I was at Cold Spring Harbor for a scientific conference, I got a phone call that I will never forget. Julianne had tragically fallen from mountaintop in upstate New York and was killed. The lab was in shock, for we all loved Julianne and truly admired her for her extraordinary dedication to her work. A few weeks later, two communities gathered together in a meadow near Yale to share our memories of Julianne: the scientific community from my laboratory and the climbing community from the local region. I can still see that meadow.

The Berlin connection: The fact that several of the colleagues who joined me at Yale were from Berlin, and specifically, the Free University of Berlin, was no coincidence. This relationship with colleagues in Berlin arose from a long-standing friendship with Randolf Menzel, a colleague, at the Free University. Randolph, a neuroethologist, was one of the leading experts in the world in honeybee behavior. In Berlin, Randolf had assembled a phenomenal group of scientists to study the learning ability of this fascinating animal. Randolph and I met at a conference in Europe some years earlier and had become close friends. Over a period of five years, for a month or so each year, I was delighted to be a guest professor in Randolph's laboratory at the Free University. I will never forget those years in Berlin, as they introduced me to a new culture; a new way to examine and think about behavior; and, most important, to a wonderful new set of friends and colleagues.

Teaching at Yale

One of the reasons that I was highly motivated to join the Yale faculty in 1983 was that it would allow me to reconnect to something I truly valued:

the opportunity to teach undergraduates. After I first arrived, I taught several types of courses and came to realize that an important dimension of a complete academic experience was missing for our students. Specifically, there was no introduction or exposure to the analysis of brain mechanisms of naturally occurring behavior. Broadly speaking, this discipline is called "neuroethology." I was fascinated by this discipline and began reading avidly to develop a comprehensive course in this area.

Writing a textbook . . . no problem! After teaching this course for several years at Yale, it occurred to me that it might be worthwhile to write a textbook describing some of the extraordinary studies that had been carried out by leaders in this field, such as Karl Von Frisch, Nikolaas Tinbergen, and Konrad Lorenz, who collectively won the Nobel Prize in physiology or medicine in 1973. Little did I know what I had in store for me. By this time in my scientific career, I had written literally dozens of original scientific papers as well as several scholarly reviews. So I thought that I was well prepared for the task of writing a textbook. How hard could it be? I will never forget one summer in Maine. We had a summer home on a lake where I spent the entire summer writing the first chapter of the book, which focused on echolocation in bats. I read avidly, collected every scholarly paper I could find, and set out to tell the story. I wrote every day and, at the end of two long, arduous months, I looked at what I had written and realized it was terrible. Perhaps the most challenging but important decision I ever made in my career as a teacher was to tear up the entire manuscript and start from scratch. So what was wrong with my work, telling the story of echolocation? The problem was simple: It was extremely scholarly, it was a very comprehensive review of the literature, and it was unbelievably boring. I did not tell a story, nor did I allow the reader to *engage* in both understanding and appreciating the amazing abilities of these animals. It was simply a dry, scholarly work best suited in the hands of a curious professional in the field, not a student's hands.

So I started over and asked myself: "How in fact do I *teach* this material when I lecture in front of students in a classroom?" I don't try to simply provide them with a scholarly perspective (although this is certainly part of the goal), but rather, I also try to engage students and allow them to come along for the ride as they learn about the phenomenal ability of bats to navigate their world with ultrasound and echolocation, using highly specialized brain mechanisms such as a neural Doppler-shift analysis to distinguish, while on the wing, between a flying moth and a falling leaf.

Once I got back on track with this kind of mind-set for the book, I worked steadily for two years, learning about a wide range of animals in their natural environment, and how the brains of these animals provided them with elegant mechanisms for successful adaptation in their world. And during this time, I was extremely fortunate to have many ethological

colleagues around the world who were experts in these specialized areas, and who were willing to read my chapters and work critically with me, to keep me on track in a scholarly domain. The book finally saw the light of day in 2000 (Carew 2000).

1990 Was a Good Year

A teaching prize: Over the years, my course, called "The Cellular Basis of Behavior" (or CBOB, as the students affectionately called it) grew substantially, often enrolling several hundred students. Teaching this course was one of the high points of my career at Yale. And I was truly honored in 1990 to be voted by the undergraduates to receive the Yale College Dylan Hixson prize for excellence in teaching in the natural sciences. This was a real honor that I genuinely appreciate to this day.

