Making Your Mind: Molecules, Motion, and Memory
Lecture 4 – Memories Are Made of This
Eric R. Kandel, M.D.

1. Start of Lecture 4 (0:19)

[ANNOUNCER:] From the Howard Hughes Medical Institute. The 2008 Holiday Lectures on Science. This year's lectures, "Making Your Mind: Molecules, Motion, and Memory," will be given by Dr. Eric Kandel, Howard Hughes Medical Institute investigator at Columbia University, and Dr. Thomas Jessell, Howard Hughes Medical Institute investigator also at Columbia University. The fourth lecture is titled "Memories are Made of This." And now to introduce our program, the Grants Program Director of the Howard Hughes Medical Institute, Dr. Dennis Liu.

2. Welcome by Grants Program Director Dr. Dennis Liu (1:07)

[DR. LIU:] Welcome to the final presentation in this year's Holiday Lectures on Science. I'm here to introduce Eric Kandel to give the fourth lecture in this series, and of course I'd prepared some very nice things to say about Eric. However, this morning we awoke to some really quite striking news on the front page of the New York Times, and that news was that Henry Molaison had died at the age of 82. Now Mr. Molaison is none other than the famous patient H.M. that Dr. Kandel introduced you to in the first, in the first lecture of this series, and I'm actually pleased to think that this program is actually a bit of a tribute to this remarkable man, who turns out to have been an extremely important figure in the history of neuroscience, and I think it's fair to say he's really the person that opened up the door to the, to the beginnings of the understanding of the role of the hippocampus in memory. So I want to thank Mr. Molaison and I want to thank his family and to give our condolences but again it pleases me that this show can be a tribute to him. Well, in his next lecture, Dr. Kandel is going to extend the story on understanding memory from beyond patients like Mr. Molaison to how model systems have helped us to understand the molecular basis of learning and memory, and once again we have a short video before Eric's lecture.

3. Profile of Dr. Eric Kandel (2:49)

[DR. KANDEL:] So I have several thoughts about science. One is that I think science in this country is viewed in too specialized a way. We see it as an esoteric occupation that a limited number of people do in relative isolation, and I think there are two or three misperceptions about that view. One is it's not a discipline that isolates people. It's an extremely social activity at every level. People interact in a lab, one lab interacts with another, there's a national community, there's an international community of scientists. It's a wonderful way to bond to people, and the social interactions of a lab are very important.

You can't escape from us. We need you at your desk.

[WOMAN:] O.K.

[DR. KANDEL:] One of the great changes that occurred in my career was the movement of women into the scientific workforce. That has just been marvelous, and for me very gratifying. And I realized that as more women came into the lab it made it much easier for any additional women to join. And now you go throughout the country, half the graduate students, sometimes more, are women. I mean it's just not an issue. In terms of the effectiveness in science it's just... and their confidence, you know, you see women doing fantastic stuff. When I think of it, it's hard to get two Nobel prizes, but the first person that comes to mind is Madame Curie.

4. Review of the systems problem of memory (4:28)

Good morning. I'm delighted to join you again for the final lecture in the Holiday Lecture series. Tom and I were told that the opportunity to give these lectures is really one of the high points of being an investigator at Hughes but we didn't quite expect the pleasure and excitement we're experiencing today. It's really been a wonderful occasion for us. In the first lecture we began the exploration of memory by pointing out that the study of memory has two parts, the systems problem of memory and the molecular problem of memory. We focused first in the systems problem and we asked where in the brain is memory stored. And we saw that memory is not a unitary faculty of mind, that it has two major forms: Implicit memory, memory from motor and perceptual skills; and explicit memory, memory for facts and events. Implicit memory involves the amygdala, the cerebellum, reflex pathways. Explicit memory involves the hippocampus and the medial temporal lobe.

5. Comparing implicit and explicit memory for molecular analysis (5:29)

We now turn to today's problem, the molecular problem of memory. We go from mind to molecules and we ask what are the mechanisms of explicit and implicit memory storage? On the surface of it, you would think that the kinds of molecules used for these two kinds of memory storages would be dramatically different, because they are different in three major ways: The nature of the knowledge stored, perceptual and motor skills on the one hand, facts and events in the other; sites of storage, reflex pathways, cerebellum on the one hand, hippocampus, temporal lobe in the other; and the strategies used, unconscious recall for motor and perceptual skills, conscious recall for explicit memory for specific events in people. This raised the question do they have any features in common and can molecular biology, with its ability to reveal homology of relationships, delineate commonalities in what appear to be very, very different memory processes. The fact is we're fortunate. If we look at these two processes from a distance we see they do have certain formal features in common. To begin with, both implicit and explicit memory storage have stages. There is a short-term memory, which goes from minutes to hours, and a long-term memory that can last for days, weeks, and the lifetime of an individual, number one. Number two, we know how to convert short-term to long-term memory. And the simplest case is repetition. Practice makes perfect. And finally we know that in many cases that have been explored, long-term memory differs fundamentally from short-term memory in requiring the synthesis of new proteins. Are there very different kinds of proteins involved in explicit/implicit memory storage or are there similar proteins involved? Do they share any features in common?

