Brain and Mind
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Eric Kandel, MD
The Storage and Persistence of Memory

Introduction by Gerald Fischbach

Gerald Fischbach: Thank you, Richard. Just beautiful. Performing that link between molecules and mind is what our goal is in creating a new neuroscience institute in many different areas.

I first met Eric Kandel in the basement of a psychiatric hospital in Boston forty years ago. He’s lost a pound or two since then but he hasn’t changed one bit in those forty years. He was enthusiastic, full of ideas, creative and full of fun then, and he still is now. I have a feeling that Eric is just beginning to take off in his career and that the best is yet to come.

His early studies, what he was doing that day when I met him at [what was] affectionately known as the Boston Psycho was studying the neurons in a very simple nervous system with electrophysiological techniques. And his goal then was to create a simple alphabet of behavior using his training in psychiatry [and] very interested from the beginning in simple behaviors—in this case simple reflexes, the withdrawal of a siphon, a structure in Aplysia californica or habituation—and trying to determine how particular nerve cells in that simple organism's brain could account for that simple behavior. Now this, in addition to a simple nervous system, is a simple idea, but like many simple ideas this is an extremely powerful one. How does the function of individual nerve cells and simple neural circuits account for our behaviors? And it has had an enormous influence on the field over the years and on everyone in the field in this room. And from that simple alphabet, Eric has moved on to ask profound questions about learning and memory even in higher organisms.

He has won many awards, many honorary degrees, culminating, if you want to consider that, culminating in the Nobel Prize in the year 2000. Frankly, I believe the committee just ought to give everyone in this day-and-a-half symposium a Nobel Prize and get it over with, but Eric's was extraordinary. And you have the feeling that now from his position as a university professor he's using that position well to expand his area of interest and influence in all directions.
He is collaborating I understand, or at least discussing, the nature of the brain and art with Jean Magnano Bollinger, who has been interested in this for a long period of time, with a recent exhibit of her scrolls. He's reaching into many different areas. He is returning to his roots in psychiatry with an interest in schizophrenia, has been supported for a long time in this effort by the Liebers and the Lieber Center, and one has the feeling of a completion of aims and ambitions begun when he was a student in Boston in those early years, and now really with escalating energy and insight in his current lab work. He's going to talk to us today about his studies of neuroplasticity and memory. Eric?

The Biology of Memory

Eric Kandel: [inaudible] . . . that Denise is here to listen to you. She would not have believed me had I told it to her in private.

People ask me why I enjoy Columbia so much. I think listening to Gerry, listening to Richard, listening to Tom, you realize why neuroscience at Columbia is so spectacular. I feel that my own good fortune in this area owes a great deal to my wonderful friendships at Columbia, and you saw really superb examples of their work as they presented it.

So in my own talk this morning I'd like to outline aspects of our current understanding of the molecular biology of memory storage, and I'd particularly like to focus at a problem that's interested me recently, that is the persistence of memory. How come one can remember certain events, for example your first love experience, for the whole of your lifetime?

Let me begin by putting the biology of memory into a little bit of a perspective for you. As you know, learning is the process whereby we acquire new information about the world, and memory is the process whereby we retain that information over time. Most of the knowledge we have of the world we have learned, so that in good measure we are who we are because of what we learn and what we remember.

For biologists' interest in mind, like many of us, the study of learning has the further appeal in that it has broad cultural ramifications. It raises some of the vital issues that have traditionally confronted Western thought. What aspects of the organization of the mind are innate? How does the mind acquire new knowledge about the world? Serious thinkers of each generation have struggled with these questions and by the end of the seventeenth century two opposing views have emerged: The British empiricists such as John Locke argued the mind is a blank slate, it does not possess innate knowledge but that all knowledge derives from sensory experience and is therefore learned. By contrast, the German philosopher Emanuel Kant argued that the mind is born with a priori knowledge, preknowledge, that predisposes it to receive and interpret sensory experience in an innately-determined perceptual framework.
In the 250 years since the founding of Columbia University, it has become clear, even to our faculty, that the methods of philosophy could by themselves neither distinguish nor reconcile these conflicting views because the issues they raise revolve around questions of what goes on in the brain when we learn. These questions require a direct examination of the brain and, as you've already learned and you will gather from the other talks at this symposium, in recent years neuroscience has done just that, and we now have a beginning understanding to some of these difficult questions.

