Learning about Learning A Course on Neurobiology of Learning and Memory

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Lecture – 17
Memory in Molecular Terms - II: Long term potentiation

Hello and welcome to lecture 17 of the lecture Learning about Learning lecture series. Just before this we have been looking at various evidences of behavior being controlled at different scales. Particularly at the level of molecules, we had a proof from dopamine measuring the dopamine levels and then correlating with the sign related this was a goal related, behavior and then even casually proving that you can actually modulate these levels and then change the animal from one way or the other. And, we also looked at the whereabouts of how the protein the role of protein synthesis was discovered and how it actually came by.

Towards the end, we were in looking at the different the neuroanatomical features of the hippocampus and then how Bliss and Lomo were planning to use those features; these features to study the plasticity in the brain in the hippocampal region. And, there we were talking about how one can actually use the different electrodes to stimulate and to record and study the throughput of the input output, or what happens to that in response to quote unquote an event. Now, we will look at that in more detail in this lecture. So, just to recap what Bliss and Lomo did were to make use of neuronal anatomy of the hippocampus and then we talked about the peripheral pathway right the inputs coming onto the dentate gyrus.

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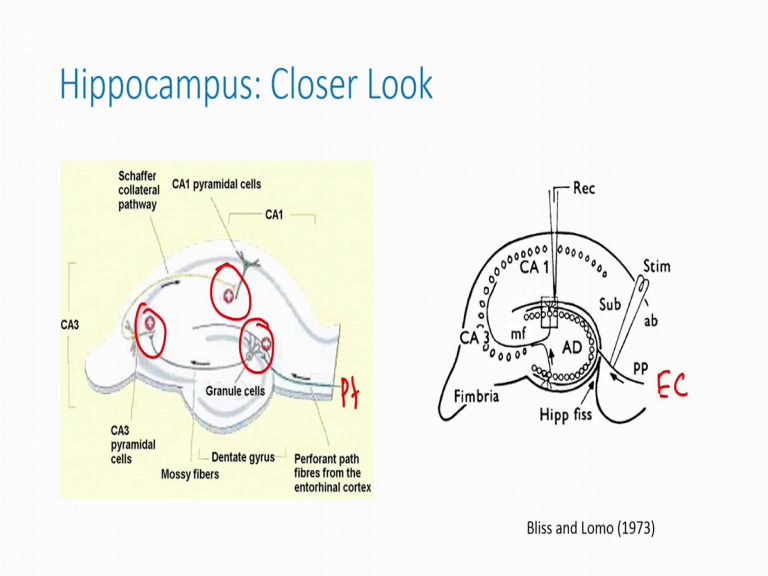

If I can go into the slide the in the peripheral pathway, these are the inputs from the internal context EC. And, they stick in an electrode the it is called a stimulating electrode and then they stick in an recording electrode in the cell body layer of the dentate gyrus sense. If you remember this is the first synapse in the hippocampus formation. And, the argument is when something like an event can be thought of as differential stimulation pattern.

So, what they were trying to do was that they wanted to look at the efficacy of the synapses, how efficient are these synapses, how much are is the how much is the throughput of the synapses in response to a stimuli. The argument is since they are globally measuring by sticking in an electron near the cell body layer, or in the cell body layer. They would be this electrode will be measuring sum total of all these responses. Now, for small stimuli that I am going to give here, I am going to look at what is the response? By that I can get a baseline level of what is the efficacy, what is the throughput?

Then, I am going to send in what the Bliss and Lomo are going to send in what they think or what they thought could be a plasticity inducing stimulus ok. We will come to the exact details of the stimulus in a minute. And, then they are going to measure the same. The logic is if by some means of changing the stimulus, they can elicit a better

reference a better response or an improved synaptic efficacy in these synapses. We would like to ask how long do this efficacy stay after removing the stimulus.

And, that is exactly what they did the way they did is in making use of rabbits anesthetized rabbits and they made use of the fact that we have 2 hemispheres in the brain, memorian brains have 2 hemispheres. And, they are we could use one of the hemisphere for an experimental condition while, the other can be used for the control condition, wherein they would not be putting in the conditioning stimuli ok. What are this conditioning and test stimuli? Now, how did the experiment go?