6. Selecting a model to study the molecular basis of memory (7:28)

Now to explore that, we'd like to take experimental systems in which we can study implicit memory storage in enormous detail, other experimental systems, perhaps the same experimental system, in which we can study explicit memory storage in some detail. The selection of the appropriate model system for a specific problem is one of the great strategic decisions one makes within biology. One is fortunate when it comes to implicit memory storage because implicit memory storage is extremely conserved. You can study this in invertebrate animals, you can study this in flies, in worms, in snails, and you can certainly study this in higher animals like mice and rats and human beings. I'm going to describe studies carried out on snail, *Aplysia*, but similar studies with very similar results have been carried out on *Drosophila* and honey bee, and actually implicit memory storage in mammals uses almost identical mechanisms. Hippocampal-based explicit memory storage requires an animal that has a hippocampus which means a mammalian animal, and as Tom pointed out to you the ideal experimental system for that is the mouse because it provides us the power of a genetic approach.

7. Demonstration: Meet a live *Aplysia californica* (8:46)

So I'm going to begin with implicit memory storage and I'm going to tell you something about the snail *Aplysia*. Now Tom demonstrated for you his lower body strength so I can't resist showing you something about my upper body strength. I'm going to show you why *Aplysia* is such a beautiful animal and why many people like to work with it. These are several *Aplysia* around, I'm going to pick one up, show you what an absolutely gorgeous animal this is as it relaxes. This is the California species of *Aplysia*. In fact you might want to touch it. Just rub its head very gently, you'll see this is a very nice animal. Would you? And you, you, all of you performed so well so you deserve this as a reward. This comes from California, it lives on the beaches of California and this is true for other animals that live on the California coast. It has reduced its life to its bare essentials: Feeding and fornicating. And some individuals are capable of carrying out both simultaneously. This animal is so accomplished because of these various things that it does that it has been publicly recognized. In fact there's a general consensus among the people who work with the animal that it's the animal that deserves the recognition and not the people who work with it.

8. *Aplysia*'s simple nervous system makes experiments easier (10:26)

But the reason one works with it is not because it's accomplished and not simply because it is beautiful but because of its brains. Your brain and mine has 100 billion neurons as you heard from Tom. *Aplysia* has only 20,000 neurons, so seven orders of magnitude less. That makes the task of analyzing the nervous system much, much simpler, number one. Number two, the cells are collected in clusters called ganglia and a single ganglion contains about 2,000 nerve cells, which controls not one but a number of different behaviors so the number of nerve cells committed to a single behavioral act can be quite small, less than 100 nerve cells in the limit. In addition to having a very simple nervous system, the animal has a nervous system that is made up of absolutely gigantic nerve cells. These are the largest nerve cells in the animal kingdom. The largest cells are a millimeter in diameter. You could see them with your naked eye, at least I could see it before I became presbyopic, I could see them with my naked eye. The cells are so distinctive because of their large size, and because of characteristic pigmentation and position, that you can return to the same cell in every animal of the species, you can identify the cell and study the same cell in naive animal and animal that has learned something. Now because of this you could give the cells names, specific names. You can call them Tom Cech, or Dennis, or Tom Jessell. We're not creative enough to think of that, so we give them very prosaic names you call them R something or other, R2, R3, R14 if they're on the right side of the ganglion, and L if they're the left side of the ganglion.

9. *Aplysia*'s gill-withdrawal reflex shows learning (12:17)

This simple animal with simple nervous system, large cells, generates a whole bunch of different behaviors, but we have selected the simplest behavior that this simple animal has. What we look at is a withdrawal of the gill. This is an external respiratory organ which it lies in a mantle shelf which is normally perfused by sea water and the gill extracts oxygen from the sea water. It's covered by the mantle shelf, a piece of skin that we've pulled back here in this drawing. That mantle shelf ends in a fleshy spot called the siphon. If you now apply a weak tactile stimulus to the siphon like that you get a withdrawal of the gill. This very simple reflex can be modified, not by one, not by two, but by several different forms of learning. Sensitization, habituation, classical conditioning and operant conditioning. I want to remind you these are the behaviors that the behaviorists, the initial students of behavioral study — so Pavlov and Thorndike, this is what they were studying. They were studying all forms of implicit memory and we can see this in the simplest animal.

10. Sensitization: A form of learned fear (13:31)

Each of these forms of learning has a short-term and a long-term memory, depending on repetition. I'm going to focus on one form of learning called sensitization, a form of learned fear. So if a horrible noise were to go off in the corner of this room, you would startle the heck out of all of us, as a result, most of us, me particularly, would be very nervous, so a modest stimulus that would have no effect at all would startle me, and so with learned fear in *Aplysia*. If you apply a noxious tactile stimulus to the tail, you frighten the animal, he has learned that it's frightened, and now when you apply the same gentle stimulus to the siphon, you get a much brisker withdrawal than you saw before. Let's see this in the real animal.

