Learning about Learning: A Course on Neurobiology of Learning and Memory
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Lecture – 18
Memory in Molecular Terms –III- Properties of a memory molecule

Hello and welcome to lecture 18 of the Learning about Learning lecture series. So, far we have been looking at and di setting out the molecular basis for the complex behaviors. More specifically, in the last lecture we were talking about the role of NMDA receptors. NMDA receptors these are a class of a subclass of glutamatergic receptors. And, we were looking at what effect do they have when on LTP, when they have been taken away from, when the gene that is responsible for generating these receptors are taken away from than animal.

Specifically, if this taking it out or the knock out is specifically localized to a region of the brain called the hippocampus and even the sub region of the hippocampus. Even the localization is so, good we can actually go down and then dissect out the molecule in only in the CA 1 region right.

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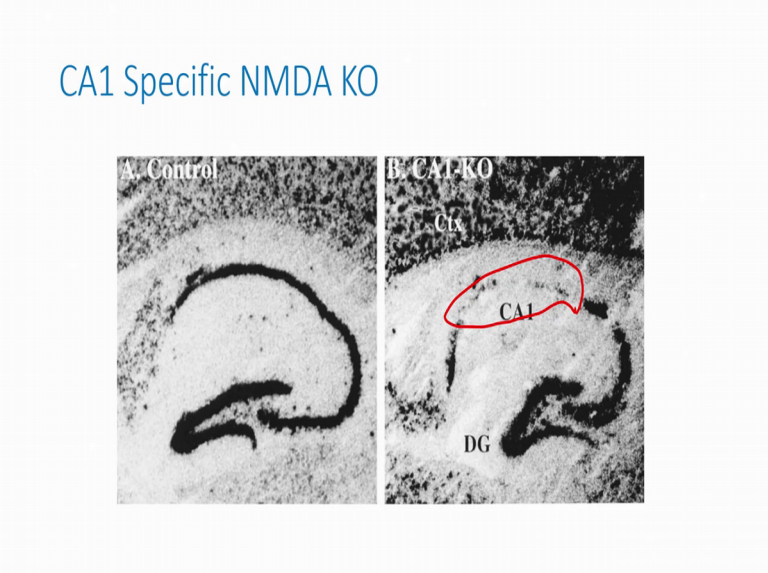

How do we know that? We saw that this picture and we where we said hey, look you can go ahead and stain for I mean, the presence or absence of these molecule in terms of its MRNE right. It is a one I mean these receptors are proteins and proteins are expressed in

a living system by translating from translation from MRNA. So, you are actually looking at the MRNA stain here and then you can see that that stain is missing specifically in the CA 1 region.

Just I want to point out one little thing here this is how deep and how good a control we have in terms of asking the questions such as what role a molecule in a region plays as compared to Carl Ashley, where he was taking our chunks of brain. See, the motive of the questions are more or less the same here right. You want to know the substrate of where the memory could be you want to know, what are all the responsible factors or what are all the factors that are responsible for forming the memory? From the days of removing chunks of brain we have gone to the place, where you can actually go down to a region of the brain in fact, a sub region of the brain identified by the differences in the cell type of the neurons that are present there.

In that region specifically go ahead and remove our gene and nothing else. Now, that causes a deficit in LTP: Long Term Potentiation. We were wondering what this means what this could mean in terms of the animals behavior as a whole. How did we how do you prove this behavior? We prove this behavior through several means and one of the behavior that we are looking at is a water mains experiment, that is and where you can where we were describing how the experiment was done.

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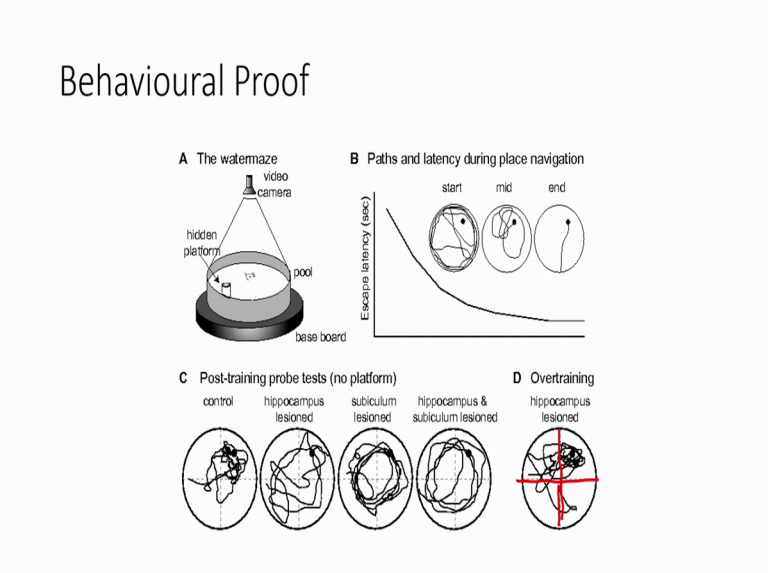