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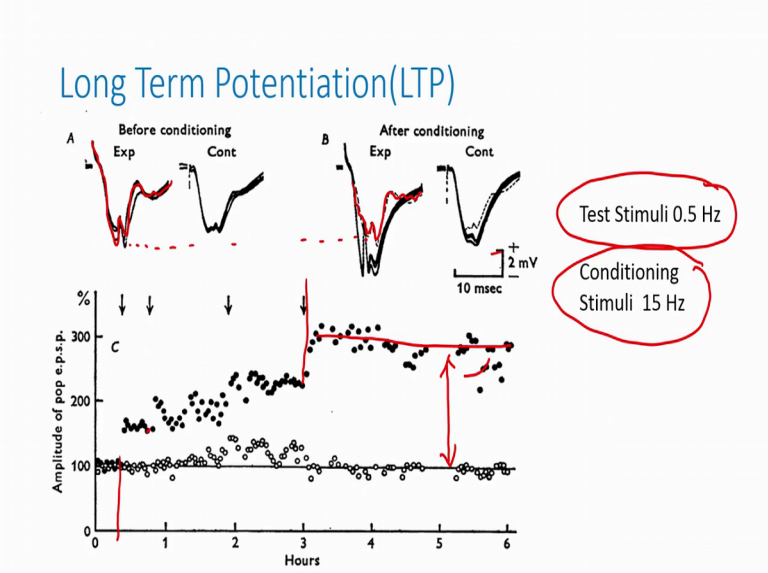

So, having implanted this recording electrodes and stimulating electrodes, what they did was to send in a test stimuli here. A test stimuli is a 0.5 hertz stimuli, that is a small stimulus, that is sent once every 0.5 seconds every 500 milliseconds. And, then they are measuring the response at the recording electrode whatever, when you do that what you will see is that a response something like that.

So now, these potentials this is an voltage responses potentials and measured in voltage. This response is from the recording that is from the recording electrode, you can characterize an amplitude, you can characterize the rise time. In this case they are actually looking at the amplitude. Amplitude of the population response and it is also called as a evoked potential. And, you are measuring that potential as a and then

normalizing it toward to be the 100 percent and then the dots, individual dots mesh tells you, what is that amplitude for that one particular stimuli that one particular pulse.

You keep doing that for a brief period of time. We establish what is your baseline level. And, they are at 100 percent making sure they are not changing in time, it is very very important. At this point in time where there is an arrow, they are giving an conditioning stimuli or a training stimuli what as I call it. A conditioning train of pulses, which is essentially the same stimuli, but then at a different frequency they are now giving like a 15 hertz. If one were to give simulate this, it is pretty much like initial they were going like tick, tick, tick, tick, tick. And then for every tick they are actually measuring how much it is a response. At this arrow they are going, at 15 hertz all of this, pulses are given in a very brief period of time.

And clearly right after that, what you see is that the amplitude has gone up in the experimental group in a control group, that is in the other hemisphere of the rabbit, they have not given that stimuli at all and as a result they are same. And, these are the amplitudes measured from the potential and the potential per say you can see is it is completely changed right, that is the level and they have increased in that amplitude right about. So, to get a measure there so, that is the scale right. So, that is about 2 millivolts scale and 10 millisecond in this axis and you can clearly see they have really gone up compared to this dotted line or the dashed line.

It is not just that they it has gone up, but it stays flat. Now, you have stop the stimuli here right you gave that brief stimuli and they stopped it and you are continuously proving how good is the synapse. Is it holding up, is it holding up that high voltage response right. You are seeing that amplitude being high for a considerably long period of time. At this point again they give and then they keep on repeating that and it is continuously going up and at this point in time they stopped giving any move stimuli at all. And, what you see is there is a steady holding up of the increased amplitude in these synapses as against the controls right.

So, remember so, this differential enhancement in the efficacy of the throughput or the increase in the amplitude they called it as potentiation, because they that the synapses are potentiated now. So, that they can actually give in a higher response for the same amount

of the input response and that lasts. So, you call it as long term potentiation that lasts much longer. So, it is about even 3 hours later.

Now, there have been cases in the there have been laps which claim that they can hold this LTP for days hour not just hours even for days. Now, the beauty of this experiment is that this for the first time allowed us to even think of what could be possible mechanisms through which a memory could be stored in the brain. Now, this does not mean memory is long term potentiation, but what it could be is that the mechanisms underlying the long term potentiation.