11. Video: *Aplysia*'s gill-withdrawal reflex and sensitization (14:24)

So we're looking at an *Aplysia* stretched out, we're going to focus in on the gill and the siphon. We're going to apply an extremely weak stimulus, so you can see the amplification with sensitization. Weak stimulus to the siphon, you see a modest withdrawal of the gill. Now we're going to frighten the animal, startle it, give a noxious stimulus to the tail. That of course causes a contraction in its own right. This contraction lasts for seconds but you can come back minutes later, the same siphon stimulus now produces a much more powerful withdrawal. We can now compare the two, normal and sensitized, and see how much more powerful the withdrawal is in the animal that is startled. The memory for this event is a function of number of training trials. So if you give a single tail shock, plotting here change in reflex strength as a function of time, the enhancement of the reflex lasts minutes, but if we give the same stimulus four or five times we get a memory that lasts a couple of days. So we have a short-term memory and a long-term memory and we can now look at the difference between them.

12. *Aplysia* long-term memory depends on protein synthesis (15:51)

We know from other experiments, more complicated cases, that protein synthesis inhibitors block long-term memory so we can see, will that work in this simple case as well? You apply an inhibitor of protein synthesis, inject it into the animal or apply it to the abdominal ganglion which controls this behavior, you selectively block the long-term process without in any way interfering with the short-term process. Moreover this memory that I showed you lasts for a few days. If you train for several days you can get a memory that lasts for weeks. So you can really in some detail compare short-term to long-term memory.

13. The neural circuit controlling gill withdrawal (16:28)

So, what we next want to understand is how does this reflect itself in the neural circuitry of behavior? Can we begin to get to the level that Tom was talking about? So in *Aplysia*, one can look at identified cells, see specific neurons and how they interconnect with one another, and we can work out the reflex architecture, the mediating circuit of the reflex. And I've simplified it for you, but there are 24 sensory neurons, they innervate six motor neurons, and the sensory neurons also interconnect with inhibitory and excitatory interneurons, the "middle managements" that Tom mentioned to you. The sensory neurons pick up directly from the siphon skin, they make direct connections to the motor neurons that move together, so this is the monosynaptic reflex of the kind that Tom talked about.

14. Temporary changes to the circuit vs. anatomical changes (17:16)

So we looked at this form of learning that we talked about earlier, sensitization, to see what happens to the reflex architecture when the animal learns. We found that when you stimulate the tail, you activate modulatory systems in the nervous system of *Aplysia* that release serotonin. The... serotonin is a small molecule that acts to affect the strength of synaptic connections between the sensory neurons and the motor neurons. Let's look at this in some detail. What happens if you give a single tail shock? If you give a single tail shock, you strengthen the connections between the sensory neurons and the motor neurons transiently. This is a functional change. You do not see any anatomical change in the

connections. But if you give repeated tail shock, you actually get a growth of new synaptic connections. So, if you remember anything about Tom Jessell's lecture, it is because an anatomical change has occurred in your brain that allows you to remember this for long periods of time. In fact, one can say, one can argue that the whole function of a series of Holiday Lectures is to allow you to walk out of this room with a somewhat different brain than you walked into this room with, as a result of the growth of new synaptic connections. So this tells us two interesting things. One is that although genetic and developmental programs specify the basic mediating architecture of behavior, they don't specify the exact strength. The strength can be modified by various forms of learning. I've shown you a form of learning in which the strength is enhanced, and other forms of learning, like habituation, the strength is decreased, number one. Number two, it tells you that the synapse has a double function. It is not simply the site of communication, it is also a site for learning and memory storage.

15. Simplifying the learning circuit in a culture dish (19:13)

Now we've looked at the reflex that is simple and we've looked at a neural circuit that is simple, but we want to go all the way. We really want to drive this analysis into the ground, and for that we want to make our task even simpler. We don't want to deal with 24 sensory neurons. We don't want to deal with six motor neurons, several serotonergic cells. Wse want to see whether we could reduce the complexity even more. We want to see whether we could take a radical reductionist approach to the study of learning and memory. So let's see if we can take a single sensory neuron, a single motor neuron, and a single serotonergic neuron and put it in a dish, and we can do that. Sensory neuron on top, forms perfectly good connections with the motor neurons and the serotonergic cells, the modular neurons, form connections onto them, and if you stimulate the modulatory neurons, you'll strengthen the connections between the sensory neuron and motor neuron. But we don't even need the modulatory neurons. We know they release serotonin, so we can substitute a pipette that simply puffs on serotonin. We now have a learning circuit, a two-neuron learning circuit in a dish, and we can now puff on serotonin, and we see what happens. If we puff on serotonin once, we get a transient facilitation of synaptic strength, we're now looking at the amplitude of synaptic strength as a function of time, lasts minutes. If we now give five puffs, brief puffs of serotonin we get a facilitation that lasts more than one day, and now we can ask something very nice. We saw that long-term memory differs from short-term memory in requiring the synthesis of new proteins. Is this represented at the elementary level? Can you see this at the connection between the sensory neuron and the motor neuron? And we could obviously do this very simply. We can apply inhibitors of protein synthesis and we find that it selectively blocks the long-term without in any way affecting the short-term process. So here we have a situation which we reduce the learning process, a component of it, a critical component of it, to a monosynaptic connection consisting of literally two cells and a puffer pipette, and we can now try to analyze the molecular mechanisms that underlie it. So I'm going to show you a blow up of the sensory neuron and the motor neuron and the serotonergic modulatory inputs.