To address these questions it is convenient to divide the study of memory storage into two parts, the systems problem of memory and the molecular problem of memory. In the systems problem of memory we ask where in the brain are the various memories stored, what different regions store different kinds of memory? In the molecular problem of memory we ask what are the molecular mechanisms whereby that storage occurs at each site? I'm going to focus primarily in the molecular problem of memory, which is the easier problem. The more difficult problem, as Richard alluded, is the systems problem, but I want to say just something about that.

**Stages in Explicit and Implicit Memory**

One of the major insights to emerge in cognitive neuroscience, in modern neuroscience, is the realization that memory is not a unitary faculty of mind, but exists in at least two major forms called explicit and declarative and implicit and procedural. Explicit memory is what you normally think of as memory, it's the recall of facts and events, it concerns itself with information about people, objects and places, it involves a particular set of structures in the brain, the medial temporal lobes, and a region deep to it called the hippocampus. And the defining feature of explicit memory storage is that it requires conscious attention for recall. This system is in parallel with a very, very different system, a set of storage mechanisms that are concerned with perceptual and motor skills, also involve simple forms of learning, such as classical conditioning, operant conditioning, sensitization, habituation. They involve a different set of deep nuclei in the brain, the amygdala, the cerebellum—the amygdala for learned fear, cerebellum for motor learning—and in the simplest cases of invertebrates the reflex pathways themselves. This is defined by not requiring conscious attention, and if you think about it much of your mental life is carried out without conscious awareness.

The existence of these two very different memory systems, which store different kinds of information, involve different kinds of brain structures and have a different logic, conscious recall and unconscious recall, raise the question, To what degree do they share features in common? Can molecular biology, with its ability to reveal homology relationships, which you learned about in the earlier lectures, delineate commonalties in these two radically different kinds of memory systems?
Now an initial clue to the fact that there might be features in common was actually suggested by William James when he pointed out that all memories have stages, a short-term memory, which last minutes to at most hours, and a long-term memory, which lasts days and weeks. And we now realize that every form of implicit and explicit memory has these stages. Moreover, we know how to convert, in most cases, short-term to long-term memory. It involves repetition, practice makes perfect, just as your mother taught you. And three, we know that in both implicit and explicit memory storage, long-term memory storage differs fundamentally from short-term memory in requiring in its initial steps the synthesis of new proteins. And indicated here by indicating ribosomes, the machinery that is involved in protein synthesis, a point I'm going to return to later on.

This feature, the requirement for protein synthesis, is extremely conserved. You find it not only in explicit and implicit memory storage, but you find it in implicit memory storage in simple animals as well as complex animals. And that conservation suggests the possibility that if the requirement is so conserved perhaps the specific proteins are conserved. And if that's so, then the delineation of proteins in any single context might reveal the schema whereby short-term is converted to long-term memory. And if one can do it in several different contexts, one might be able to get a general idea of a principle of a mental process of how you convert a short-term to long-term memory.

And I would like to consider that with you by using two examples, one the marine snail *Aplysia* to consider a very simple form of implicit memory storage, that of a learned fear, and the other spatial memory in the mouse, the opposite end of the spectrum, if you will, an extremely complex behavior.

**Sensitization and Memory in *Aplysia***

Let me begin with studies of *Aplysia*. This is the marine snail *Aplysia*, this is not only a very beautiful snail but you can tell at a glance a highly intelligent animal. This is the sort of animal any one of you would select for the cellular study of learning and memory. The reason it is so attractive is not only because of its physical exterior but because its brain is remarkably simple. Your brain and mine is made up of a million nerve cells and, as Tom pointed out to you, these are interconnected in a set of complex ways. By contrast, the nervous system of invertebrates such as *Aplysia* consists of 20,000 nerve cells. The nerve cells are collected in clusters called ganglia, and each ganglion contains about 2,000 nerve cells. Moreover, a single ganglion controls not a single behavior but controls a set of behaviors, so the number of cells committed to a single behavioral act can be quite small, and the behavior I'm going to describe to you involves less than a hundred nerve cells.

Not only are there few cells involved in generating the behavior, but for reasons that one doesn't quite understand in the marine snail *Aplysia*, one encounters the largest nerve cells in the animal kingdom. These are gigantic nerve cells. Before I
developed presbyopia I could see them with my naked eye. The largest cells are a millimeter in diameter. Because they are so large after a while you can recognize them and you can give them different names, Gerry, Tom, Richard, you can go down the line—David Cohen—and you can return to the same cell in every animal of the species. So you can look at the same cell in a naive animal, an animal that's trained, and see exactly what has altered in the brain.