Paying the bills: When I arrived at Yale in 1983, I was extremely fortunate to already have extramural funding. As I mentioned previously, while at Columbia I received a Career Development Award, which I took with me to Yale. It lasted until 1987 and was extremely important in the early years. After that, I was fortunate to maintain continuous funding through several other grants, mainly from NIMH, and also crucially, for many years from the National Science Foundation. But in 1990, I received a surprise: a letter from NIMH that I opened late one night in the lab. It looked to be pretty routine; so I just scanned it. Then I looked again. I remember walking down the hall to my postdoc Bill Wright's office and saying, "Would you read this . . . does it say what I think it does?" In Bill's inimitable way he said, "Yep." The letter said that I had been awarded a MERIT Award from NIMH that provided 10 years of uninterrupted funding. It was a good night.

An endowed chair: To cap off 1990, at the end of that year, I was awarded an endowed chair, being named the John M. Musser Professor of Psychology. Being recognized at Yale in this fashion was quite special.

A Visit from Jim McGaugh

It was common at Yale, as at most major research universities, to have famous scientists come to give one or more talks about their work. Thus, it was no surprise that a leading figure in behavioral neurobiology, James McGaugh, came to give a talk. Jim was not just a pioneer in developing this field at UCI, but also one of the founding fathers of the university itself. He also happened to be an old friend from my graduate school days at UCR. So, when he visited Yale, Mary Jo and I invited him to stay at our home rather than stay in a hotel (not an unusual occurrence in our family). After his talk and the usual dinner with other faculty, we came home and sat around the

fireplace chatting. Jim had a funny grin on his face and said, "You know I feel a little awkward being a guest of Yale and asking you this question." That said, pressing on, he asked whether I would be interested in the possibility of coming to UCI to be the chair of the Department of Neurobiology and Behavior. Having held this department in very high regard for many years, I found this a seriously intriguing possibility. To make a long story short, I made a couple of very productive visits to UCI and truly enjoyed meeting the faculty and students. After a third visit, when Mary Jo and I returned from Irvine to New Haven, we broke out a bottle of wine to think it over, and we decided to take on this new adventure. So we packed our bags and headed back west.

The University of California Irvine Years

I was extremely fortunate to have several of my Yale colleagues join me in my move to UCI. These included my long-term colleague Carolyn Sherff, and a new postdoctoral fellow, Stephane Marinesco. In addition, four graduate students, Mike Sutton, Angela Purcell, Adam Bristol, and Joanna Schaffhausen also elected to come along, and a very talented undergraduate at Yale, Martha Bagnall, who had worked in our laboratory in New Haven, also opted to move with us. Finally, a critical member of our Yale group, our lab manager Paul Hofstadter, also elected to join us, rounding out our UCI research team.

In large part thanks to all of these colleagues, the move to UCI was extremely easy and efficient. The moving van arrived on a Monday, and by Friday the lab was, for the most part, up and running. We had only one rule: Everyone had to replicate the last experiment they did in New Haven to make sure that they still worked on the West Coast.

In addition to the colleagues who moved with me from Yale, I was joined at UCI by a new, highly talented postdoctoral colleague, Shiv Sharma, and a few years later, by a second postdoctoral fellow, Lu Pu. Finally, I was extremely fortunate to have several very bright graduate students join the lab during the UCI years. They included Kathryn (Kate) Reissner, Justin Shobe, Gary Phillips, Shara Stough, Xiaojing Ye, Soren Fischbach, and Ashley Kopec.

Chairmanship of Neurobiology and Behavior

Before I discuss the scientific programs in our laboratory at UCI, I want to spend a moment discussing a new role for me in my career: being the chair of a major neuroscience department. I had indeed been chair of the Department of Psychology at Yale for six years. I thoroughly enjoyed that experience and learned a great deal from it. But Yale had a tradition going back more than 350 years, whereas UCI was only 35 years old when I joined it. Thus, I

had the opportunity at UCI to deeply engage in the continued development of an exciting, young department, the Department of Neurobiology and Behavior (NB&B). Moreover, I was privileged to receive an endowed chair at UCI, being named a Donald Bren Professor of the University. There were only two other Bren Professors at the time: F. Sherwood (Sherry) Rowland (who won the Nobel Prize in Chemistry in 1995), and Franciso Ayala, a world-famous evolutionary biologist and philosopher . . . heady company, indeed.