16. Animation: Molecular activity in *Aplysia* short-term memory (21:36)

So sensory neuron is on top, motor neuron is at the bottom. On the left-hand side you see the tail activating serotonergic cells. Let's look at the basal condition. In the absence of tail stimulation, we generate an action potential in the sensory neurons. As Tom Jessell pointed out in his lecture, that propagates to the presynaptic terminal, it opens up calcium channels, allows calcium to come into the presynaptic terminal, and calcium is absolutely required for the exocytotic release of transmitter. It allows vesicles to bind and to be released. What happens if we now stimulate the tail? We activate serotonergic cells. These release serotonin, serotonin is recognized by a serotonergic receptor in the presynaptic sensory neurons. These engage an enzyme called an adenyl cyclase that converts ATP to cyclic AMP. Cyclic AMP is a second messenger. There are lots of different second messengers. They all have the same function: They carry information from the membrane surface to the molecular machinery

inside the cell. Cyclic AMP activates a particular molecular machine called the cyclic AMP-dependent protein kinase. That is abbreviated protein kinase A, PKA. Cyclic AMP-dependent protein kinase has two subunits, a regulatory subunit and a catalytic subunit. I've indicated the regulatory subunit as a spindle-shaped structure and the catalytic subunit as a oval-shaped structure. The regulatory subunit inhibits the catalytic subunit under normal circumstances. When the level of cyclic AMP rises, the regulatory subunit binds cyclic AMP, undergoes a conformational change, frees the catalytic subunit, it now can move into the presynaptic terminal. It acts on ion channels, it phosphorylates them, alters their properties, allows calcium to come into the presynaptic terminal, so now an action potential has more calcium coming in, more vesicles can bind, more vesicles can be released and you have enhanced transmitter release, and that is the mechanism of short-term memory for synaptic facilitation. And this is occurring at every single sensory neuron that is involved in the reflex.

17. Animation: Molecular activity in *Aplysia* long-term memory (24:05)

What about long-term memory? When we give repeated tail shock, we activate the serotonergic cells more, they release more serotonin, increases the level of cyclic AMP more, activates the cyclic AMP-dependent protein kinase more persistently, so now the cyclic AMP-dependent protein kinase can do two jobs, not just one. Number one, it goes to the presynaptic terminal where it enhances transmitter release as it did in the short term, but now it does something very interesting. The catalytic subunit moves into the nucleus. So one of the reasons you need repeated training is to allow second messenger kinases to move into the nucleus and there they activate transcription, they act on the transcription factor and Tom told you what transcription factors are. They're proteins to bind... that bind to specific sequences in DNA. This transcription factor is called CREB, cyclic AMP response element binding protein, and it binds to the sequence in DNA called the CRE, cyclic AMP response element. When it becomes phosphorylated by PKA, it can bind to that sequence, activate genes, and these give rise to the growth of new synaptic connections, and it's the growth of new synaptic connections that is the maintained form of memory storage. This growth is what is responsible for the maintained strength of synaptic connections and if you leave this room remembering Tom's lecture you will actually have an anatomical change as a result of this.

18. Gene-expression, long-term memory, and autism (25:44)

So there are two really interesting things that I want you to think about. The first is that long-term memory differs from short-term memory in involving the expression of genes, altering gene expression. Now you heard from Tom and we generally think of genes as being the controllers of behavior, the determinants of behavior and that is true, they're extremely important. But what is not also appreciated is that genes are also the servants of the environment. They respond to environmental contingencies, so when you interact with each other for a long period of time and affect each other's behavior, you are producing alterations in gene expression. And gene expression is particularly important because it is the gene expression that gives rise to the growth of new synaptic connections. The gene products that are produced are responsible for the growth of new synaptic connections and they are absolutely required for that. If you block CREB, you don't get a long-term memory, you don't get a growth of new synaptic connections. Now, what are the kinds of proteins that are required here? Well two of the proteins that are required are neurexin in the presynaptic neuron, and neuroligin in the postsynaptic cell. Have we heard about these before? Yes. Tom brought them in as being zippers, important in developmental synapse formation. And these studies show they're also important in the new growth associated with learning, and that suggests the possibility that one of the things that is defective in autism, in which these particular genes mutated in certain individuals, is that they lack the capability to learn certain aspects of social interaction.