That is the enhancement in the evoked potentials, that you see in this experiment could be same as that of one that happens when you form memory alright. So, granted it is a laboratory phenomena the LTP, its I mean there is no there is no necessary when, there is no equivalent necessarily for the out word there is no equivalent stimuli in an outside world, but that is realistic that can actually generate such kind of a response in the brain. But, the fact is when you do things like this, you are seeing an enhanced amplitude and that stays.

And your ability to and it involves the neurons ability to enhance or modulate it is response, and be able to hold that modulation for a long period of time. And, the mechanisms involved in this could I mean they have similar property similar time characteristics. So, it is tempting to correlate that they could be same as that of the one that are formed in memory. Now, this really opened up a huge field of study, because now you have a way of simply testing, which are all the drugs, which are all the chemicals. That can enhance LTP that does do the enhanced memory a (Refer Time: 12:09) and you can talk about mechanisms, that can be underlying this.

What protein? What gene? What not, you can actually really dissect out go ahead and dissect out various aspects of this regional in fact, the field did expand and people did use this to the best part of then to now. As with any good things there is always this issue of the debate about is LTP really memory. What would like to say is that it is very highly correlated, that does not mean it reflects memory often this fails because people say that hey, look there are several mechanisms, which actually enhance or impair which affect LTP, but that does not seem to necessarily affect memory.

So, if it is really that meaning this equals that then that should be reflected in this correlation that should really show that right. So, to this we will talk about this particular review later my good friend (Refer Time: 13:18); he post the question in a slightly different way. Instead of asking the question of all does all the factors that affect LTP do they affect memory? He asked how many times have you seen a memory deficit or an enhancement without a change in LTP. It turns out that correlation is extremely tight than the other way around ok. Again, alluding to the point that could be a, these gives us an opportunity to investigate possible mechanisms, that may be common between LTP and memory.

And, it is that way it is a fantastic candidate and it is probably one of the extremely highly studied candidates in the field of learning and memory and for a good reason as I was stating that. But however, good the reason be, however, good the candidate be you would like to know what might be a possible reason, why this is happening right. What would be a possible molecular correlate? Now, in order to do that we need to understand a little bit so, this is clearly happening at the synaptic level and we need to understand a little bit more about the synaptic communication themselves.

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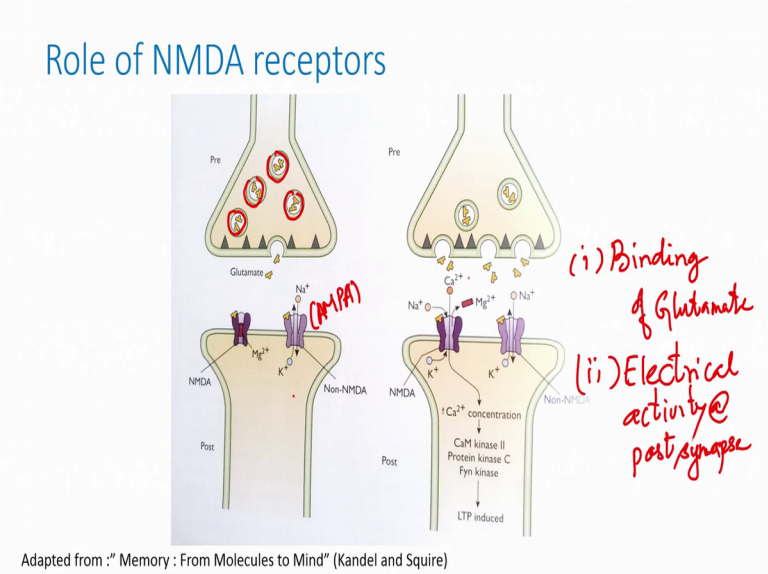

Now, if we look at the synapses here adapting a picture from this famous book called Memory, From Molecules to Mind by Kandel and Squire; a very good introductory prelude to anybody who is interested at the molecular details of memory and behavior to

some extent. So, what do you, what happens is that as and when the nerve impulse the action potential, we talk about when you are talking to a neuroscientist. And, when the available an action potential reaches the presynaptic bouton in response to this action potential the presynaptic bouton releases the neurotransmitters, that are packaged in vesicles.

So, these round entities that are shown here are vesicles that contain neurotransmitters. We have seen this neurotransmitter before dopamine is one of them, but that is not the only one there are many such neurotransmitters present in the brain. And one of the prominent one is glutamate with these neurotransmitters glutamate for example, here then goes and binds to receptors that are present in the postsynaptic membrane, which depending on the kind of receptor it is can signal the postsynaptic membrane quite a few things of which one particular thing being changing the conductivity of a class of ion channels.