The faculty colleagues who I was privileged to work with in NB&B were a highly collegial group and were deeply committed, not only to their own work, but also to the continued growth and excellence of the department. I was also delighted to become a member of the Center for the Neurobiology of Learning and Memory (CNLM) that was established by the University of California Regents in 1983, with Jim McGaugh as its founding director. The CNLM was a lively and exciting group of UCI faculty and students who all shared a deep interest in learning and memory.

In my position as chair of NB&B, I learned a great deal, not only about different areas of neuroscience that were represented in our department, but also about the importance of consultation, transparency, and collaboration in guiding the department. I also learned a lot about myself. For example, I learned that I thoroughly enjoyed the process of recruiting highly talented new young faculty to the department, providing them with as many resources as we could muster to ensure their success, and then getting out of their way and watching them flourish. This was one of the most rewarding aspects of my 11 years as chair at UCI.

I also enjoyed having the opportunity to learn how a major research university actually works, at the level of higher administration. During my time at UCI, I was extremely fortunate to have a wonderful chancellor, Ralph Cicerone; a highly engaged and talented provost, Mike Gottfredson; and a very collegial dean, Susan Bryant, to help me learn the ropes as I developed as chair of NB&B over the following decade or so.

Now back to science. During our 11 years at UCI, my colleagues and I continued some of the principal work that we had begun at Yale and also set out on some new scientific paths to explore.

Temporal Domains of Memory

One of the major themes that we had established at Yale, and we continued to pursue at UCI, was an exploration of the mechanisms underlying different temporal domains of memory. An important collaborator in this enterprise was my graduate student Mike Sutton. Together with Martha Bagnall and others members of the lab, Mike carried out a number of seminal studies examining different phases of memory in *Aplysia*. One key finding in these studies was that different forms of memory in exactly the same

temporal domain could be clearly distinguished by their underlying molecular mechanisms. We were especially interested in a temporal domain called "intermediate-term memory." We found that there were actually *different forms* of intermediate-term memory for sensitization that were mediated by different molecular mechanisms. For example, one form that is induced by repeated trials (either tail shock in the intact animal, or serotonin in cellular experiments) required both the second messenger PKA and protein synthesis, whereas another form, produced in an activity-dependent fashion, required a different second messenger system, PKC, and did not require protein synthesis (Sutton et al. 2001, 2004). Further understanding of the temporal domains of memory was provided by Justin Shobe together with other colleagues in the laboratory. Building on the work of Shiv Sharma in the lab, Justin found that the temporal phases of activity-dependent plasticity and memory in *Aplysia* are mediated by a form of compartmentalized "routing" of the critically important second messenger, mitogen-activated protein kinase (MAPK), in the sensory neurons (Shobe et al. 2009).

It's all in the timing: Another theme that we began to explore at Yale, but took up in earnest when we moved to UCI, was the importance of the patterning of training trials in producing long-term memory for sensitization.

A key finding produced by Mike Sutton and other colleagues in the lab was that long-term sensitization in *Aplysia* behaved just like virtually every other form of long-term memory in the animal kingdom; it is extremely sensitive to the temporal pattern of training trials (Mauelshagen et al. 1998; Sutton et al. 2002). Thus, trials spaced out in time are typically far superior in producing long-term memory than the same number of trials presented in rapid succession. This is called the "massed-spaced effect," which was first described more than a century ago by the renowned learning psychologist Herman Ebbinghaus (1885).

Another seminal finding in the examination of patterning in memory formation was made by Gary Phillips and others in the lab. Specifically, Gary found that long-term memory in *Aplysia* could be induced by only two sensitization training trials (tail shocks), if those trials were spaced by a critical temporal interval, 45 minutes. Surprisingly, the same two trials spaced either by 15 or 60 minutes, were ineffective in producing long-term memory. Only the 45-minute interval provided a "permissive window" for the induction of long-term memory. Gary went on to show that the transient activation of MAPK also was confined to a narrow temporal window that exactly aligned with the behavioral observations: MAPK was significantly activated only in the 45-minute window (Philips et al. 2007; Philips and Carew 2013). This not only provided important insights into the importance of trial pattern in memory formation but also paved the way for critical future experiments examining the role of growth factors in long-term memory.

