Learning about Learning: A Course on Neurobiology of Learning and Memory

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Lecture – 19
Memory in Molecular Terms - IV: Remote memory and its characteristics

Hello and welcome to the lecture 19 of the Learning about Learning lecture series. In the previous lecture, we learned about the molecular representation of memory, if you had to do that. And what are all the characteristics of given molecule should possess, given the fact that there is a molecular turnover and how can we transpose this problem such that the memory can be encoded at the level of individual molecules, at the same time robust enough to not being getting muddled with the turnover right, not getting spoiled by the molecular turnover. And we hypothesize that are we actually proposed that such a molecule should have certain characteristics.

And then we were looking at alpha CaMKii that is calcium calmodulin kinose 2. And the alpha is a form of that could be a possible candidate for involving in this kind of a function like encoding, I mean preserving or maintaining that memory in the molecules at the molecular level. Now, the question that we raised was that can this alpha CaMKii if you take it out from the mice can this alpha dip deletion in the CaMKii show up as a loss in memory. Now, that is the question Alcino Silva working with the Susumu Tonegawa asked way back in 1992. So, they generated the alpha CaMKii knockout mice and then first thing, you want to study is that in doing so you are not affecting the neuronal conduction right.

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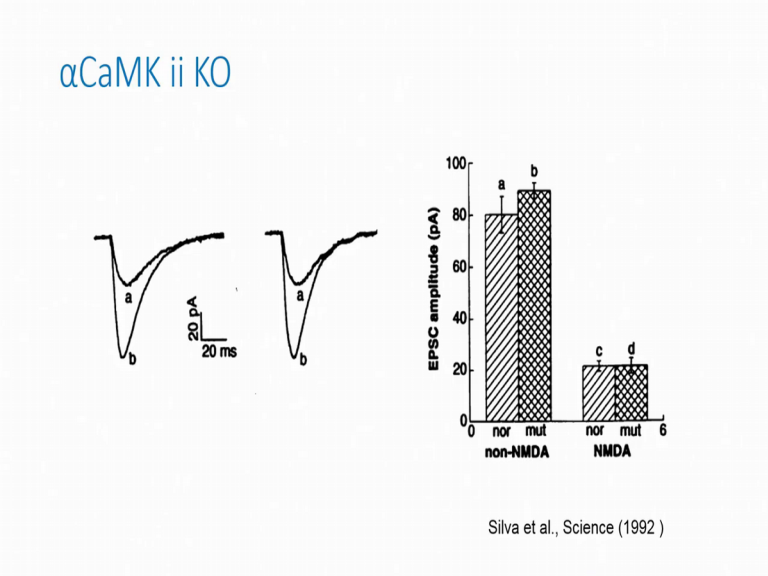

That is pretty important, because if you had to affect the neural conduction, it is much to do with the information, in come information itself is not coming in. So, there is no point of including it. So, it is, it should not be affecting that and clearly, they show a for as can be seen in this responses, the evoked responses what we are seeing here are there worked responses in the knockout just in the control and they are classifying to, they are measuring two different kinds of currents.

The NMDA receptor dependent current and non NMDA receptor dependent current; the reason I mean how you would do, that is you just block the receptor the NMDA receptor and then measure whatever is happening and that is the background current and you can subtract that and you can actually generate even the NMDA current characteristics. So, these are in, these inserts are that NMDA currents in both the normal as well as the mutant. In fact, you cannot even make out which is which, that identical they are.

So, the inputs are all right, there is no problem with the input. Now what happens to the L T P right, the first thing that you want to ask is that, do they express L T P and they asked the same question and then when you perform this experiment depending on whichever the measure that you do with the Timon and Bliss and Lomo study that we have discussed. We talked about measuring one thing, which is the amplitude. You can actually measure the amplitude as a function of the percentage based on line conductance as is done below in this graphs.