Using these advantages we focused in this simple animal with a simple nervous system on the simplest behavior that the animal has, a simple withdrawal reflex like the withdrawal of a hand from a hot object. The animal has an external respiratory organ called a gill, which is covered by a sheet of skin called the mantle shelf, which ends in a fleshy spout called the siphon. If you apply a tactile stimulus to the siphon you get a brisk withdrawal of both the siphon and the gill. This simple behavior, it turns out, can be modified by several forms of learning. And one of the principles that came out of it is even the most elementary behaviors are modifiable by experience. And with each form of learning there's a short-term memory, which does not require protein synthesis, and a long-term memory, which does.

I'm going to focus on a particular kind of learning called sensitization, a form of learned fear, in which an animal learns about the properties of an aversive stimulus and learns to enhance its reflex responses. So if you give the animal a weak tactile stimulus of the siphon you get a modest withdrawal of the gill, but you now give the animal a shock to the tail, the animal recognizes this as being offensive and it enhances its reflex responses in preparation for escape. So the same weak tactile stimulus that previously produced a moderate stimulus, after a noxious stimulus of the tail that same weak stimulus will now produce a much more powerful withdrawal. And the animal will remember that offensive event as a function of number of repetitions. If you give one stimulus you have a short-term memory, which lasts minutes, doesn't require new protein synthesis. If you give five trainings or more, you produce a long-term memory that lasts anywhere from days to weeks, and this requires new protein synthesis. So clearly we want to understand how do you set up the short-term memory and how do you convert it to long-term memory?

The first thing we did was to work out the neural circuit of the reflex, and I show you in simplified form what is already a very simple neural circuit. There are 24 sensory neurons that pick up from the siphon skin, they make direct connections to six identifiable motor neurons, and indirect connections to those motor neurons through inhibitory and excitatory interneurons. When we examined this neural circuit in some detail we were struck, as Tom first pointed out, by the invariance of these connections. There were always 24 sensory neurons, there were always six motor neurons specifically identifiable, and certain sensory neurons always connected to certain motor neuron and to certain interneurons. We saw here in reductionist form Kantians' view of the world. We see that built into the architecture of the brain is preknowledge, the neural knowledge for basic behavior.
But in turn, this raised the paradox, how do you reconcile this with the fact that this behavior can be modified? In order to see what happens with the modification, we looked dynamically on line, in time, what happens in the nervous system when the animal learned sensitization. And we found that when you stimulate the tail you activate modulatory neurons of which the most important component is serotonergic, a transmitter also involved in your brain, and that modulatory system acts in the sensory neurons, including the presynaptic terminal, to strengthen the synaptic connections between the sensory neurons and the motor neurons. If you stimulate the tail only once there's a functioning strengthening, which persists for minutes. This doesn't require new protein synthesis. But if you stimulate repeatedly you release more serotonin and that activates genes in the sensory neurons, which ultimately give rise to the growth of new synaptic connections. This step requires new protein synthesis, the turning on of genes gives rise to proteins that are essential for the growth of new synaptic connections. So we see here Locke's contribution to the thinking of the gill-withdrawal reflex, and a reconciliation in a radically reductionist form of these two points of views; that is, built into the brain is the capability for neural action. But what is not specified in the genetic and developmental program is the exact strength of these synaptic connections and what environmental contingencies—such as learning—play upon is the ability to modify strengths. And you can do that with different forms of learning in both directions.

Molecular Mechanisms of Implicit-Memory Storage

So what is the molecular underpinning of this? In order to consider that with you, let me show you a blowup of the connections between the sensory neurons and the motor neurons. This is the sensory neuron. This is the motor neuron. Tail stimuli activate serotonergic connections. These serotonergic connections act on a receptor in the sensory neurons to engage a system of intracellular signaling mediated in this particular case by a second-messenger system called the cyclic-AMP system. That activates an enzyme in the cell—this is a way of carrying information from the cell membrane into the cell—it activates the cyclic AMP-dependent protein kinase, an enzyme in the cell, and that acts in the presynaptic terminal to strengthen the connections by releasing more chemical transmitter in the way that Gerry Fischbach described it for you. This does not require new protein synthesis, doesn't engage the nucleus of genes, this is a transient change, which lasts for minutes.