G-proteins: A final critical observation that gave us further insights into the importance of patterning in memory formation came from the work of a gifted graduate student, Xiaojing Ye. Along with other members of the lab, Xiaojing showed that critical small molecules, "G proteins," exhibited clear pattern sensitivity during MAP activation, both in the induction of long-term synaptic facilitation and long-term memory (Ye et al. 2008). These results critically informed our understanding of the way the nervous system uses its molecular tool kit to induce long-term memory in a pattern-sensitive fashion.

Behavioral, Synaptic, and Molecular Analysis of Long-Term Memory

Mitogen-activating protein kinase: A recurrent theme in some of the studies that I have described emphasized the crucial role of a fundamentally important second messenger MAPK in memory formation in *Aplysia.* MAPK is also known to be critically important in memory formation in a wide range of other species, from invertebrates to mammals. Our understanding of MAPK was significantly advanced by the studies conducted by Shiv Sharma in the lab. Shiv, together with Carolyn Sherff, Martha Bagnall, and Mike Sutton, discovered a differential role for MAPK in three distinct phases of memory for sensitization (Sharma et al. 2003a; Sharma and Carew 2004). Shiv also showed that MAPK was responsible for removing a critical molecular brake (the phosphatase calcineurin) in the induction of both intermediate-term and long-term memory for sensitization (Sharma et al. 2003b).

The neuromodulator serotonin: Earlier work in the Kandel lab had uncovered the critical role that the neuromodulator serotonin plays in the induction of long-term synaptic plasticity in *Aplysia.* A new postdoctoral fellow, Stephane Marinesco, joined my lab to further explore the role of serotonin in memory formation. Stephane developed an electro-chemical detection system using carbon fiber electrodes to directly measure the release of serotonin, in real time, in the central nervous system of *Aplysia* (Marinesco and Carew 2002a). Using this approach, Stephane identified a "distributed serotonergic network" that was activated by the sensitizing stimuli that produce long-term memory. He went on to show that the regulation of behavioral and synaptic plasticity by serotonin was accomplished through its release within local "modulatory fields" in the nervous system of *Aplysia* (Marinseco and Carew 2002b). These studies significantly advanced our understanding of the ways in which the neuromodulator serotonin exerts its diverse effects in the induction of both synaptic plasticity and memory.

Synaptic plasticity underlying long-term memory: In parallel to the behavioral and molecular studies described earlier, another focus of the lab was the study of synaptic plasticity during memory formation. One key study

was carried out by Carolyn Sherff, in which she examined the *coincident induction* of long-term facilitation at the sensory-motor synapses of *Aplysia*. She was able to identify critical factors both on the presynaptic and the postsynaptic sides of the synapse (Sherff and Carew 2002, 2004). In parallel, another important study was carried out by Kate Reisner, a new graduate student who moved to my group from a purely molecular lab at UCI to dive into neuroscience, and by Joanna Schaffhausen, who came with me from Yale. Kate and Joanna discovered a novel *postsynaptic mechanism* for the *sharing* of the critical cation calcium in the induction of short-term plasticity at the synapse (Schaffhausen et al. 2001; Reissner et al. 2010).

Collectively these and other synaptic studies rounded out the three principal levels of analysis (behavioral, synaptic, and molecular) that we routinely used to study learning and memory. Combined, these three levels allowed us to establish a better understanding of the diverse mechanisms used by the nervous system to produce long-term memory.

Growth Factors

A final research program we began at UCI deserves mention. These studies were focused on growth factors, a topic I introduced in discussing our research at Yale. Continuing the rationale that motivated our Yale studies, we became very interested in the possibility that growth factors, in addition to being important during development, might be retained throughout the life of an animal and used (if you will, recycled) in the service of establishing long-term memory. This idea was certainly not novel. For example, the basic notion was suggested in another form by the pioneering Spanish neuroanatomist Santiago Ramón y Cajal, who won the Nobel Prize in 1906.