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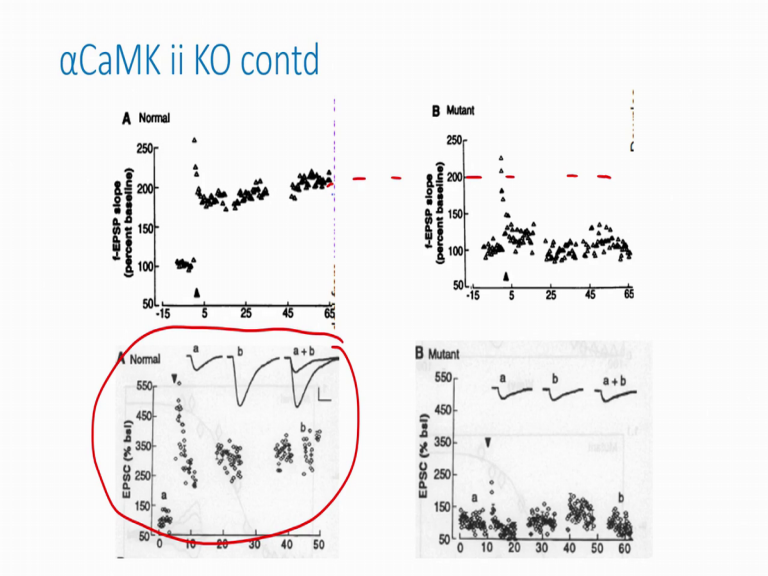

This guys or you can measure the rise the; if the rise of the potential how fast the slope. So, to speak of these potentials and whatever you measure what you see is that in the normal animals they are pretty intact that is that is exactly what you would expect. However, in the mutant even though they get this conduction that is normal right. So, you would get that that is there, but they are not able to maintain that higher level, that that is where it is right. It should have been, but it is not there it is actually down reduced pretty highly down.

And in fact, you can I go ahead and again measure the NMDA versus the non NMDA currents, what you see is that again that you can do it in the amplitudes. What you see is that the alpha currents that have the non NMDA currents that have gotten enhanced, in these guys we do not see that there right. After the induction of the L T P that is not happening there at all clearly the ability to maintain the higher conductance is not there is lost and the only thing that you have done is you have taken away the gene for making alpha CaMKii protein, in this mice.

That is astounding, that is really astounding at in when this was done at 1992 for the first time. You can actually show that you can knock out a simple molecule at that gene for a simple protection of a molecule and these organisms becomes like H M. We are not removing the hippocampus; we are not lesioning out any brain region. We removed a gene and the resultant that you have is a loss of memory or inability to form and maintain

that memory. what I have shown you is that they do not have L T P, if I had to make the jump of memory, we need to actually do a behavioral task. The task is again or what remains experiment.

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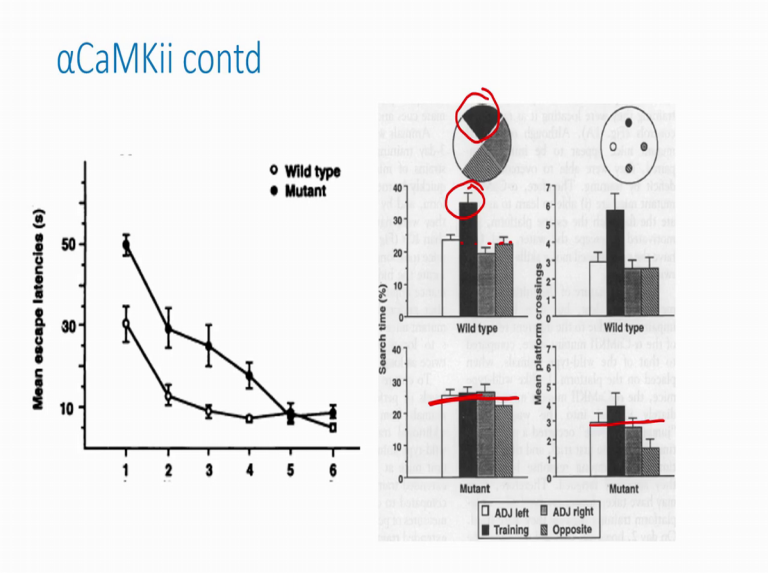

Clearly, you can see, they can learn, they are mean latency does come down; however, when you actually ask the question of how well they do in the probe trial. Remember probe trial is the trial where we are actually taking away the platform, all right taking away the platform and asking where does the my spend most of its time searching ok. So, that legends for this different graphs are given down. So, this dark is the training quadrant, right above here and rest of them are the quadrants surrounding that and you can actually see the wild type mice. That is the mice with everything being normal, no gene deletion they search significantly more in the target quadrant, while a compared to the rest of the place, while the mutant mice do not do that at all.

They search equally well in it, when they do not distinguish the target quadrant. In fact, it becomes even more striking, when you make slightly different measure. Here now, you are looking at you are saying the plats, you are actually measuring platform crossing. This is a measure where you are saying hey, look if hypothetically, if the platform had to be present in this in the other quadrants right. You can draw out the platform in those regions and then ask, how many times that place has been crossed by this mice ok.