19. Synaptic growth from learning in *Aplysia* and humans (27:30)

In *Aplysia* the growth is really quite dramatic. So this is a sensory neuron, here is a motor neuron before learning and here after long-term sensitization training you see a outgrowth, a doubling of number of synaptic connections. In the short term the change is presynaptic. Any long-term process involving growth has to be coordinated. So it's both pre- and post-synaptic. Now you can argue, this is only a snail. Does this apply to you, does this apply to me, and I want to convince you that this is happening in your brain. This is the motor strip in your cortex. We talked about this yesterday in which every movement of your body is represented. There's a hand representation. And when I was a medical student I was taught that the hand representation in my head is fixed. It cannot be changed. And we now know this is not true. That if you simply do what Tom had you do, oppose your thumb to your finger and you do this all the time you get better at it, and that is because you're actually changing the size of the representation of the fingers in your brain. And we're going to show this very nicely in an experiment by Traub, in which he looked at the representation of the left hand involved in fingering the violin, and he looked at that representation in control people who did not play the violin, and in string instrument players, and he found that the string instrument players who fingered the violin all the time with their left hand, did the fingering, had a much larger representation than those who don't play a string instrument. And, and this answers one of the questions that emerged earlier, if you do what your parents told you to do, you start playing early and practice a lot, your representation will be much greater than if you start later on in life. So what does this say? We have reason to believe that you can continue to learn new stuff throughout your whole life, and you can continue to grow new connections throughout life, but the capability to do that varies as a function of age and it's magical in the early years. This has several implications. It tells you that Michael Jordan is Michael Jordan and Mozart are Mozart not simply because they have the genes for the genius, which they do, but also because they began to practice their skills very early in life. You have an enormous capability and this still applies to you at this point, to acquire new information. These are the magical years for growing your brain, number one. Number two, it means that every single person in this room has a different brain than every other person in this room, if only because you've had different social interactions. Identical twins with identical genes will have somewhat different brains because they've had different kinds of intellectual experiences.

20. Q&A: Are new connections added only to existing partners? (30:41)

So I will go on to consider explicit memory storage with you but I will stop now to take a few questions from you. Yes?

[STUDENT:] When the neuron forms new synaptic connections, is that only with the neuron that it's already associated with?

[DR. KANDEL:] That's right, that's a very interesting question. All of the evidence available so far indicates that it forms stronger connections with the neurons that it was originally in contact with. People are beginning to see whether in some cases it would extend beyond that. It's quite likely that it happens but it hasn't been seen as yet.

21. Q&A: Do neurons vary in the ability to create new connections? (31:16)

Yes?

[STUDENT:] Well you mentioned for the element of long-term memory that you need to have cAMP activating the catalytic subunit of the PKA, and since some sensory neurons would be larger, you would have to have more PKA activation to reach that critical level at which you get DNA transcription. So would that mean that some sensory neurons are less able to be stimulated to perform long-term memory versus others?

[DR. KANDEL:] That's a very good point. Most of the sensory neurons that we're dealing with are approximately the same size, but the point that you make is a very well taken one. Neurons have to adjust for their size and there are compensatory homeostatic mechanisms that take the size into consideration. In this particular situation that has not been a significant biological issue, but it could certainly be an important issue in other contexts.

22. Spatial learning and explicit memory storage in mice (32:07)

So let me thank you for your questions and let me continue by turning to explicit memory storage. This is the magic memory storage that allows us to recall memories from the past consciously. Now, again, one can study this in a number of animals. It's turned out to be extremely convenient to study this in mice. Mice have a hippocampus, they don't recognize people but they're very good for spatial memory and they also recognize objects. So I'm going to describe to you a series of experiments on spatial memory, which has been commonly used. In mice, one can study this in a spatial maze of the kind that I've illustrated for you here. You put a mouse on a open platform that has 40 holes, and you shine lights on it and you play rock music, and this is the kind of thing that you and I enjoy immensely. Mice hate it. They don't like rock and roll, they only like to hear Schubert, they are not comfortable with this stuff, and they want to get away from the surface, and the only way they can do that is to find the one hole out of 40 that leads to a hidden escape tunnel where they can safely hide, and at first they do this in a fairly random way, or a serial, they go from one hole to another, but then they notice that the escape hatch is in relationship to a spatial marking, and they say ah-ha, it's in relationship to that marking on the wall, and boom, they go to it.

23. Video: Mice navigate a Barnes maze to test spatial learning (33:36)

So let me show you a video clip that illustrates this point.

[MS. MACH:] Now mice have an aversion to bright environments, to noisy environments, so this task really capitalizes on those aversions in that, the maze is placed in the center of the room which they don't like. So they're extremely motivated to find an escape tunnel. So the maze has 40 holes, and underneath one of the holes we put the escape box, and for each mouse the escape box is always located underneath the same hole.

[DR. KANDEL:] So this is a naive mouse looking around. The escape box is in relationship to the red landmark. Do you see that in the right-hand side? Boom. You do this now two or three times to the mouse and watch what happens. Looks up, sees where that red thing is, boom. And now Mary Elizabeth does something very interesting. She took the box and she moved it to the left, so Harry no longer finds the box where it was, looks around...

Beautiful, found it again. If you do this one or two trials, automatically he would get there.

24. Hippocampal circuits involved with explicit memory (35:10)

So mice can learn this very rapidly and they do this using the hippocampus. This is the hippocampus. This is a slice of the hippocampus. It shows you three distinctive synaptic pathways within the hippocampus. The hippocampus gets an input from the cortex. The information that travels around these, this three-neuron arcs and the details of this are not important for you, and then the output goes back to the cortex so it's like a loop that amplifies information. And we can ask a very nice question. Implicit memory storage involves a change in synaptic strength, what about explicit memory storage? Does it use the same class of mechanism? And the answer is yes.