Tyrosine kinase: A key observation that contributed to this research program was made by Angela Purcell, along with Shiv Sharma, Martha Bagnall, and Mike Sutton. Angela found that an important signaling molecule, tyrosine kinase, along with MAPK, is critically involved in the induction of long-term facilitation in long-term memory (Purcell and Carew 2001; Purcell et al. 2003). It had been well established in other systems before Angela's work that tyrosine kinases are critical molecular brokers of the actions of growth factors in the nervous system. So Angela and her colleagues opened the door for us to begin to study the actions of growth factors at a molecular level in *Aplysia.*

Cystine-rich neurotrophic factor: Another critical step in our understanding of growth factors was taken by Lu Pu, a postdoctoral colleague in the lab. Lu cloned and studied a growth factor in *Aplysia* called *cysteine-rich neurotrophic factor* (*ApCNRF*). She found that this growth factor both

facilitated the growth of neurons in *Aplysia* and also activated MAPK during the induction of long-term synaptic facilitation (Pu et al. 2014).

Growth factors in time and space: A final research program focused on the interaction of different growth factors in memory formation in *Aplysia* was initiated by a graduate student, Ashley Kopec. I will discuss these findings a bit later.

In summary, the analysis of the role of growth factors in memory formation was a central focus in our research program at UCI. The basic idea underlying these studies is that *nature never throws anything away*. Thus, growth factors, which are critical for building brains in developing animals, appear to be "recycled" or "repurposed" in adult animals in the service of memory formation.

Unexpected Honors along the Way

While at UCI I had three lovely surprises. First, soon after arriving, in 2001 I was delighted to learn that I had been elected as a fellow of the American Association for the Advancement of Science. A few years later, in 2005, I was elected chair of the Neuroscience Section of the AAAS.

Second, I had a real treat in 2004 when I received a phone call one afternoon telling me that I had just been elected to the American Academy of Arts and Sciences. This was both completely unexpected and a genuine honor.

Third, in 2007, I was elected to serve as president of the Society for Neuroscience (SfN). Over the years, I had served on numerous committees and as an elected councilor in the SfN, but being elected as its president was truly an honor. Now it would be disingenuous to say that this truly falls under the heading "unexpected honors," as I did, indeed, agree to run for the office. Where the "unexpected" part comes in is that I certainly did not expect to win. That said, it sure was fun to receive the phone call from the then-president and my old friend David VanEssen, telling me of the results. My three years in the leadership of the SfN (as incoming president, president, and past president) was a wonderful experience, one that I will always deeply appreciate.

A Call from Tony Movshon

One day in the early spring of 2011, I received a phone call from an old friend, Tony Movshon. Tony is a distinguished faculty member at NYU, where he is a university professor and a Silver Professor, and at the time, was also the director of the University's Center for Neural Science, which he founded in 1987. He told me that he was on the search committee seeking to fill the position of dean for the Faculty of Arts and Science (FAS) at NYU, and he asked whether I might be interested in applying for the position.

The notion of possibly joining NYU in this position was at the same time daunting and exhilarating. So I said that I would be happy to chat with folks at NYU to explore this possibility, at least informally. After a visit or two, and a long and engaging interview with the search committee (consisting of distinguished faculty, university leaders, students, and administrators), I was hooked: This could be a terrific job. And I was fortunate enough to be offered the position. But should I take it?

A little help from my friends: One of the huge attractions of NYU was its central location in the city of New York, which boasts one of the strongest neuroscience communities in the world. In addition, as luck would have it, an old friend from my Yale days, Richard (Dick) Tsien, had very recently joined NYU as the director of the NYU Neuroscience Institute at the NYU Medical Center. I remember calling Dick one snowy evening from Cleveland, Ohio, where I was on a scientific team reviewing the Neuroscience Program at Case Western Reserve. Dick and I chatted for more than an hour. I appreciated his telling me that he was very happy in his new position, and he encouraged me to take the job. The next afternoon, Susan Amara, another old friend from my Yale days (who was on the same review team) and I got snowed in at the Cleveland Airport. Over a beer, Susan strongly encouraged me to take the position, essentially saying, "Take the chance and go for it!" Susan took her own good advice: A couple of years later, she left a leadership position at the University of Pittsburgh to become the Scientific Director of NIMH. Another friend who helped a great deal was Cristina Alberini. Cristina also had been a postdoctoral fellow of Eric Kandel many years earlier, and although we didn't overlap in Eric's lab, we had become close friends. Cristina was a professor of neuroscience at Mt. Sinai Medical School, so she was already in New York City—a real plus. She also strongly encouraged me to take the position. Finally, I checked in with my former mentor and friend, Eric Kandel, and he too thought it was a terrific opportunity and encouraged me to accept the job.