That is another measure right, because if the animal were to remember really where the platform at is the, is present, then you would expect that such a crossing to be higher in the target quadrant compared to the rest of the quadrant. And in fact, that is what you see in the wild type; no doubt the black bar is the tallest among the rest of them.

So, they that is clearly higher, but when it comes to the mutant, they are not making the distinction at all. They are doing, they are moving around that place almost as if, as the other how much of they are moving in the other quadrants clearly demonstrating that they do not have memory, they do not have the ability to form the memory that is known to be dependent on hippocampus. This is memory is known to be dependent on hippocampus.

So, with this I have taken you through a reasonably long journey of starting from H M, where we said memories are formed in hippocampus, a particular kind of memory are formed in hippocampus and if you remove that region like completely taking away that region what happens to those memories and what happens to the H Ms ability to form those memories. From there to a place, now we go ahead and manipulate our gene and remove a gene and show that the animals ability to form these kind of memories affected. Now, it is time to look back and ask what else can we learn from H M.

If you are wondering so, what, what aspects of the CaMKii, we talked about the aspects of CaMKii that really identify that really can identify as memory molecule, but what other aspects of CaMKii can be serving the neuronal function. There are several hypotheses that has been put forward ranging from insertion, I mean stabilization of the AMPA receptors that are present already in the postsynaptic neuron to inserting more, activating more, AMPA receptors to inserting more AMPA receptors and so on and so forth.

But there is one very very important character that is going to prove it to be extremely crucial, that is differential distribution of CaMKii alpha CaMKii across the brain. Hippocampus seem to be having a higher concentration of this alpha CaMKii compared to cortical regions. Now, keep this in mind, we will come back to this very fact while we are revisiting H M.

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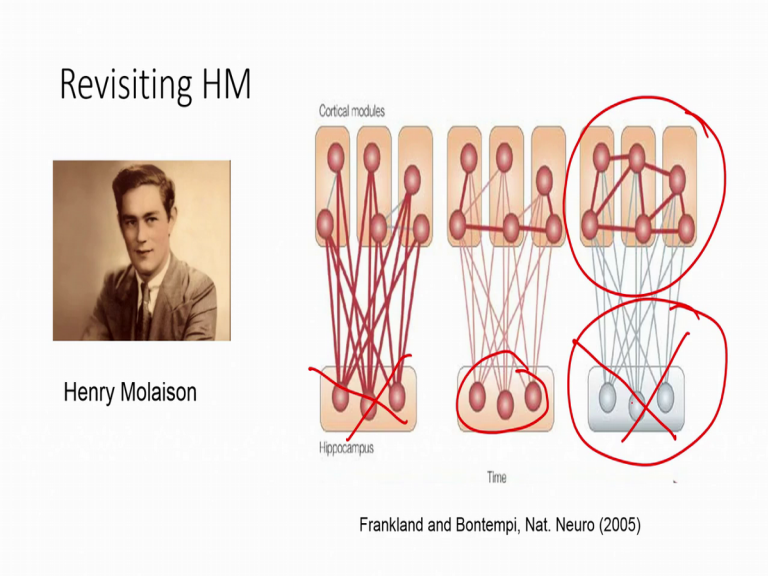

Let us look back at H M, we remember this picture. This is a picture of a H M Henry Molaison, who went through neurosurgery, I mean brain surgery to cure his epilepsy, where we have removed, I mean people have removed the medial temporal lobe. From there on he lost his memory, he lost his ability to form new memories, not of all, not of all kinds of memory, but a specific kind of memory. There by we came up with this wonderful classification of memory scheme and there, there on we went on to relate it to how we learn and so on and so forth.

But there is one aspect, that we did not touch upon. What we did not touch upon is the fact, how he was able to retrieve his old memories. The memories that required hippocampus for its initial formation, but now know now, the hippocampus has been removed and still he is able to retrieve say for example, he is able to retrieve what his name is, what his family is, where is his home so on so forth.

What could be happening here, that introduced the notion of a process called systems consolidation, wherein the hypothesis is that initially the information coming from various different cortical modules are getting integrated in the hippocampus and form a integrated picture of an event or a memory in the hippocampus, but over a period of time the memory that is formed in the hippocampus is such that it is able to assist or help the cortical modules to form an independent, a reasonably independent trace of memory for this event.