But what happens if you stimulate repeatedly? If you stimulate repeatedly the level of cyclic AMP goes up more, and this enzyme, the cyclic AMP-dependent protein kinase, recruits another friend, another enzyme, and they both translocate into the nucleus in order to activate a transcription factor, a control of gene expression, of the kind that Tom Jessell described for you. That transcription factor acts on genes that ultimately give rise to proteins that are responsible for growth of new synaptic connections.
So there are two points here that I want to emphasize for you: One is that learning recreates a program that Jessell described for you in development, whereby signaling pathways activate genes in order to give cells a sense of identity. Here in learning we're seeing how the outside world acts on signaling mechanisms to activate genes that give you a change in state of a preexisting neuron. So one thinks of genes as being the controllers of behavior. It's also important to realize that they're also the servants of the environment, they respond to external stimuli, and learning experiences produce long-term changes by altering gene expression. So insofar as you remember anything in these lectures—you probably want to forget my lecture—but insofar as you remember Richard's or Tom's or the subsequent lectures it's because gene expression is being altered in your brain. This is not heritable, you don't have to worry, your kids are not going to be contaminated by this, but genes are going to be altered in their expression in the brain. And the reason that alteration is important is because it gives rise to the growth of new synaptic connections.

And this is such an interesting point I just want to elaborate it for you. This is what Craig Bailey did in which he labeled individual sensory and motor neurons before and after learning. And you can see in Aplysia where the change is particularly robust that you see an outgrowth of processes in both the sensory neuron and the motor neuron. If you actually count the number of synaptic connections you see it doubling in the number of synaptic connections. So this is really quite profound because, to elaborate on my metaphor, insofar as you remember anything in these talks it is because altered gene expression gives rise to anatomical changes in your brain, so you're going to walk out of this symposium with a somewhat different head than you walked into this symposium with, and all this without taking any drugs. Think of this, think of this, every single person in this room has a somewhat different brain than every other person, if only because of the environmental experiences. Identical twins with identical genes will have different brains because they've been exposed to somewhat different learning experiences.

Explicit Spatial Memory in Mice

So, so far I've talked about the simplest form of memory storage, implicit memory storage. What about explicit memory storage? How does it work and does it use any of this molecular machinery? Is growth also involved? Now simply to remind you, one of the reasons our fondest memories are recruited through explicit memory storage is because it recruits conscious awareness and it allows for mental time traveling. You know, I can sit back and I can think, forgetting about space and time, what it was like to be a kid in Vienna. I can remember coming to the United States. Each of you can sit back and recall events that occurred many, many years ago. You can remember specific places that you were and you can think of exactly what it felt like to be it, it's a remarkable experience how you can really overcome geographical and temporal barriers in order to reach back in your mind to these early events.
And I want to focus in a particular example of that, spatial memory, how one recalls space. A perfect example of mental time travel is to ask a London cab driver. I would not recommend you do this experiment with a New York cab driver. London cab drivers know how to get around the city, and if you ask a London cab driver just to think in his head, or her head, how to get from Hyde Park in the south to Primrose Hill in the north of London, they will close their eyes and if you image them it will light up their right hippocampus, the region that is involved in spatial memory. Moreover, and this will come as no surprise to a sophisticated audience like you, if you ask experienced cab drivers compared to relatively new cab drivers and image their hippocampus you will find that experienced cab drivers will have a larger right hippocampus than will inexperienced drivers. So even in people there is an enlargement of the structure with continued use.

We can explore this in experimental animals. The mouse has a perfectly good spatial memory system, in fact it's exceptionally good considering it's a mouse, and it has a beautiful hippocampus, which is a minor structure of your own. So the animal can find, for example, one hole out of forty that leads to an escape hatch. And it finds it very readily. And we can really begin to try to understand how information about space gets into the hippocampus. And this is really quite interesting as it follows naturally from what Richard told you before. There is for each classical sense modality, for touch, for vision, for olfaction, a topographical, a map-like recreation in the brain, an internal representation that is organized so that neighborhood relationships are preserved. But space is a fiction, space is not a single modality, space is a composite that is put together only in the head so you can reconstruct it. And it requires a number of different senses, visual, tactile, position senses, as well as olfactory. This is put together in a series of cortices, and finally projected into the hippocampus where the map is combined in the most coherent way in a particular subregion called the CA1 region. You can record from single cells in the hippocampus, as John O'Keefe first did in 1971, and see how the spatial map develops. And it's really quite remarkable.