So once again, back in California, Mary Jo and I pulled out a good bottle of wine and thought it over. We decided that, indeed, we were we both up for a new adventure. So we packed our bags and headed back east. By now, it must seem that, geographically at least, my career looks a bit like a bicoastal tennis match, with the net somewhere in Kansas.

NYU Redux

One of the important conditions of my move back to NYU as dean was the clear agreement that I would be able to maintain my scientific program. This was in no way a problem, as the NYU leadership at the time, President John Sexton and Provost David McLaughlin, were both very supportive of my keeping an active laboratory. To be sure, I downsized considerably. But

I was indeed most fortunate that Ashley Kopec, my graduate student from UCI, decided to come with me in the move to NYU. She was amazing in her energy and high standards: The lab was up and running in no time. In addition, soon thereafter, Gary Philips, my former graduate student from UCI, who had just completed a postdoctoral fellowship, returned to the NYU lab to finish up a long-standing project that was central to our work. Some new colleagues joined us as well. They included another graduate student, Anamaria (Ana) Alexandrescu, and a postdoctoral fellow, Nikolay Kukushkin, who came to us from Harvard. Finally, we were joined by Anastasios (Taso) Mirisis, who initially assumed the role of our lab manager, but now is a full-fledged member of our research team with his own independent project.

In our research program at NYU, we continued two of the major themes that had been the focus of our work for years: One was timing and pattern in the induction of long-term memory, and the other was the role of growth factors in memory formation.

Timing and Pattern in Long-Term Memory

A molecular context: Recall that in his earlier work in the lab at UCI, Gary, along with other colleagues, discovered the importance of a narrow temporal window (45 minutes) that was permissive for producing two-trial long-term memory for sensitization (Philips et al. 2007). Why did the second trial have to arrive exactly in that 45-minute window? In his work begun at UCI and completed at NYU, together with Xiaojing and Ashley, Gary found that the first trial set in motion a chain of events that established a "molecular context" for the second trial to "inherit" at 45 minutes (Philips and Carew 2013). Specifically, the first trial caused (a) the protein synthesis-dependent translocation of activated MAPK into the nucleus of the sensory neurons; (b) the activation of a transcription factor, CREB, that is well known to be critical for long-term memory formation in *Aplysia* and many other species; and (c) the induction of an immediate early gene in *Aplysia*, CCAAT/enhancer-binding protein (*c/ebp*), which is synthesized in response to CREB activation and plays a critical role in long-term facilitation and memory in *Aplysia* (Philips et al. 2013). These were seminal observations, as they illustrated the complex molecular choreography engaged by different patterns of training trials that ultimately support memory formation.

Growth Factors

Time and space: In discussing our work at UCI, I briefly mentioned the work of a highly creative graduate student, Ashley Kopec, who began her project there and continued it at NYU. Ashley was interested in the general question: Might different growth factors play different roles in the

induction of long-term memory? The foundation of this question was as follows. Several growth factors have been implicated in long-term memory, but no single factor can support all of the plastic changes that occur during memory formation. Because growth factors engage highly convergent signaling cascades that often mediate similar functional outcomes, the relative contribution of any particular one to long-term memory is difficult to ascertain. To explore this question, Ashley determined the unique contribution to memory formation of two distinct growth factor families: one signaling via tropomyosin receptor kinase B (TrkB) and the other via transforming growth factor beta (TGF-beta).

Ashley found that TrkB and TGF-beta signaling are differentially recruited during two-trial training in both *time* (by trial 1 or 2, respectively) and *space* (in distinct subcellular compartments). These growth factors independently regulate MAPK activation and synergistically regulate gene expression. She also showed that *both* trial 1 TrkB and trial 2 TGF-beta signaling are required behaviorally for memory formation (Kopec et al. 2015). Specifically, Ashley found that the immediate early gene *c/ebp* is upregulated in sensory neurons in a TrkB signaling-dependent manner by the first trial, and these nascent transcripts are subsequently stabilized by a TGF-beta signaling-dependent mechanism following the second trial. These data are highly instructive, as they support the view that growth factors engaged in memory formation are interactive components of a complex molecular network (Kopec and Carew 2013).