Such that now, when you go ahead and remove this hippocampus much later you have these memories, that are formed in the cortical region that help you to retrieve that. We retrieve this event, but remember to form this you need the hippocampal memory and that record that is where the hippocampus is really required. I mean if you remove the hippocampus right here, there is no integrator; there is no formation of that episode or an event in your life.

So, they there by you there is no memory at all; however, over a period of time. Now, you can actually go ahead and remove this, because this is independent of that, you can retrieve that independent of that. That is an hypothesis. How would you test it? Now, you could test it, I mean we already have some evidence that H M is actually able to retrieve this memory, but the point is, you want to have this model outside of this patient right, then that is really proof of the idea that you can this is what is happening.

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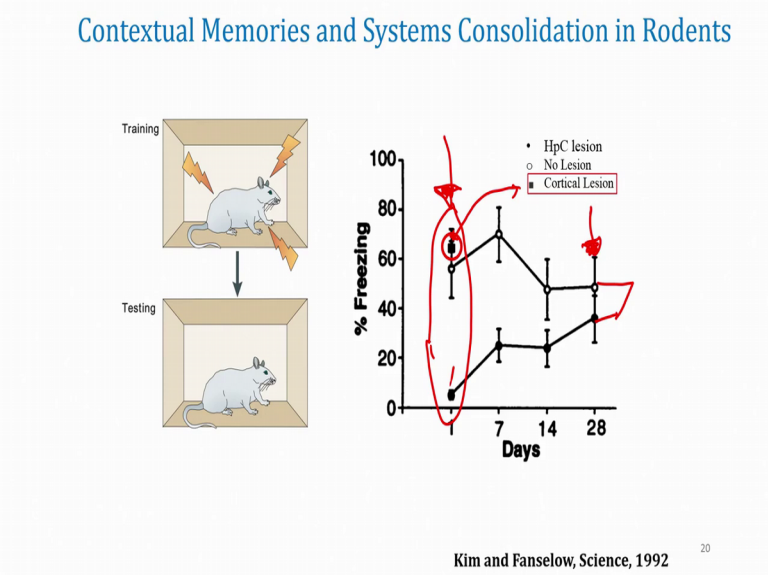

Once it, once you have this model, then you can start to prove various characteristics of this model, of this hypothesis and the model per say came from again in 1992, from Michael (Refer Time: 15:19) lab at UCLA, what they did was that they made use of the fear conditioning paradigm. Specifically, they made use of contextual fear conditioning paradigm. Wherein, if you take this animal's rodents and then puts, put them inside a box, we have seen them before and present them and foot shock. Now, there is no

apparent Cs, you are not presenting any stimulus externally apart from placing the animal in the context or in that box.

This in the absence of such we have seen already that what we used to call it as a background or in this specific case what we, we can call it as a context the setting of this box the lighting, the various all factory and light cues and all together form an integral representation of the context here, which is the cs. The animal makes an association with this context to the shock. Now, what will be the result? Later on you bring this animal back to this context and you test it the animal expresses fear and you can score that fear expression of the fear by measuring freezing and then ask how well does the animal remember. This is very good.

Now, there what they showed is that such a remembrance or such retrieval is dependent on hippocampus, but that dependence is very short lived. So, what you could do is that when you are actually; so, what you are seeing is that you are measuring the freezing as a function of number of days, after which you are performing a hippocampal lesion ok. So, that there are two different controls here; one is cortical lesion, I mean just to remove a similar amount of volume, just to show that it is nothing to do with the act of lesioning and handling the animal through the surgery and all that stuff removing the equivalently.

This called the double dissociation experiment, where you do that lesion in some other region and then you really show that that really does not affect it; however, if you do the hippocampal solution right there, in about a day you do not see the memory clearly showing this data clearly shows, that the contextual fear expression is dependent on hippocampus. If you remove that campus there is no expression of contextual fear.

So, that later on they have gone to show that what is stored in hippocampus particularly the dorsal hippocampus here, is really the representation of the context. So, that context memory is gone, but it is gone only if you do this lesion in about a day. If you were to wait for long enough period of time, say about 14 or 20 28 days and then do the lesion. The hippocampal lesion guys are as good as the control. They both can express fear for the given context, for the training context, almost equally well. Here, is our model of a H M.