This is a replication that we did of the O'Keefe experiment he first carried out in rats. We worked on mice because we can do genetic manipulations, but this is essentially what O'Keefe found. You can image an animal, a mouse or a rat, moving around in an enclosure with specific markings so it orients those markings, and you can record from a number of different nerve cells simultaneously in the hippocampus. And as the mouse moves around you will find that different cells fire when the animal assumes different positions in space, so some cells will fire here, bur-bur-bur-bur-bur-bur, others will fire when the animal is here, bur-bur-bur-bur-bur-bur-bur-bur, others will fire when the animal is here, bur-bur-bur-bur-bur-bur-bur-bur, and if you bur-bur-bur-bur-bur-bur-bur enough you will be able to see that every single position in space is occupied by one cell that is responsible for that. And if you record from a hundred cells, as Matt Wilson and others have done, you can predict where the animal is in space from its firing pattern.
And I just show you three examples. The yellow pseudocolor means that the animal is moving around in this space but the cell does not fire, so cell number one fires when the animal is at six o'clock, cell number two fires when the animal is at nine o'clock, and cell number three fires when the animal is at twelve o'clock and somewhere in the center. So this is very nice. What is fascinating about this is that unlike other sense modalities every time you move in a space you have to learn the space de novo. And, in fact, you find if you put the animal in an enclosure, within 15 minutes it forms this internal representation, and in optimal circumstances, and I will define for you in a moment what optimal means, that animal will be able to retain that map over a long period of time. You take it out of the space, keep it out of there for several weeks, bring it back into the initial space, it'll replay exactly that same internal representation. You can take it out of the space in which it's familiar, put it into a new space, it'll form a new map with some of the same cells and some different cells, put it back into the original space it'll replay the original map. So this is quite interesting, really a learning process that's going on here and it's distinctive for each space.

Attention and Spatial-Memory Stability

And now we can ask, How does attention fit into all this? Is attention important in the formation of the map, or is attention important for the stabilization of the map in a long time? So it is important for first forming it, or is it important for maintaining it? In order to explore that ability Cliff Kentros developed a graded series of attentional demanding tasks. The first was one just basal attention, the animal walks around without your doing anything particular in order to draw its attention. In my case it would be like my walking around in a fog, as I usually walk around. The second thing is you throw food pellets into the enclosure so the animal's attention is aroused a little bit. A third degree of attention is you in addition to having the animal move in its usual space you introduce a discriminating space, and finally you can really draw the animal's attention by having it do a spatial task. And that is the animal walks around in its enclosure, all of a sudden noises and lights come on. The animal hates that, mice are not like neurobiologists, they don't want the attention, they don't want the publicity, they want to get away from that, and the only way they can do that is to sit onto an unmarked goal region and sit on it for a couple of minutes. That turns all these stimuli off.

And now we ask, How does attention affect the map? And we found that irrespective of the degree of attention, the map always forms and is stable in the short-term. So even basal attention is sufficient to give you a formation of the map and stability in the short-term. But what attention is required for is the long-term stability of the map. And I can illustrate this with one example: So these are two cells taken from the two extremes that I showed you before, from the basal case and the optimal spatial-attention task. If you take the animal that is just basal attention, just barely paying attention, you can see that this cell fires in a particular position, you take it out, three hours later it fires in very much the same position. But in the absence of attention, if you now look at the same cell the next day, the
next day, the next day, the next day, you see that it shifts every day in firing a different position. In contrast, if the animal is paying optimal attention, not only does the map form and is stable in the short-term, but it's like a cookie cutter, every day it fires in exactly the same position. And if you look at the systematic relationship you see that the long-term stability of the map absolutely requires that the animal pays attention, that the map is not stable, as I will show you later on, the animal cannot, without a stable map, remember spatial locations in the long-term, but if the map is stable it remembers the locations in the long-term extremely well.