ELAV: In light of the findings just described, that distinct growth factor families are recruited in unique spatial and temporal domains during the induction of long-term memory, we next explored the critical mechanisms and cellular processes that are dependent on this coordinated signaling system. Taso Mirisis, an important member of the lab, together with Ashley, has taken on this task. As mentioned earlier, the immediate early gene *c/ebp* is upregulated by a single training trial, and these new mRNA transcripts then are stabilized by a TGF-beta signaling-dependent mechanism following the second trial (Kopec et al. 2015). A bit of background: a specific region of the mRNA for *c/ebp* contains AU-rich elements (AREs), which function as binding sites for RNA-binding proteins, which modulate the stability of their target transcripts. A well-described member of a family of such binding proteins (*ELAV*), binds to ARE-containing transcripts and increases their longevity by (a) hindering the ability of destabilizing proteins to bind to the AREs, and (b) shielding the mRNA from degradation. Taso's preliminary studies have implicated the coordinated binding of an *Aplysia ELAV*-like protein to *c/ebp* transcript as a prerequisite of long-term memory formation. Why is this important? The novel possibility that growth factor–mediated regulation of mRNA transcripts can be critical for the induction of long-term memory significantly broadens the horizon of

possible molecular mechanisms that are deployed in neurons to form lasting memories.

Cystine-rich neurotrophic factor (ApCNRF): Another avenue of investigation of growth factors in the lab is being carried out by a thoughtful and dedicated graduate student, Ana Alexandrescu, whose work is building on our earlier findings that *ApCNRF* both facilitated the growth of neurons, and activated MAPK, during the induction of long-term synaptic facilitation (Pu et al. 2014). In extending this work at NYU, Ana is exploring mechanistically the *spatial contribution* of *ApCNRF* signaling at the sensory-motor synapse during the induction of long-term synaptic facilitation. Ana has already shown that *ApCRNF* is expressed in *both* the sensory and motor neurons. She is now exploring its mechanisms of action in both these pre- and postsynaptic compartments by taking advantage of the high spatial resolution of the sensory-motor neuron coculture system that was first developed in the Kandel lab many years ago and is widely used in the *Aplysia* community. Considerable evidence indicates that synaptic activity regulates the synthesis, secretion, and signaling of growth factors, which in turn can induce changes in synaptic efficacy and morphology to support long-term memory formation. Ana's experiments are examining the cellular sites of *ApCNRF* synthesis, release, and actions during the induction of synaptic plasticity, using the monosynaptic "microcircuit" of sensory neurons connected to motor neurons in culture. Why is this important? This detailed analysis of a well-identified microcircuit, of known behavioral relevance, can significantly contribute to our understanding of how secreted molecules like growth factors are tightly regulated *specifically and locally* in the synaptic compartment during the induction of a long-term memory.

Insulin signaling: A final component of the growth factor research program in our laboratory is the province of a postdoctoral colleague, Nikolay (Niko) Kukushkin. Niko initiated a research program exploring the growth factor insulin, and his results are already highly informative. Specifically, Niko was interested in the fact that insulin-like signaling is ubiquitous in virtually all animals, but its role in the nervous system is largely unexplored. A critical exception is the recent work of my friend and colleague Cristina Alberini, who by the way, we were fortunate enough to recruit to NYU soon after I arrived. With her colleagues, Cristina identified an insulin-like growth factor (IGF2) that serves a critical role in memory consolidation and, in addition, is a molecule that can act as a potent memory enhancer (Chen et al. 2011).

Niko's work has two focal points. The first is the insulin system itself. He shows conclusively that *Aplysia* not only possesses insulin-like receptors and ligands related to their mammalian counterparts but also exerts effects reminiscent of a "hybrid" insulin/insulin-like growth factor functionality: on the one hand, control of metabolism; on other hand, stimulation of

growth. The second focal point Niko's work examines is the neuromodulatory effects of insulin-like signaling. As a model system, *Aplysia* is most useful for studying neural phenomena at multiple levels of analysis, from molecular to behavioral. In his work, Niko has obtained evidence that implicate the *Aplysia* insulin-like system in a variety of effects that reveal a common theme across these levels: decreased activity and excitability in response to nutrition. Because these effects range from tyrosine dephosphorylation and reduced neuronal excitability to reduced locomotor activity of the fed animals, they paint a clear picture of a systemic, coordinated "rest-and-digest" response directly controlled by insulin-like peptides.