Or, the class of receptors called the AMPA receptors here is called non-NMDA receptors, but we know that now the basic or a default conduction neural conduction is taken care of by a set of receptors what we call it has non named (Refer Time: 16:41) here in this figure is actually AMPA receptors. They are actually receptors for the neurotransmitter glutamate, but you can differentiate them by using these drugs I am for and NMDA. And, they selectively bind to one fraction of the glutamine receptors and the other one finds to binds to the other. So, thereby classifying them into AMPA and NMDA receptors, that besides that point what you have is there are 2 classes of receptors I mean there are many classes, but that in here we are looking at 2 classes of receptors AMPA and NMDA.

While, AMPA takes care of the routine neuronal conduction NMDA does a slightly different job. The job here is that it is a receptor for glutamate at the same time, it is also a sensor for an voltage. Now, normally when they when you look at these receptors, their conductance is blocked by binding of magnesium ion in it is cavity. However, when the glutamate comes and binds to this receptor and within a certain period you have an electrical activity in the postsynaptic neuron that, releases this magnesium thereby letting the calcium ions flow through this NMDA channels.

Now, such a kind of a behavior right which is in contrast to the AMPA right; AMPA we are talking about just a simple regular conduction just ion channel it is a simple ion

channel. I mean simple receptor where it binds to the glutamate and then you have the opening up of that pore and then sodium potassium exchange happens. So, neuronal conduction happens. However, in here for this pore to open this receptor to open it is ion channel activity it needs to have 2 things; I binding of glutamate, II electrical sufficient electrical activity at the post synapse. Now, why we will be talking about this? Now, such a character right, such a character of this receptor puts it in a very unique position.

It can actually detect simultaneously the activity in the pre as well as the postsynaptic neuron right. It detects the activity in the presynaptic neuron because it needs the glutamate to bind to it. It detects the activity in the postsynaptic neuron because without that electrical activity the magnesium would not be released I mean without the electrical activity. And so, it needs to be both that the glutamate binding along with the postsynaptic activation, opens up these receptors for neuronal conduction, and when it opens up calcium ions being the being positively charged, they having higher concentration outside than the inside, they flow through these channels thereby triggering a biochemical cascade.

Now, again this activity of NMDA receptors puts them as a very high chance or very good candidates for detecting or coincident activity. They are called coincident detectors thereby that have a ability to encode associative memory.

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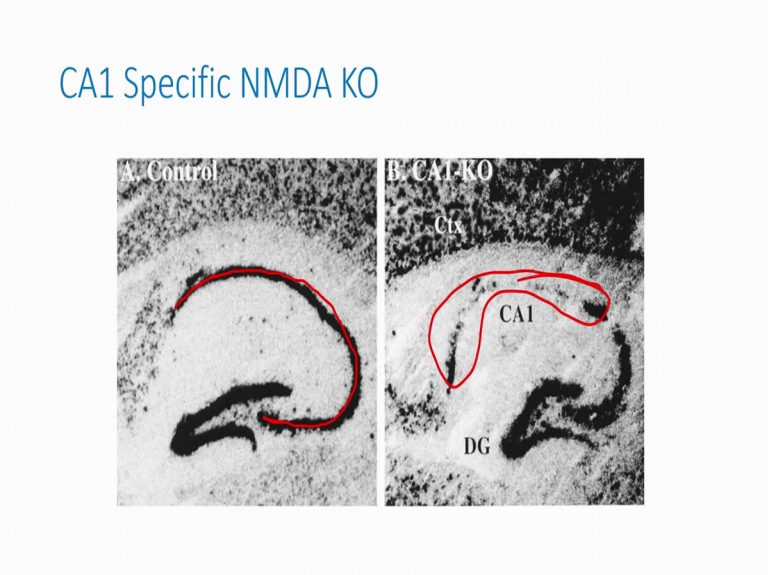

And, from that is the argument of people from Cisco Susumu Tonegawas lab used to generate a kind of a mice in which these receptors were absent, this receptor were absent specifically in CA 1 region alright. So, if you not if you take away these genes from the mice the result in mice you generate you call them as knockout mice. And, you can generate these knockouts in various different ways. One of the ways is this is the region specific knock out of a given gene, and you can actually test them may sit region specific, then in here what you are looking at as the images of expression of the MRNA. This is a stage before the receptors are proteins themselves.