25. Long-Term Potentiation (LTP): A form of hippocampal memory (35:58)

These pathways use slightly different mechanisms, but I'll focus on one because it's the most interesting one and it's the output connection of the hippocampus, it's called the Schaffer collateral pathway. So the Schaffer collateral pathway is the presynaptic terminal, they end on the target cell which is the CA1 pyramidal cell. If you apply a single set of stimuli to the Schaffer collateral pathways, you facilitate synaptic transmission for one or two hours. That's called long-term potentiation, LTP. And this is the early phase, so it's called early LTP. If you get repeated stimuli, exactly like with training, repeated training, practice makes perfect, on the synaptic level as well you produce a facilitation that lasts for a much longer period of time, over a day and sometimes for many days. And now we can ask again the question. We know in implicit memory, long-term memory differs from short-term memory requiring new protein synthesis. Is this again with explicit memory present at the synaptic level? We can apply an inhibitor of protein synthesis easily to the slice and we find selectively blocked the long-term but not the short-term process. What about the molecular details?

26. Animation: Molecular basis of early LTP (short-term memory) (37:21)

Here we can focus directly on the input neurons, the Schaffer collaterals ending on the CA1 pyramidal cells. We now see on the right-hand side, this is the post-synaptic cell, notice we're now focusing on the post-synaptic cell. The early change for explicit memory storage is going to have a post-synaptic target rather than a pre-synaptic target. The Schaffer collaterals come in, they release glutamate just as we saw in the sensory neurons in *Aplysia*. They act on two different kinds of receptors to glutamates, a receptor called NMDA receptor and a receptor called the AMPA receptor. Under normal circumstances only the AMPA receptor is active but if you give a train of stimuli, the NMDA receptor becomes active, it flexes calcium, allows calcium to come into the cells. Calcium activates a second messenger kinase but a different one than the cyclic AMP-dependent protein kinase, a calcium-sensitive kinase called the calcium calmodulin dependent protein kinase. It acts by inserting additional AMPA receptors into the post-synaptic cell, and that is the mechanism for short-term synaptic plasticity in LTP in the hippocampus. A postsynaptic mechanism involving Cam kinase inserting new AMPA receptors.

27. Animation: Molecular details of late LTP (long-term memory) (38:48)

What about long-term memory? What happens if we stimulate repeatedly? When we stimulate repeatedly we reach the threshold of a modulatory system which releases dopamine. Dopamine is a modulatory transmitter like serotonin. It acts to alter synaptic strength in neurons in the brain, and it is altered in its activity in a number of diseases, Parkinson's disease for example, and in schizophrenia. When dopamine gets recruited, it acts on a dopamine receptor that is coupled to an adenyl cyclase that increases the level of cyclic AMP, activates the cyclic AMP-dependent protein kinase, it activates CREB, CREB acts on downstream genes, and that gives rise to the growth of new synaptic connections. So the long-term memory shares a number of components in implicit and explicit memory storage.

28. PKA-deficient mutant mice have a reduction in late LTP (39:45)

In mice, this is the reason one goes to mice, one could do something very nice. You can do genetic manipulations, so you can see what are the consequences of affecting one component and the other. A number of these have been carried out and I'm going to show you only one. What happens if we block the ability of PKA to function in this system, if we compromise it? So we can do this by expressing a transgene that shuts off the cyclic AMP-dependent protein kinase. It's called R(AB), it shuts off the cyclic AMP-dependent protein kinase. If we look at the physiology we find that in both wild-type and mutant mice the early phase of LTP, which doesn't involve cyclic AMP at all is perfectly normal,

superimposable, normal in wild-type. But if you look at the late phase, which requires the cyclic AMP-dependent protein kinase, it is significantly compromised.

29. The PKA-mutant mice perform poorly in the Barnes maze (40:36)

And now we can go to your friend, the Barnes maze, and we can see how these mice do. Wild-type mice do extremely well in this task, most of them acquire it very rapidly, but the mutant mice that have PKA compromised act as mice equivalent of an H.M. syndrome. They cannot handle the spatial task of the long term. So this tells you that, in memory, as in development, as in motor systems, you can use the mouse very beautifully for genetic manipulations to tap in one or another process.

30. Summary of the contribution of molecular analysis to memory (41:11)

So let me step back and summarize what I originally tried to question. What are the similarities between short and long-term memory? What are the differences between implicit and explicit memory? And to what degree has molecular biology enlightened the study of mind and helped us in understanding these processes? When you look at short-term memory, molecular biology allowed us to see that the details are quite different, that in implicit memory storage we activate the cyclic AMP-dependent protein kinase, it's a presynaptic mechanism, and it's reflected in enhancement of transmitter release. In explicit memory it's a post-synaptic process. It involves insertion of new postsynaptic receptors. If you look at the general principles involved, despite the fact that the details are different, there are certain common similarities. In both cases, you're recruiting a second messenger kinase, you're producing covalent modifications, that is, phosphorylation of substrate proteins and you're changing the functional effectiveness of previously existing connections. What about long-term memory? Long-term memory in both cases involves activation of genes leading to the growth of new synaptic connection. In almost every single form of memory storage that's been looked at, CREB is absolutely critical.