This is the point at which H M has lost his hippocampus, but is able to retrieve his old memories, because it is in mice, it is 28 days old. This is the point in H M, where he, he

is not able to form any new memories, because you are listening or the, the whole experience in, the whole training paradigm was one day old, it is new. So, through very simple, but yet a powerful demonstration of lesion and the behavior, they have established a wonderful model that mimics this process of systems consolidation in rodent animal, paralleling H M.

This started us sprout in memory research of particular kind, a memory, it is called the remote memory. These are memories, we call it me remote memory, because these are memories of the events that are that happened remotely, that happened some time ago. What kind of research of the, first thing you want to ask is yes, it is good that it can actually retrieve this memory, but you want to ask how good are this memory compared to the original memory or their exact same details is there something different.

Now, if it tried to be the fact that you are, there is a there is a different learning system and there is a different substrate than the original substrate, what is the likelihood that the different new substrate that is representing this memory older memories is going to be same as that of the, original memory. If it is so, why even have that kind of duplication Brian Wilson, in Alcinous Silver Slab asked that a question of how similar this memories are?

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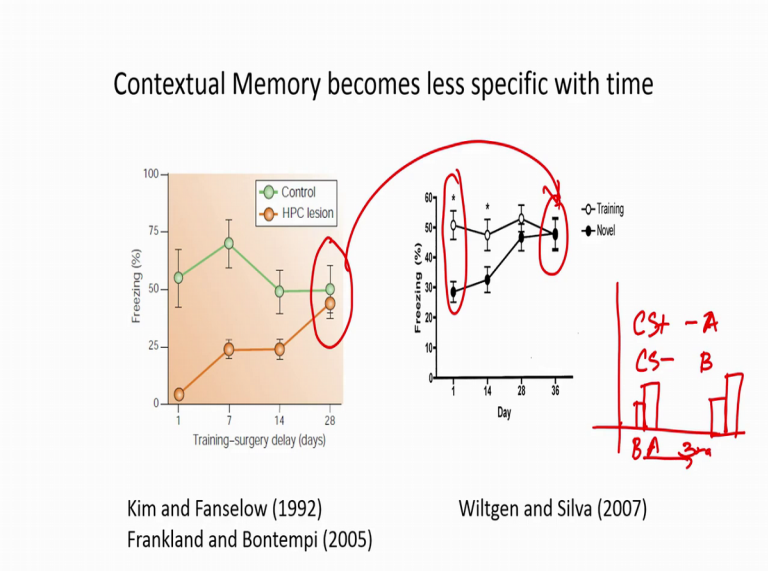

In order to do that task what he did is, he took these animals all right, he we know that if you lesion out the hippocampus they are, they show the time dependent consolidation

effect, but then he asked a slightly different question. What I am going to do is that I am going to put these animals in a slightly different context in the training context ok. So, the slightly different is the keyword here. How slightly, slightly? It is slightly that when you actually look at this put these animals and ask the question, do you remember this context or this context looks familiar to that.

Then the response of that animal would be it depends, it depends on what it depends on when you ask the question. So, if you had to ask that question that is you put this animal in our training, in a novel context that is similar to a training context. If you had to put that animal right after, you have trained them within a day's gap they can clearly tell the difference between which is a training context and which is the novel context you see this difference. However, that difference diminishes away as you proceed in time.

Similar time scales as that of the system consolidation, you see at about 30 day time point when you ask this animal, can you say if it is a training context or the novel context. They say HM I do not know. It is not that they cannot differentiate the two contexts. See that is different from telling apart in which context they got a shock. See these two are different. I want to make that distinction clear, it is one thing to say A is different from B and it is other thing to say I am not sure it is the A or the B which is relevant, is the second type of memory that memory loss or memory deficit that this animals are showing.

How do we know that? We can actually put them in a different context A context C, which is dissimilar to A and B you will see they are at baseline. And in fact, what you can actually also do are a few other cool experiments, you can give a small remainder somewhere in between and then they will definitely tell the difference between A and B. You can also do a few other characteristics which tells if they can separately separate the A and B well, that seem to be unaffected at all. What seem to be affected is their ability to say in which context they got the shock, not to say which contact it is.

This put together with the fact that you see the hippocampal dependence vanishing away in similar timescale. Let us put these two graphs together simultaneously, one after the other. If you put these two graphs right next to each other what you see is that similar timescale and similar characteristic suggesting that the memories that you retrieve after hippocampal lesion, from the cortex may be having this property that is to say they have

the animals seem to have generalized. Thus C S on which they got trained they are called stimulus generalization that they are, they were generalized. They have transferred the fear to a novel context the ability to discriminate in which context they have got trained is lost.