**Dopamine as a Candidate Mediator of Attention**

But that of course raises the question, Can we use this as a radically reductionist approach to studying attention? How does attention work, how does it play itself out on the neural system, how does it work on the cells in the CA1 region of the hippocampus? And to do that one can take advantage of the fact that we actually know a modest amount of the system's properties of the hippocampus, how association cortex feeds into the hippocampal formation, and how it feeds into this particular region. And one of the characteristic things we know about this region is that this region is extremely important for spatial memory. Larry Squire has shown that if patients have a lesion restricted to this area they have a profound deficit in explicit memory storage, including spatial memory. Moreover, the pathway into the hippocampus, the Schaffer collateral pathway into the CA1 region has been extensively studied, and it shows a characteristic alteration in synaptic strength not too dissimilar [to] what I showed you in Aplysia. This is called long-term potentiation [LTP], and we know a lot about it, and we know that if you interfere with that you interfere with spatial memory.

Now a large number of labs have contributed to working out the signaling transduction pathway for LTP in these hippocampal neurons. And in brief it begins with calcium inflow into the postsynaptic cell, which engages again a kinase that gives you a transient strengthening of synaptic connections. The details differ from implicit memory storage but the principle is very much the same, a functional change in synaptic connections. But if you activate it repeatedly the calcium activates this enzyme that synthesizes cyclic AMP, you recruit the cyclic AMP-dependent protein kinase, you activate genes—actually many more than I indicate here, this is a simplification—and it gives rise to the growth of new synaptic connections. What is also fascinating is that an absolute requirement for turning on genes is the conjoined action of a modulatory input, analogous to the serotonergic one, a dopaminergic input that is required to turn on gene expression.

So we can ask the question my gosh, this looks awfully similar to the salient signal mediated by serotonin. Is this how attention mediates part of its action? Does it come in through the dopaminergic input to the hippocampus? So Cliff Kentros asked this question, he took animals in a completely nonattentive mode and gave them a drug that stimulates these receptors specifically, and he was able to improve this attention deficit disorder in the mouse. Moreover, if you took animals
that paid slightly more attention and compromised this receptor with an inhibitor of the receptor, you could compromise the stability of the place cell even more. So here you could increase the stability of the place cells, and here you could decrease the stability of the place cells.

And now we could do something very nice, we could say, "Look, let's use the power of genetics in order to explore this, let's affect one of these steps and see how this affects the spatial map." And also, since we can use the animals for multiple purposes, we have lots of these mice, we can see how it affects spatial memory storage. And what Ted Abel did when he was in the lab was to produce a line of mice that were compromised in the cyclic AMP-dependent protein kinase, so the whole later steps, all the later steps involving the turning on of the genes and the growth of synaptic connections, could not occur. If you looked at the spatial map in those cells in those animals you found that they formed perfectly well within one hour, because that does not require the dopaminergic input and attention, but it was unstable at 24 hours. If you now in these same animals asked, "To what degree is the spatial map necessary for spatial memory?" you find that spatial memory also is impaired. Perfectly good in the short-term when the map is stable but compromised in the long-term.

So this is really quite interesting because it suggests that despite the fact that explicit memory storage and implicit memory storage are radically different in terms of the neural systems that use them, and the nature of those neural systems, the storage mechanism per se shares core features in common. In each case a signaling system that involves importantly the cyclic AMP-dependent protein kinase activates genes, they give rise to the growth of new synaptic connections, and a modulatory system is importantly recruited to trigger the long-term process.

What is interesting and really sort of struck us as we thought about it is we even saw a fundamental difference between implicit and explicit memory. Think of it, implicit memory, like explicit memory, uses a modulatory system in an important fashion. But we would argue that the difference between the two is how that modulatory system is recruited. In implicit memory storage it's recruited in a bottom-up fashion, unconsciously, if you will, by activating the tail, the tail sensor neurons contact directly the serotonergic cells. By contrast, in explicit memory storage, we know that the information comes from the cortex itself, from the prefrontal and posterior parietal cortex, then projects down in the dopaminergic system, so you have a top-down influence. So the difference between implicit and explicit is between unconscious attention, if you will, and conscious attention.

**Local Protein Synthesis and New Synaptic Growth**

But given the fact that in various forms of learning, and this sort of schema has now been shown to apply to a number of different learning processes, both implicit and explicit, it really raises a fascinating question that I want you to think about. If long-term memory involves gene expression and therefore the nucleus, an
organelle that is in principle in contact with every synapse of a neuron, does that mean every time you turn on a long-term process in your brain it must necessarily involve every single synapse, that it’s a neuron-wide process? If that was so it would limit tremendously the computational power of the brain. A single neuron has not one but a thousand different synapses, which contact a number of different target cells. Does that mean every time you throw a switch for the long-term process you throw the switch for all of these, or can you use a transcriptional mechanism and restrict expression to some synaptic terminals and not others?