So, a stage before the protein expression is the MRNA presence of the MRNA; MRNA gets translated into the proteins. And, you are looking at probing the levels of MRNA here. And, the dark lines and the black contrast here indicates the level of MRNA. And, you can clearly see that in the control mice the NMDA are receptors MRNA is for them are beautifully expressed and it is very high, while that is not so, in here in the knockout mice ok. And, it is specific to the CA 1 region you can actually go ahead and look at other regions.

For example cortex or in DG or in CA 3 they are all fine they are not very different. Having generated this they wanted to ask now I am going to study the LTP not the LTP the kind that I mean it is a kind that a Bliss and Lomo established, but in a different region. So, Bliss and Lomo were looking at the DG 2 I mean internal cortex to the DG right. They were looking at recording they were recording electrode of the cell body layer of the DG. But, they are now here what we are going to look at is the LTP coming in or to the CA 1 region right them and then what do we see.?

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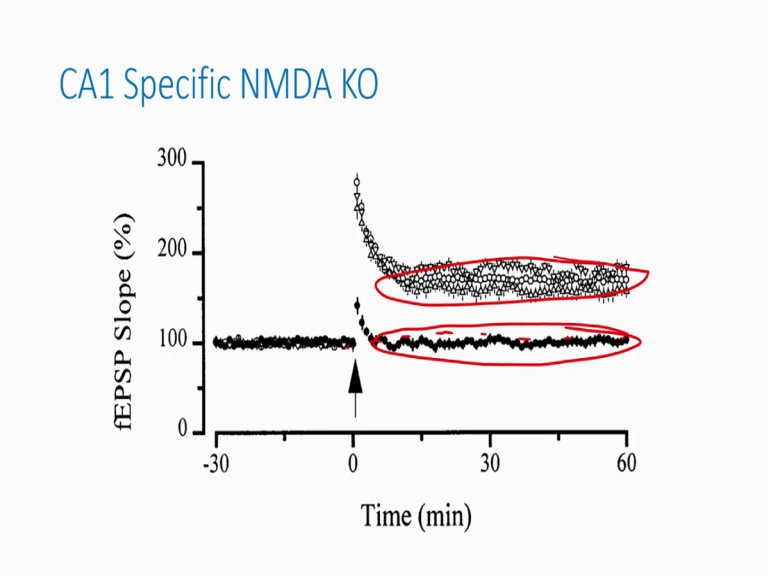

If you look at the LTP, now the open symbols represent the control animals. There are 3 I mean there are 3 or 4 different controls, but does not matter that is not worry about the difference in the controls but, the idea is there all controls ok. For various different aspects, when you are doing a knock out study it is important to establish quite a few things are not the reason why you are seeing this affect.

And, the bottom line here is you can do all those controls and it turns out they are all not the reason. Why you seeing this profound effect, where in the open circles I mean you can, wherein you can see this the LTP in the knockout animals represented by the dark circles, and the filled circles is much lower. And in fact, they do not they do not have any LTP at all, they do not show any LTP at all how do we know that, you see this response here right. That is at the baseline level right after you stopped I mean it comes back to the baseline level right after you stop this means; it goes up a little bit, but then it comes back down ok. Compared to the controls where it goes it goes up and stays high at a much higher level than the baseline.

So, having the NMDA receptors at least in this a CA 1 specific knockout in the CA 1 pathway seems to be necessary for expressing LTP and inducing LTP and being able to maintain it. Now, we have granted we have not separated the idea of induction and the maintenance, but the point is that you need in a without this NMD you are not going to

have that LTP. So, there is no potentiation the CA 1 region without the NMDA long term potentiation without, NMDA receptors being present there.

Now, that is again it is a lab phenomena right that I said it is LTP is a lab phenomena and a laboratory induced phenomena; how relevant is it in terms of the behavior? So, clearly any experiment that we one does to unravel the role of these different molecules in the behavior particularly the memory, and then related to LTP need to test the memory to. And, the way you do that is through behavioral task this is called as a water maze or a task.