Ending on a theoretical note: "*Memory takes time.*" A final recent theoretical piece from the lab deserves brief mention. Niko and I had great fun recently writing a theoretical review (Kukushkin and Carew 2017), in which we consider the *temporal structure of memory*. We suggest that memory is an adaptation to particular temporal properties of a past event. In neurons, this adaptation can be understood in terms of a hierarchical system of molecular and cellular time windows, which collectively retain information from the past. We propose that this system makes various timescales of past experience *simultaneously* available for future adjustment of behavior.

The basic theoretical notion we advance is this: Memory is represented in the brain much like a "Fourier transform of experience." Just as sound is broken down by the auditory system into many discrete bins of frequencies that are perceived simultaneously, Niko and I suggest that an experience as a whole is parsed by the brain into many "time windows" that collectively represent the past. Most memories last seconds before they are forgotten, but some last a lifetime, and yet at each given moment *both kinds of memory* coexist with ongoing experiences on the same terms. For example, a familiar musical piece is experienced simultaneously through the short-term memory of the few notes just heard and the long-term memory of listening to the piece in the past. Both retain information about the past and both shape perception in the present.

Being a Dean

Soon after I took the job as dean of FAS at NYU, many of my science colleagues would ask me: "What's it like being a dean?" The question was always quite polite, but often was asked as if I had just moved to another solar system, and they wondered what the weather was like there.

It's a big job: Now, indeed, being dean of the largest School at NYU, the FAS, is no small undertaking. There are about 1,000 faculty, 13,000 students, and in excess of 400 staff in the School. Moreover, the FAS spans a broad range of disciplines, which include the humanities, the social sciences,

and the sciences. In addition, there are numerous institutes, centers, and programs with extremely diverse interests and goals. So it's a complex academic landscape, indeed.

It's a fun job: There are three things that I especially enjoy about being a dean of such a large and complex enterprise. First, I am extremely fortunate to have an amazing staff. These colleagues have, as their daily jobs, the management of matters ranging from budgets, to faculty hiring, to human resources, to overseeing physical facilities, to making sure that the dean's office is an efficient and collegial operation. Another group of colleagues within the FAS who also make a huge difference are the three divisional deans (who attend to the humanities, social sciences, and sciences, respectively), the dean of the college, and the dean of the graduate school. All six of us work together as a coordinated team to ensure that the School stays on course.

The second source of great satisfaction is the extraordinary intellectual reach of the job. In a single day in my office, I will be visited by a poet, then a physicist, and then an historian. Typically, these colleagues want to discuss a proposal they have in mind or perhaps a new hire for their department. But I have (a bit playfully) put in place a modest "intellectual tax" for any visit. Before the colleague discusses the actual purpose of his or her visit, I ask that they spend 5–10 minutes telling me about their own scholarly work. Thus, I am privileged to have the opportunity, on a daily basis, for an ongoing tutorial across an amazing intellectual landscape.

The third extremely rewarding aspect of the position is the ability to actually contribute to making a difference in higher education, through the lens of NYU. There are enormous challenges to be sure. Let me highlight just two. First is the critical importance of increasing diversity, inclusion, and equity across all levels of the university, from faculty and students to administrators and staff. A second pressing concern is affordability. There are simply way too many extremely qualified students across the nation and around the globe who cannot afford to go to college. Both of these are of the highest priority in the FAS at NYU. And although there are absolutely no simple quick-fix answers to these complex and critical issues, having the chance to work with faculty, students, and administrators in addressing them is, in and of itself, extremely rewarding.

A few trade-offs: There are, indeed, trade-offs in being a dean. I cannot spend nearly enough time in my laboratory to give my research team the attention they fully deserve. Another trade-off is that being a dean limits the extent of my contact with undergraduates. I still teach an advanced undergraduate course in molecular mechanisms of memory each year, but this brings me into contact with only a modest cohort of students. Nonetheless,

while the number of students I teach is certainly reduced at NYU, their curiosity and boundless energy remains a great source of satisfaction.

Coming full circle: So, here I am, having come full circle professionally from NYU, to Columbia Medical School, to Yale, to UCI, and back to NYU. Each of these institutions has afforded me the opportunity for exceptional professional and personal growth. And over these years, the very best part are the amazing friends and colleagues who have come my way. They make it all worthwhile.

Epilogue

No mom, I haven't finished my experiment yet.

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