So, the briefly the experiment is done by placing the mice in a pool of water and asking it to find a platform that is hidden inside that pool. Now, over a period of time you will see that the time required for the mice to find, or any rodent, or mice or the rat to find the platform will go down. But, more importantly if you actually section out or divide that entire pool into 4 little quadrants. And, then ask where the mice is searching predominantly when you remove a platform. This is a probe trial dream and you remove a platform and now you are asking where are this is the mice searching now predominantly, you see that they are searching in the quadrant where the platform is usually present.

Now, the definition of these quadrants comes from the fact that this is a platform. And, then the mice know this is where the platform is because, not because it can see the platform, but it is actually computing where the platform could be based on the cues that are outside of the maze. So, it needs to make through two things here. One it needs to understand, what are all the cues that are present outside of the maze is called the extra maze cues. It needs to remember that in relation to those cues where the platform is. Now, that is exactly what you are testing and that is some memory here.

And, we said this is also dependent on hippocampus. If, you lesion out the hippocampus in a regular training the mice fails to figure out where this platform is. Of course, you can over train them and then make them learn that it is; make them learn to search in that particular quadrant. But, the point is in a regular training they would need a hippocampus and if you lesion out them, if you lesion of the hippocampus they will not be able to localize locally searched this quadrant.

Now, what happens to these mice in whose CA in CA 1 region lacks NMDA receptor. Now, can we actually take these mice and then put them to this behavior protocol. And, then if you do that they clearly learn you see that latency coming down, but the important point that as I was stressing is that, you want to test their memory right. A memory is tested when you do not in this particular behavior, when you do not have a CS equivalent of a signal here right, you remove the platform and you asking the mice to actually figure out sorry yeah there is no US present there.

So, you remove the platform and then you are asking the mice to probe where I mean probing the mice can it actually figure out where as the platform would be. When you do that, that is what you see.

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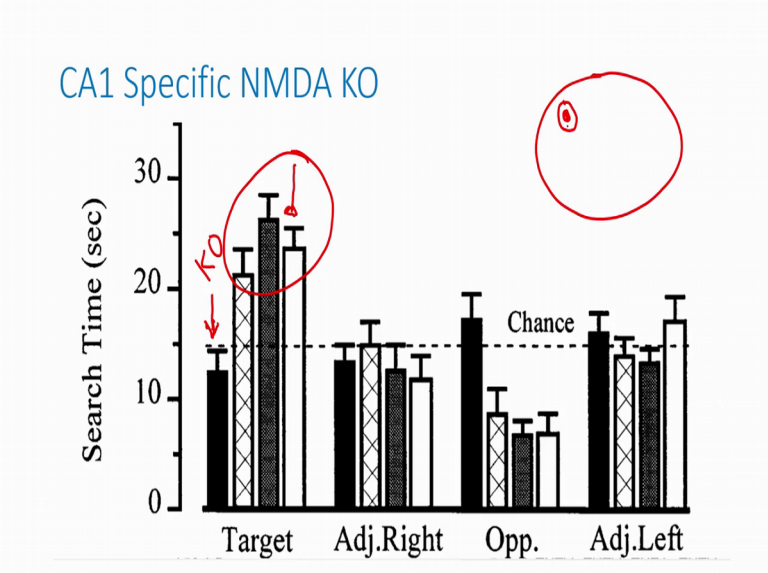

What we are plotting here is the search time right in the probe trial ok. And, then we are asking how much of the time the mice is actually spending in each of the quadrants ok. I want to draw your attention to the black bars here; they represent the knockout animals. Similar to the LTP measurements where, we had a few bunch of controls which I said do not have to worry about we just concentrate on the meet of this experiment, where the bunch of controls are done to ensure that we are probing the right thing right. So, you can think of them all meaning you can take a regular control that is the in this case it is the open bar.