Now, why is that happening is that that they lost the details themselves or something else is happening it turns out. You can do a different experiment here. What you can do is, you can do a discrimination training. So, right from the beginning you trained them in a as a shock context all right, remember our discrimination training is the C S plus and C S minus training. So, if you had to say A is C S plus and plus C S minus is B as a function of time. So, in the recent time you actually do this. So, they learn that for B they freeze less, for a they freeze more and then you come back after 30 days and then ask how will they remember.

They clearly remember, A is the shock context and B is a novel context are, is a no shock context. So, there is no problem in their ability to discriminate A and B, if you teach them to discriminate, they will discriminate. They will remember this details very beautifully, no problem at all, but left to themselves the details seem to be getting muddled. To explain this one of the proposition was from Franklin Lam that if you are having multiple overlapping memories or multiple exposures to a same event then, what the system consolidation process might be doing is, they may be picking out a commonalities between all these events as against those things that are different. If they are picking out the commonalities, then that is one way you why you may be generalizing and not discriminating at a remote time point.

Now, how do you test it first thing is to prove that they are capable of picking out these commonalities. So, they went back we go back to the water mace experiment and now, they said hey look, I am going to make a small modification to the water mace experiment.

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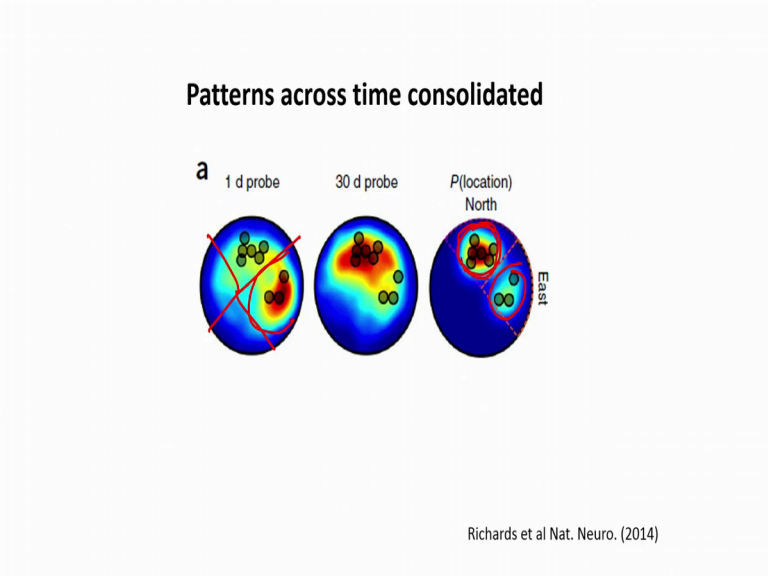

Now, I am not going to place the platform in one particular location ok. What I am going to do is that I am going to move this platform around a particular probability distribution that is one time I will be here, I mean ten times I will be here, one time here, one time here like that. So, these are the HM exact locations, where the platforms of placed. So, you can clearly see a dominant amount of platforms where placed in this quadrant a small amount were also present here and then you train them. Mice it is inordinately difficult, but the mice learn that today, this is where the platform is.

How do we know that? You come back a day later. So, they have initially trained them in this set of platforms and then they got into this introduced this platform in a new quadrant right these three places. Now, they come back after a day and then ask where are the mice searching? You can clearly see they are searching in the quadrant that they have been trained on lately, that is recently right, one day. This is the last training that they have received and that is where they are actually searching. However, you wait for about a month or so and then now, you ask where are they actually searching?

What you see is that they are predominantly searching in the quadrant, where there is a highest probability of finding the platform. It is not about which quadrant, but it is about the which quadrants do I have a highest probability of locating a platform. Clearly, giving in the cues that they are capable of extracting the commonalities given enough

time and that is probably what is happening when you are giving enough time and then when you are forming these representations over time, across time.

So what ? So we, what address the question of what happens to this memory over a period of time. Now, we can step forward and ask, is there a molecular correlate here to? This is where the statement that I made right at the end of the alpha cam k to study becomes important. I told one of the beautiful characteristic of this alpha CaM Kinase is that a differential distribution of this molecules across the brain. These are hippocampus is highly enriched in the alpha CaMKii compared to the cortex. So, given this, they said all right. Now, when we knock out a gene in an animal, we know that the genes come in two copies.