This is a question Kelsey Martin addressed when she was in the lab. She reconstructed, really based on a methodology that Gerry Fischbach developed, the gill-withdrawal reflex in dissociated cell culture. She took a single sensory neuron, two motor neurons, quite distant apart, the critical elements of the neural circuit of the gill-withdrawal reflex in *Aplysia*, and puffed on serotonin and showed she could simulate the learning process perfectly well in the dish. When she stimulated once briefly she simulated the short-term facilitation, lasted minutes, restricted to this synapse, nothing there. More surprisingly, when she gave five pulses she produced a long-term facilitation that lasted days. This required new protein synthesis. This required alterations to the gene expression. If you block CREB in the nucleus you didn't see this; moreover, it involved the growth of new synaptic connections. But if you looked here you saw nothing. So you can get a transcriptionally-dependent long-term process with the growth on new synaptic connections at one set of terminals and not others.

And that raised the question, How does this come about? And Kelsey figured out that a signal goes back to the nucleus to activate gene transcription. Gene products are sent to all the terminals, but only those terminals that are marked by the short-term process can utilize those gene products productively, those proteins and messenger RNAs that are coming down productively, in order to grow new synaptic connections.

So that raised the question to the next level: What is the nature of the marking signal? And Kelsey made very good progress in working even this step out. She showed that when you activate a set of synapses with serotonin you send a message back to the nucleus, gene products are sent to all processes but only those that are marked can utilize that successfully for the growth of new synaptic connections. And the mark has two components to it: A cyclic AMP-dependent protein kinase, the same kinase that I showed you before, is necessary for the growth of new synaptic connections. But there’s another surprising component. We’ve known for many years that there is, in addition to the cell body, there is in each synapse a machinery for locally synthesizing proteins. And her study made it clear that this machinery is part of the mark, that if this is not activated this growth occurs but is not maintained. So even if this growth occurs by just marking the cyclic AMP-dependent protein kinase, if you don’t allow protein synthesis to occur that growth retracts in front of your eyes. So if you block protein synthesis you will see that within a day or two that synaptic growth retracts. Now this is a remarkable
result because it made one realize for the first time that setting up the long-term process, growing the next synaptic connections, is only part of the machinery for maintaining something through the lifetime of an organism. You need a local protein synthesis in order to maintain that process, to perpetuate it over time.

**A Prion-Like Mechanism to Carry Memory**

And that of course raised the final question: How is this perpetuation achieved? And this is where Kausik Si entered the picture a couple of years ago. He found that what maintains the local protein synthesis is a protein that's a regulator of local protein synthesis, it's like a transcriptional factor is to genes, this is to protein synthesis. It has a funny name this protein, it's called the cytoplasmic polyadenylation element-binding protein. He found that there was a new form of this protein in the brain, and this was absolutely essential for activating the protein synthesis necessary to do this, and if you blocked it you grew connections but they were not maintained. That was very nice, it gave one an insight as to what regulates protein synthesis, but then he asked himself the question, What does this have to do with maintenance, how do you get from here to maintenance? And he looked very carefully in the protein and he found amazingly that this protein looks like proteins that are involved in prion formation. Now prions, as you probably know, are horrible things. They cause mad cow disease, they cause Jakob-Creutzfeldt disease in people, they are proteins that can maintain themselves through self-perpetuation. And the reason they maintain themselves is because they cause death and destruction.

Why would you want to find something like this working in the brain? Well he found that it works in the brain in a very different way, it works to do good, it uses the same mechanism but for beneficial purposes. But first let me tell you something about how prions work and then I will show you the novel variation that's been found in this context.