And, compare to the open bar here you can see these mice the knockout animals do not search in the target quadrant at all in fact, that search the search in all the quadrants almost equal with equal probability just at the chance level. Compared to the controls where they specifically search on the target quadrant. This is significantly more compared to the rest of the quadrants and that is again a point or a proof that these animals, when you knock them off of NMDA receptors, specifically in CA 1 region is unable to form a memory of where the platform is located right. You can do some quite neat variations of this experiment here, where you remember the water maze and the

platform, but you can actually do is you can place a visual cue like a post, like a flat post there.

You will see the beautifully learn to good navigate to that flat post and then launch out to the platform. So, there is no problem of understanding how to reach there that is not the problem here. They simply do not know where is the platform. Now, that is very convincing that you need these coincident detectors these receptors that can active, that can get activated only in response to both the pre and the post neurons activation. Those you need these receptors to form this associative memory. And, we also know that they are required for forming the LTP: Long Term Potentiation in this region.

Now, this is all about forming or inducing the potentiation right. Now, I have described to you the function of these receptors. Clearly from just by this function there is no simple way for you to extrapolate to the fact how after removing the stimulus and once that is gone forever, how it can they maintain their higher level of throughput right. The memory is not just about changing the throughput, but being able to maintain that over a period of time right, that is same is true with LTP too right.

It is not just sufficient to induce it induction it is not just similar to the memory, it is induction together with the maintenance you need both of them. Now, how do you I think that is that can be can that be done at a different level or can that be done by another molecule. Now, that is a big question because whenever it comes to maintaining a molecule or a memory through a molecule, then you are talking about maintaining over time scales that are larger than the molecular turnover time scales ok.

Typically, if let us say if a memory were to be encoded by a molecule A. You can say I am going to increase the levels of the molecule A and that molecule then that level of that molecule should probably include that memory. Now, the problem is each and every molecule in our body in a living system has a finite lifetime, what happens to this memory after this lifetime, it is not infinite just like our lifetime is much longer than this molecules lifetime.

So, there they do get turn over much faster much much faster in fact, then compared to our anybodys longevity here. So, in to solve this seemingly contradicting problem so, one thing to say is maybe the memories are not represented in the molecules, but somewhere else. Or to come up with an real proposition real idea how the molecules can

act like a switch almost to say, you turn on a even turns on the switch and it maintains that state until something else happens ok.

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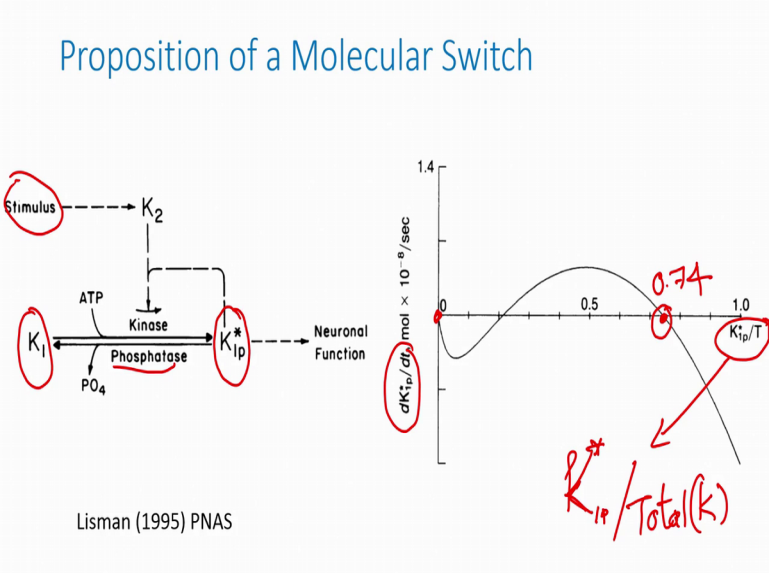

How can you do that? Without being susceptible to this molecular turnover, in 1995 John Lisman proposed this who came up with this unique idea. By, which he said hey we know in a biology many of this enzymes go from activated to an inactivated state through a signaling mechanism called phosphorylation, there is addition of a phosphate group to the enzyme or a molecule by a living system.

Now, if that were that were to be possible then maybe something related to that can be utilized in here to store the memory too. Again the main problem is the mechanism that we are going to propose need to be robust enough, and need to be overcoming the overcoming the problem faced by the turnover. So, it cannot be just the phosphorylation, because the phosphorylation phosphorylated enzymes are at some point going to be decomposed and going to be taken care of by the living system.