31. Aging, memory loss, and Alzheimer's disease (42:36)

Tom pointed out to you that one of the reasons molecular biology has really revolutionized biology and revolutionized the study of the nervous system is, not only does it allow us to understand normal processes, but it allows us to understand disorders of function, including disorders of mental function. And I want to end my discussion by reviewing with you what we know about aging and memory. If you take 100 people, age 70, with no evidence of neurological disease, no evidence of psychiatric disease, no obvious evidence of memory loss, and you give them very, very subtle tests of memory you find that 40 percent of them do quite well, 60 percent show a minimal defect in memory. Initially, you cannot distinguish between them, but if you follow them over time, you see one continues to progress at a very, very slow rate. People forget where they put their keys, where they, you know, parked their car. This is called benign senescent forgetfulness, normal age-related memory loss, or non-Alzheimer age-related memory loss. The other 30 percent shows the early stages of Alzheimer disease, and that's a progressive, terrible disease of memory storage in which people, after they forget where they left their keys and where they parked their car forget, you know, words that they want to recall, they forget names that they want to recall that never come back to them. After a while they forget, tragically, the name of their partner. After a while they fail to recognize their partner. They turn to their partner after 50 years of marriage and say, who are you? And then the disease progresses even more, and people become physically incapacitated. Alzheimer disease begins in the hippocampus. It begins with things that look like Clive Wearing and H.M. It begins with a synaptic disorder in the hippocampus. It goes on to kill cells in the hippocampus, wipe out the hippocampus, and ultimately it affects the whole brain.

32. The Alzheimer-associated Aβ peptide is toxic to neurons (44:57)

There are a number of contributing factors to Alzheimer disease. A critical and a very, very important factor is the abnormal processing of a particular transmembrane protein called the amyloid precursor protein. If it's processed abnormally a peptide is released into the extracellular space right outside the neuron. It's called A-Beta. And this A-Beta peptide is toxic to neurons. There are a number of factors that lead to this increase in abnormal processing. One that is particularly devastating are mutations in the gene that leads to increased processing. If somebody has a mutation in this gene, they get Alzheimer's disease at a very early age. Kids with Down syndrome that have an extra copy of this gene get Alzheimer's disease at a very early age.

33. Aβ protein shuts down PKA activation (46:05)

Let's see what the consequence of A-Beta deposition is. In the early stage it just chokes the presynaptic terminal. It damages synaptic transmission very severely. With time, plaques form, aggregates precipitate out, and these plaques kill neurons. Ultimately, since this begins in the hippocampus it wipes out the hippocampus. It produces exactly the syndrome that H.M. had. People can no longer convert new short-term information to new long-term information and ultimately, most tragic of it all, it just propagates throughout the brain. The sulci, the grooves within the brain, become gigantic as the gyri shrink back. We know a fair amount about the biochemistry of Alzheimer's disease. We know that there's terrific accumulation of A-Beta and there are lots of therapies that are directed towards reducing the A-Beta load, either by developing antibodies to A-Beta or by stimulating other kinds of processing steps that get rid of A-Beta. But recently a very interesting finding was made by somebody who asked the question, what is the toxicity of A-Beta? How does that work? And they found it turns on the inhibitory constraints in the neuron that prevent the cyclic AMP cascade from being activated. In particular, they shut down the adenyl cyclase, prevent PKA from being activated, prevent CREB from being activated.

34. Lowering cAMP breakdown activates PKA , reverses synapse loss (47:46)

If you were to try to design something that would help Alzheimer's disease, you'd increase the cyclic AMP system in one way or another to get CREB activated, and you can do this in a number of ways. One way is to prevent the breakdown of it. It's broken down by an enzyme called a cyclic AMP phosphodiesterase. There are drugs available off the shelf that you can use, Rolipram is one of them, it inhibits it, allows the cyclic AMP to hang around for a longer period of time, and that allows the cascade to work and to give rise to the growth of new synaptic connections.

35. Alzheimer mice increase synaptic connections after Rolipram (48:21)

Mike Shelanski, who did this work, now took these findings into the mouse. You can generate a mouse model of Alzheimer's disease by putting in this mutated gene that we talked about, that invariably causes Alzheimer's disease in people, it also causes Alzheimer's disease in mice, and you see in the early stages loss of synaptic connections in the mouse. If you now give the mouse Rolipram, you restore the synaptic connections. As you might expect the animals also have a memory deficit, and Rolipram reverses that. So the point I want to make is that we're obviously in early stages in an extremely complex disorder. We have just a beginning understanding of it but lots of scientists are working on this because there's really a tremendous public health problem, and we're beginning to get some insight into it. And so the molecular biology here is helping us not only understand normal processes but also understanding disease states.