Prions are distinctive—and this is what Stan Prusiner first pointed out—for existing in two conformations, A and B. They could convert from one to the other, they're interconvertible, and one form, and I indicate this in yellow, is dominant, it can act on the recessive form and cause the recessive form to convert into a dominant form. And the dominant form tends to form little aggregates. Now this is interesting because it turns out that in this context, and we have reason that something similar might be occurring in the mouse as well, the dominant form is in fact the normal functioning form of the protein. The other form is the precursor form. So the way we think of it now is indicated in this diagram. You give five puffs of serotonin and you set up the long-term process, but only in the synapses that have been marked—and I'm going to focus on this one—can you utilize the gene products produced by the expression of these genes effectively. And the way that works is that a marking pulse causes the synthesis of this protein, CPEB, and most of the copies of that protein are produced in an inactive precursor conformation. But either stochastically or with the help of chaperone proteins conformation A is
converted to conformation B, and serotonin is critical for that. And once it's converted to conformation B, which is the prion active form, the dominant form, it feeds back on these inactive forms and causes them to become functional proteins forming these small aggregates. And it is these aggregates that bind to messenger RNAs that leads to their modification so they can be translated and be used to give rise to proteins that stabilize the synaptic growth.

So when you think of it this is really a very nifty mechanism. You can change at will, if you will, certain selected synapses and neurons without touching others, and you can do that by using a perpetuation mechanism that in principle can carry this a very, very long period of time.

What is fascinating about this is the study of the prion in a completely different context, in the study of memory storage, has revealed a new class of properties about prions that was never previously anticipated. And that is that a physiological signal can regulate the conversion, and that the form that is the self-propagating form is not a killer form, but it is the good, healthy, functional form of the protein that allows the memory to be carried forward in time. And it suggests that the possibility that we may be describing here one part of a sort of larger class of proteins in which the self-propagating form of a prion mechanism is used to create a functional protein that can exist in its self-replicating form for long periods of time, and that might be involved in steps of development, in neuronal identity, in persistent gene regulation, and in a number of other contexts.

So one thing you obviously want to ask yourself is, this is the way it is utilized in this one example in implicit memory storage is Aplysia. Does it also apply to explicit memory storage? Is this how attention stabilizes the memory? And we obviously do not as yet know the answer to this. But we have some preliminary clues. Martin Theis has found that there is a homologue of this form including an amino acid sequence that looks like it might have prion capabilities that is present in the mouse brain. It is present in the hippocampus in particular. Moreover, we have found that dopamine alters the level of expression in the mouse brain—Martin has found this. So this suggests the very interesting possibility. I only give it to you as a suggestion because the evidence for it is extremely weak at this point, that dopamine, again working like serotonin, not only mediates an attentional mechanism but this attentional mechanism might be able to stabilize place fields by perhaps recruiting a mechanism very similar to one I described to you in Aplysia in which a prion-like mechanism acts to stabilize synaptic connections in the brain and allows you to remember spatial relationships for the long-term.

**Conclusion**

So I've described to you how molecular biology—and molecular biology, which I really learned from the hands of these two masters that you saw in front of you—has really been able to take a complex mental problem and in reductionist systems
use it in order to see the generalities of biological problems, a theme that you've heard repeatedly this morning and will hear again this afternoon.

What is difficult in memory storage is to work out the systems problems, which Tom and Richard began to address. And this is one of the reasons we're very pleased about the Kavli Institute, which is going to be concerned with the systems problems of neurobiology. We want to know in detail, How do the various sense modalities actually come in and combine in the hippocampus to give rise a sense of space? This is an enormous challenge and we're just at the foothills of what is a great mountain range.

So let me simply conclude by pointing out the colleagues in my lab: Naveen and Cliff were involved in the spatial map studies; Kelsey, Maurizio, and Amit Etkin were involved in the synapse specific facilitation; Martin Theis was involved in the recent work on the mouse; Kausik Si single-handedly opened up this self-propagation CPEB story; and, we were fortunate to have the collaboration of Bob Muller, Craig Bailey, and Susan Lindquist. Susan was involved in the prion story. And I've been very fortunate to have the long-term support of the Howard Hughes Medical Institute and recently Columbia's been privileged to receive the Kavli Institute.

Thank you very much.

**Question and Answer**

I'd be delighted, if you are not exhausted and starved, to answer any questions that you have.

[Question inaudible.]

You have both the body and the . . . first of all, neglect is a posterior parietal cortical problem, it is not a hippocampal problem. And you really have an external image. You know the classic experiment of standing at the steps of the cathedral in Milano in which the patient can recognize all the landmarks on one side, can't recognize it on the other side. You now ask him to stand in the other side of the piazza, he reverses what he does. So he's got the knowledge in his head, he just cannot handle the spatial relationships.