So, clearly you can take away one of the genes and leave the rest, leave the other or you cannot take away anything, how you can take away both of them. Depending on that you will call it, call them as heterozygous wild type and homozygous mutants. The alpha CaMKii that we looked at were all homozygous mutants that is, the alpha CaMKii has been deleted completely, both the copies of the genes have been removed now, if there is a differential distribution of this concentration. Now, if you go back to the heterozygous then we can see, we have an ability to specifically look at the effects, the concentration affects, much more in a, much more pronounced manner.

So, Paul asked this question and then said hey look, we know cortex takes part in remote memory formation and we have a way of modulating this differential response through alpha CaMKii heterozygous mutants. Since, cortex has less anyway now, if you remove even half of it, that can bring down the alpha CaMKii concentration below the levels that are required for plasticity formation. So, they may not be good enough that the levels may not be good enough to form remote memory while on the other hand in hippocampus anyway it is enriched in alpha CaMKii if you reduce by half maybe you get away with that.

Now, how do you test it? You take this heterozygous mutants and then run through the behavior right and what do we expect? We expect hippocampus dependent memory that is the recent memory to be intact, because if things were too normal and that is a half concentration is sufficient to see through, you should be able to see through. And in fact, we know that half may be sufficient, not because the, remote memories to form in the

cortex, while at the cortex that may fall below the critical level, making the animal not able to form the remote memory. This is inverse of the HM problem right. The animal would be able to form the recent memory, but will forget will not be able to keep it for longer period of time ok. What do we see?

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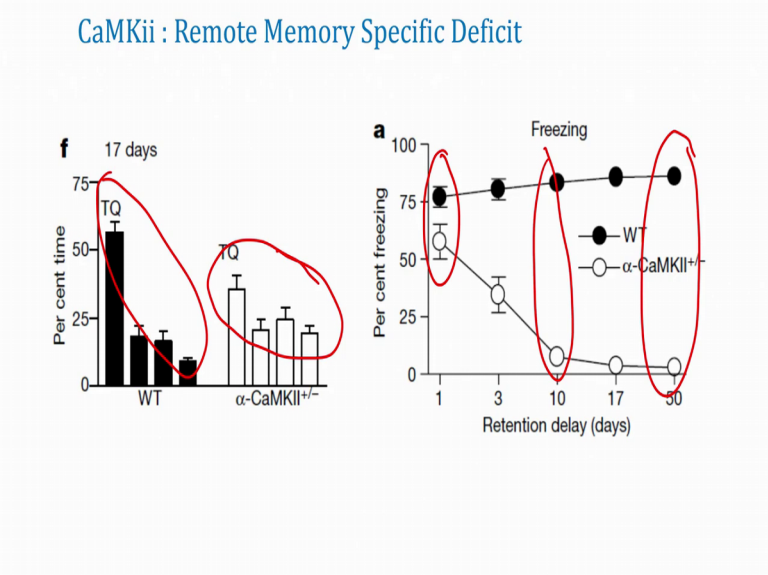

So, they took these mice so and then subjected to the water mice experiment, when you do that you can actually see these are the comparisons. We are talking about wild type versus, the alpha CaMKii heterozygous mutant the plus slash minus represents that, if it is totally minus it is a homozygous plus it is a wild type, plus means the gene is being present minus means it is not present. So, when you are looking at the heterozygous mutant what you see is at the end of the training, at the end of 3 days they are beautifully learning, no problem at all.

There is specifically searching in the target quadrant pretty much like the wild type. However, when you bring them back after 17 days, you do not have to even wait for a month you bring them back after 17 days, what you see is that compared to this. Not just the water maze, you can actually do this experiment on fear conditioning to and that is what you see. In the recent time point they remember very well, no problem at all. However, if you wait long enough time or in fact, if you wait for a reasonable time, any reasonable time right after 10 days they fail, to retrieve this memories.

Here, we have created a new kind of memory deficit, we are in the recent memory, hippocampal dependent recent memories absolutely fine; however, the ability of the animal to retrieve thus over a long period of time the remote memory is lost. With that I would like to end this lecture now and then we will see you in the next lecture and I will describe to you there, what are all the recent advances and how and where we have reached to the point where we can actually start to manipulate this memory what I mean neurons participating in memory. How do we do that and what effects do you see when we do that will be the part of the next lecture.

Thank you.