36. Summary (49:25)

So let me step back and try to sort of pull together these several kinds of issues that Tom and I have tried to address in these four lectures. I think perhaps one of the main themes that has emerged is that neuroscience is a relatively new discipline. It is just come of age recently, and during our career we have seen a vindication of Broca's vision. Broca was the first one to try to put psychology and neuroscience together, science of the mind and the science of the brain. With the aid of molecular biology, a new science of mind has emerged, and this new science of mind allows us not only to understand normal processes but also disease states, and there are several principles that have emerged from it. You should be familiar with them by now. One is that mind and brain are inseparable. Every action of mind is a process carried out by the brain. Two, that each mental function is carried out by separate neural circuits in different regions of the brain. Three, that all neural circuits are made of the same class of signalling units, the basic building block of the brain is the nerve cell. The synapse serves a double function, it's not only the point of communication between nerve cells but it's also the site of memory storage. And finally the synapse, tragically, is also the target of disease, not only Alzheimer's disease, not only age-related memory loss but schizophrenia, depression, almost every disease of the nervous system to one degree or another is likely to involve synapses. So we can't help but come to the following conclusion. We realize that we are who we are in good part because of what we learn, what we remember. Learning and memory involves our synapses, so in a highly simplistic sort of way, we realize that, to a great degree, we are our synapses. Let me end with a couple of final thoughts. I'm grateful for many people who have given me the opportunity to participate in this lecture but it's been a particular pleasure to work with Tom Jessell, a long-term friend, a fantastic colleague, in preparing these lectures, and I also want to thank all of you. This has been an exhilarating experience for Tom and me, and one of the reasons there are so many unsolved problems in neuroscience is we think that we don't want to deprive you of the opportunity to stand up here in the future and give your, give you a chance to give the Holiday Lectures with your contributions.

37. Q&A: Is serotonin being used to enhance memory? (52:04)

So I'll be delighted to answer any questions that you have. Yes?

[STUDENT:] Is serotonin currently being used to increase memory ability in any way?

[DR. KANDEL:] Are there any drugs being used to enhance memory? Is that what you asked?

[STUDENT:] Like, specifically using serotonin.

[DR. KANDEL:] To enhance serotonin release. Serotonin release is useful for enhancing the gill withdrawal reflex. There is no reason to believe that giving this systemically, in a way, would necessarily enhance all other behaviors in *Aplysia*, and certainly no reason to believe that it would do so in mice. It is interesting that, in depression, there is a reduction in serotonin activity, and by increasing serotonin activity or serotonin release, you can be tremendously helpful in depression, and one of the consequences of depression is the hippocampal-based memory loss, and that reverses as the therapy becomes more effective. So, serotonin does have some function in memory storage in mammals but it isn't the general solution to it.

38. Q&A: How does Aβ spread throughout the brain? (53:05)

Yes?

[STUDENT:] So you talked about how A-Beta plaque, like, degenerates the hippocampus and then you said it could affect the rest of the brain afterwards. How does it affect other brain function?

[DR. KANDEL:] That's a wonderful question. How it propagates, one doesn't know. We actually, and a number of other people are trying to do this now in an experimental way, to actually infect the brain with A-Beta and follow the propagation. One doesn't know how this occurs.

39. Q&A: What accounts for varying memory abilities? (53:33)

[STUDENT:] What types of factors can cause individual variances in short-term memory and retention? We find that some people don't have to study for a test as long as others or...

[DR. KANDEL:] Yeah, yeah, yeah, yeah, yeah, I mean, one doesn't really know that. There are obviously differences between memory capabilities in all of us. The reassuring thing is, having a perfect memory is not what you want. You want a good memory, but not a perfect memory. My guess is, part of it is, and also in part practicing, working a little bit at trying to remember things. One way you encode things, one thing, one way to remember it is to encode it better, to pay perfect attention and to try to really focus in on what's happening. One of the things that happens to elderly people, in addition to the fact that the basic memory process gets weaker, is that their attention is no longer as fixed on what they need to do. When they meet somebody, they don't, you know, attend to their name as carefully, maybe, associate the name with something they're familiar with. Learning to associate new information with old information, it's like classical conditioning, is very powerful for retaining it. So the initial encoding process, repeating things to yourself, is very helpful.

40. Q&A: Can repetition improve social interactions in autism? (54:50)

[STUDENT:] In autism, we see that people are socially deranged so, by repetition, by making those people go into such gatherings and become socially active will that actually decrease their...

[DR. KANDEL:] When people become socially engaged and socially active, it improves their brain. It's like the enriched environment that, when you put young animals in, it really stimulates the growth of new synaptic connections. I should tell you, in continuation of the discussion yesterday and in line with your point, I think there's every reason to believe that the social engagements work because they strengthen synaptic connections and cause new growth. We discussed yesterday how there's increasing evidence that you can pick up abnormalities in the brain of people with depression for example or post traumatic stress syndrome, and insofar as they're reversed in psychotherapy, that abnormality is reversed. So my guess is when we really begin to understand this, and psychotherapy is really a controlled example of a social interaction, we will see that all of these processes really produce alterations in the brain, that mind and brain are inseparable. I think we've heard that before. Pleasure having a... we have to stop unfortunately, pleasure having a chance to interact with you, on behalf of Tom and myself, thank you very much.

41. Closing remarks by HHMI President Dr. Thomas Cech (56:27)

[DR. CECH:] So, thank you, Eric Kandel, for another most stimulating lecture. It was very generous of you to leave a few problems in neuroscience as unanswered for this next generation to investigate. Thanks again to Tom Jessell for a wonderful pair of lectures, and thanks to all of the staff here at Howard Hughes Medical Institute who made this series possible. Now, next year the series continues with Professor Bonnie Bassler, who is a Howard Hughes investigator at Princeton University, and Toto Olivera, an HHMI professor at the University of Utah. Their topic will be discovering medicines from nature. And now, to all of you and all of those in our webcast audience, goodbye and have a wonderful holiday season.