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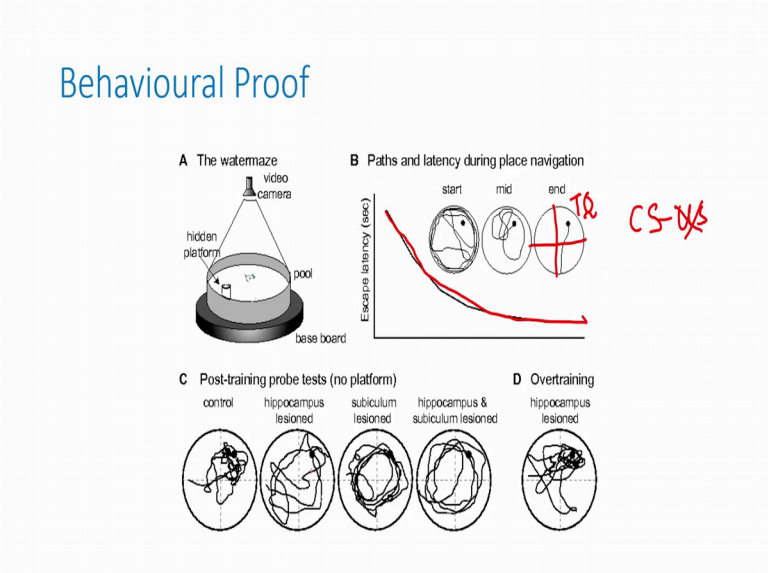

The waterways experiment involves taking an animal rodent here. And, then putting it inside a big pool of water a big a big tub, in which it is filled with the tub is filled with water and you place the animal inside the tub. Rodents do not like to swim, but they are capable of swimming; they do not like to be in the water though. So, they would want to very very first instance when they get an opportunity, they would like to come out of that water and stop swimming.

And, you put them in the pool they will start they will keep afloat by continuously swimming, but inside that you hide a platform. What do we mean by hiding? You place a platform just about the level of the water. So, if you at the level of the water and you will not be able to see it, added to the fact the water itself is also opaque, you make them opaque make the water opaque by adding milk powder or a some turbit, turbidity

inducing substance. As a result you will not be able to see through the platform. So, you do not know where the platform is. However, the rodent have to figure out where the platform is that is the task.

So, you train them over repeated trials to learn where the platform is. So, how would it learn? What are the cues? The cues are placed as objects present outside the maze; these are extra maze cues that are placed on the room around the water the tub. And, using that cues it has to figure where that platform is not just that, it has to figure out and remember. So, that the next time; when it comes back it can go to that same place. Now, the mice takes some time to learn this task and you can measure the performance the progression in this task by asking, how long a time the mice takes before it actually reaches the platform. And, if you do that that is called as an escaped latency or the latency to actually reach that platform'

And, when you measure that you see a you will see a continuous progress and then latency comes down. And, you can also look at trace it is path because you can you are recording through the video. So, you can actually trace it is path initially it is does not have any clue it is a circling around all over the place. Over a period of time it develops a strategy and develops an association between the cues and the position of the platform, there by slowly refining it is path such that towards the end where it really has learned the task, you can actually reach that platform in a very quick time. Again, whenever you are testing memory you actually want to delineate the training which largely inverts the learning aspect from the probing of the testing, which actually involves which largely inverts the memory aspect.

See both the training and the testing involves a combination of learning and memory it is never only one or the other. But what we are talking is, what you are talking about here is, we bias such we are such that the training is dominantly learning and testing is dominantly memory retrieval ok. So now, what we are doing here is that we had we talked about the training face here and then how do we probe the performance. We want to know how do we actually test the memory capacity, the way you do that is by doing a probe test right. And, just in our traditional CS-US experiments, we would remove the US and I ask, what is the response for the CS? Right same way here what we will do is we will remove the platform and then say what is the mice doing now.

If, the mice is actually understanding or remembered that this is where the platform is then it will selectively search a larger fraction of the time, that is given to it in the place around the where the platform is. And, you can measure it you measure it by dividing this entire circular pool in 4 quadrants and measuring what fraction of the time a mice spent in each of these quadrants. In short when you do this, you turns out that if the animal has learned, it will end up spending a large amount of time in the platform quadrant, it is also called as a target quadrant.

Now, this behavior depends on hippocampus how do you know it? You can lesion it out, if you lesion it out what happens is that the mice searches all around the quadrants it is just a moving around all around. And, it is not that it cannot find it at all you have to understand that, in for it to find you need to really over train them, but the point is at a moderate the regular training it needs, if you lesion out the hippocampus it cannot find the platform.

In the next lecture, we will see how this group use this behavior to test the requirement of NMDA receptors in the CA 1 region in forming the memories for this task.

Thank you.