So, it cannot be simple phosphorylation, if it cannot be simple phosphorylation then what else could it be. He said there can be a generic mechanism phosphorylation being one example, but a generic mechanism where in there is activity that is turning from one state to the other. Like the phosphorylated to the unphosphorylated state, that is very much same as what you have been talking about ok. Now, let us look at this proposition here,

where he says you have a molecule K 1. And, the molecule the molecule K 1 gets phosphorylated to become active molecule K star.

Now, in biology we in living systems we have both these reactions possible, that is forward reaction where this molecule gets phosphorylated through addition of a phosphate group and becoming activated molecule. And, they can also be dephosphorylated by a set of enzymes called phosphatases. The kinoses which adds the phosphate to make the molecules activated, then there is a set of free enzymes that can take away this phosphate and then revert it back to the original stage.

If you were to hypothesize that the activated molecule are responsible for neuronal function; number one assumption of the hypothesis. It is activated molecule that are responsible for neuronal function like maintaining the plasticity and so on and so forth. Second the crucial point being the activated molecules themselves can self-catalyze their own phosphorylation that is the key here. If, you can these molecules themselves right? So, can act as their own enzymes, their own kinases, such that this molecule becomes active they can actually in turn activate more of their fellow beings and so on and so forth.

It can be an infinite cycle, because there are also reverse reaction possible the phosphatase is possible right. So, in such a system you will fast achieve an equilibrium, but that equilibrium can be affected by arrival of a stimulus S is hiding here. So, stimulus that that stimulus. And, the stimulus when it comes through some other kinose or some other some other mechanism, the momentarily increase the kinose activity momentarily that is the key here. That increase is sufficient to flip this whole equilibrium from one state to the other ok.

Now, that is all good and saying it in words, but I the beauty of this is that it is such a simple reasonably simple chemical reaction, you can go over go ahead and write down the rate equations and the concentrations of K 1 K 1 activated p as a function of time. And see, how is the behavior of the system? So, what is it I am looking for? What I am looking for is let us say the fraction of the molecules the total molecules the K here. We are talking about since it is it is it can add phosphorus phosphate group on it is own, they are kinoses we are looking at kinose class of compounds which can auto phosphorylate itself.

And, we are looking at specifically the concentration of the activated kinose the fraction of the activated kinose and how it is changing or how that concentration affects the subsequent rate of formation or deformation. Why I am interested in this subsequent rate of formation or deformation? What I want to know is that I want to look at stable states? What do you mean by stable states, when we are looking when you are plotting these rates as a function of this K 1 the activated state concentration. I want to look at a play a place or a multiple places where the concentration of the activated state does not change; it takes a lot of pushing or pulling to flip from one level of concentration to the other.

And, it turns out you can actually go ahead and calculate write down these equations and calculate this particular rate. The rate here we are talking about is the rate of decomposition or rate of change of K 1 activator state as a function of time. So, that is what we are actually plotting here. As a function of the fractional this is actually K capital K activated estimated as a fraction of the total K all right now what I am looking for here. So, now let us look at a few of the points here a 0.0 here and then a point right about here.

They are characterized by these stable states. Why I am saying that. If, if the concentration were to increase around this point a little bit. Then, what is going to happen is the rate is negative and it increases really fast here. This negative increase in the rate which is the deceleration so, to speak will pull down the concentration back to 0. Because, you are actually reducing the rate at which the K 1 activated state is forming and the phosphatase is good enough to bring it back to 0.

Same is the case here. Instead of now bringing it to 0 it brings to somewhere in between it is about 0.75 if you act 0.74, if you actually go back to this paper and look at the number. So, except for these 2 points nowhere else this is not a good point because depending on whether you are high on this side I mean you are deviating from this side or that side it is actually rising slope right. So, it is going to this is going to pull it forward and; that means, that it is a reduction in the negativity. And as a result the whole slope is positive we are actually looking for a negative slope around that point. So, that it can actually pull it back.

From these 2 states actually form a very good stable states and in fact, now you can go ahead and ask if they are forming this stable states, can I simulate a stimulus arriving and

then monitor or plot the concentration of the case to have peak one peak as a function of time; when the stimulus is arriving and look at that behavior.

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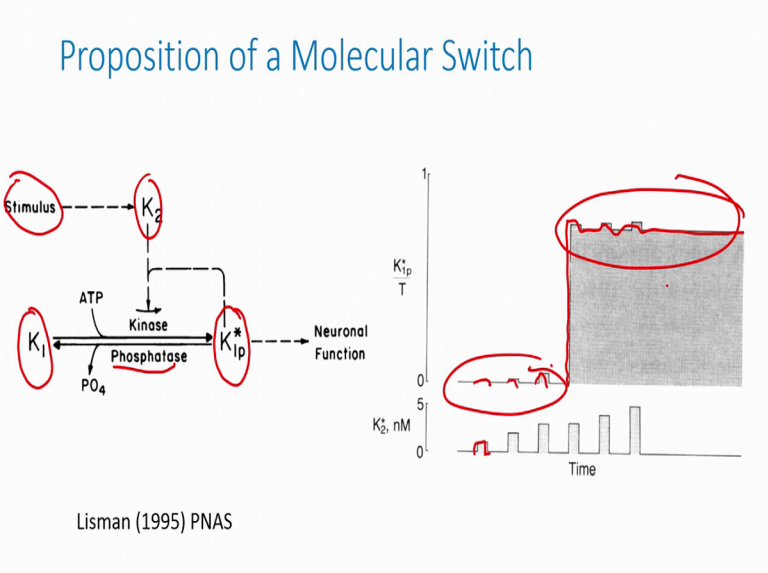

And, they did that in that paper and you can look at this now, where we are plotting as a function of time 2 things. Number 1 the stimulus the K 2 in terms of the activated K 2 here, that is in terms of nano molars does not matter it is the concentration of the stimulus. So, you are giving that stimulus and clearly the system is responding the fraction of the K 1 activate state is going up a little bit little bit until a certain point right about here, where it completely goes up and then stays there. Now, that is memory it went from this state to that state. Now, it no longer you are giving the stimulus and it is continuing to maintain it there, you have flipped the switch from one state to the other state.

All that by identifying a way in which there is a self-catalysis of a reaction of an activator so, to speak and that is taking care of much of your neuronal function that is all we are told. Of course, implicit in this entire assumption is this the reverse reaction is catalyzed by some other enzyme and that is happening all by itself there is we. Now, there are interesting reasons why this would the system would behave the way it is, but that is purely enzyme kinetics really chemical kinetics there. And, but the bottom line message that I want to drive is that, we can if you want to look for a molecule we want to

look for a molecule that needs to have self-activation or a self-catalysis and that need to be some kind of a kinose equivalent of that molecule.

And, that can have a function why I am talking about the kinose here, because it needs to have a ability to modify the neuronal function it needs to activates few other molecules down there, which can actually carry forward the function which increases the neural conductivity of the efficacy.

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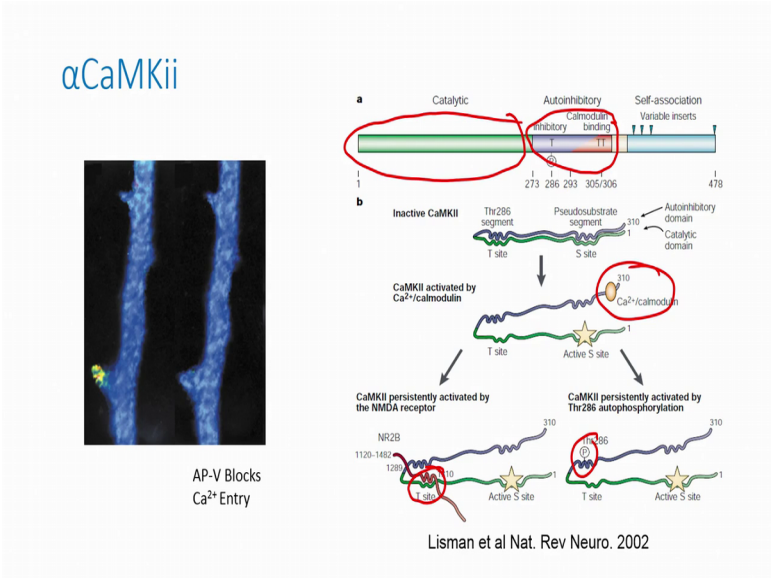

People looked for those kind of molecules and one potential candidate happened to be alpha cam K 2 abbreviated as alpha cam K 2, it is also called alpha calcium calmodulin dependent kinose 2 alpha is a one form of a this big protein. And, they hypothesized that the lisman again they hypothesized that this could be a potential target for maintaining I mean for maintaining the memory. So, alpha cam K 2 the calcium dependent calmodulin kinose 2 what does it do?

Number 1 we need to make sure that we are able to respond to the NMDA signal right the NMDA receptor signaling right. We have opened I mean the NMDA receptors act like coincident detectors they have opened up and then the calcium flowing in. So, now, first thing you want to make sure is that, alpha cam K 2 this enzyme is physically present in a place where the NMD are signaling mechanism is happening and you can actually detect this, this, this enzymes need to be able to respond to that. And, the first thing that they saw was that you can actually mean they are the alpha cam K 2 you can actually do

a similar localization study like the staining that we talked up where we saw in the NMD or knockout case we can actually do that.

And, then when you do that you see that they are highly enriched in the synaptic mean spines dendritic spines. These are the first I mean these are the regions of the postsynaptic neuron. And, through which the postsynaptic neuron receives the information there this is where the NMD are localized to. And, what we want to see is that when we are doing the NMDA or base signaling they are localized to the synapses, I mean the dendritic spines and that is what it is been shown here? So, what they have done is they have taken this neuron and loaded this neuron with the calcium dye. And, they went there near the spine and then locally uncaged through a photochemical process you can release a bunch of glutamate molecules.

These are molecule these are compounds which form through a chemical bond cage the glutamate I mean they do not let them move free. So, they connect them and then keep it there, but when you send in a photo active pulse, which is basically a light pulse and when you do that that bond gets cleaved. And, then the glutamate is now free to move you need to do that because you want to get that spatial localization. So, that you can go to the spine and then release just there and not anywhere else, and when you do that what they see is that these stimulus potentiating causing stimulus is releasing when it is released near the spine, it is activating the spine and it is dependent on NMDA receptors.

How do we know it? Because, you can use a drug A P 5 and that blocks the NMDA receptor and you do not see this calcium signal for this stimuli anymore. So, clearly saying that they are NMDA receptors are localized in spines. Through others chaining we know that the cam K 2 are also localized in the spines. Now, physically they are in the same place. So, that problem is solved, but that is our alpha cam K 2 qualify the criterias that we have post that disposed by Lisman, John Lisman.

In order to answer that question let us take a closer look at the molecule itself and it is structure when you look at that. So, it is a protein it has multiple domains or multiple structural elements each doing it is own function. There is a large portion of a catalytic domain and there is also calmodulin binding or an inhibitory domain and so and so on and so forth ok.

So, now if you actually that is when you are actually lining up all the constituents of this protein and then lining them up and then asking what these functions are, but if you actually look at the structure you can kind of approximate or you can a kind of draw this hairpin like structure to them. What you see is that there are specific areas? And, this specific areas gives us rises the possibility that this can be behaving the way John Lisman has hypothesized in his 1995 paper what is that?

Normally, they exist in this closed state. When they exist in this closed state then the active site right, the site which is used by the enzyme to phosphorylate or to carry out it is activity you call that as active site, that state is not accessible for other molecules. As a result you can think of that enzyme is existing in an inactive stage. However, when it binds to calmodulin or when you have phosphorylation, what you have is there is a steric hindrance, whereby the molecule by per say cannot close itself and letting the active site exposed. So, that now it can remain in an active state by itself.

Now, this can also happen when it binds to some other protein that that can happen through another site. So, this can also be because of another calmodulin binding or some other protein binding there. So, that these are the key aspects of the calmodulin molecule, that allows us to postulate probably I mean calmodulin kinose I mean cam K kinose 2 molecule. That allows us to postulate maybe these molecules are useful in preserving the memories in maintaining their memories. So, summarize quickly the molecule exists in 2 states active state and inactive state, and going from inactive state to active state request phosphorylation or binding to some other protein molecules of it is own kind or some other kinds. And, it can happen the phosphorylation can happen by itself meaning by another molecule of it is one kind right.

So, that happens at this particular residual I mean in sight it is called a thrown in 286 site, when it does it goes into that active site constant active site no. So now, this is telling it is likely that these molecules can take part in encoding that memory and maintaining them. But, the proof of the pudding comes when you actually can really prove without this molecule you cannot maintain the memory. And, that is what we have been and guess what we can actually do that and people can do that not now. As, you can actually guess from the way we have progressed in the lectures. You can go and specifically take away this particular molecule and ask what happens to the memory. And, that is what we will be doing in the next lecture. And, show that really this molecule why really this molecule

is a good candidate and it is considered as a represent as a possible correlate of our possible substrate for memory storage. I will see you in the next lecture.

Thank you.