Learning about Learning A Course on Neurobiology of Learning and Memory
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Lecture – 20
Memory in Molecular Terms V Selective labelling of memory encoding neurons and their manipulation

Hello and welcome to the lecture 20 of this Learning about Learning lecture series. We started off this lecture series with the idea of with the promise of saying we will look into the learning and memory in living organisms. And what are all the various different factors that affect the learning and our ability to retrieve these memories that are stored in response to the learning, the act of learning.

We have come to the point where we have showed that one can trace these aspects of learning as well as the memory formation all the way down to the molecules. The examples are how the dopamine levels can actually modulate your behavior expression of your behavior and how a particular class of receptors, NMDR receptors presence and absence of them in a small region of the brain or small sub region of the brain.

In fact, can modulate your ability to form an associative memory and that is an NMDR knockout. And, while type study that we talked about and then come all the way to the point where you can remove a particular gene that too in a differential amount, when you do that the effects that you see are very very profound. When you remove them a lot then you lose your ability to form and maintain that memory for a long time.

I mean the memory does not get formed at all. On the other hand when you remove them in partially, then the recent memory seems to be ok. But your ability to remember for a longer period of time seems to be affected. And we said that that this is because of the nature of the memories and what happens to them as we as it evolves in time.

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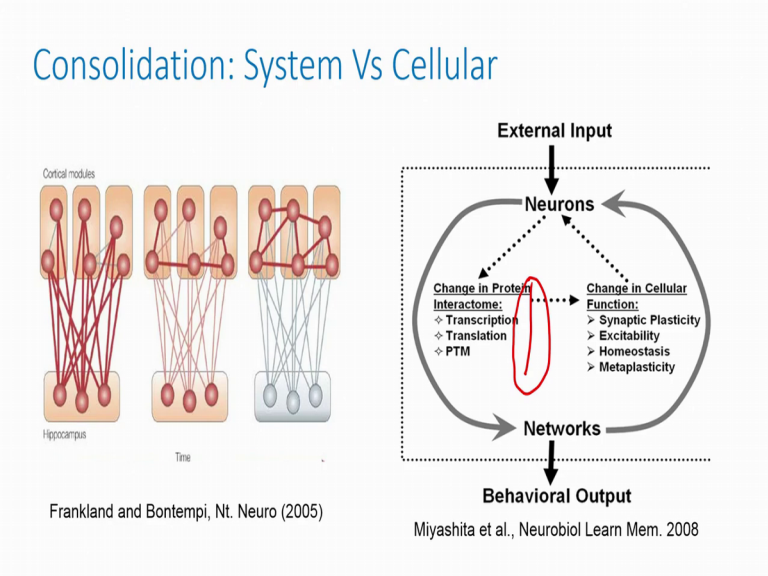

Quickly though the model that we proposed is that initially for the memory the initially the informations are getting integrated at the hippocampus to form a representation or an event and that is a memory that is staying in hippocampus itself. When you retrieve them very initially that is where you are retrieving it from. And if you remove the hippocampus you cannot integrate it and definitely you are not forming that memory.

So, that is so, called the sheet of memory is hippocampus and if you remove them you do not form that memory at all. However, if you wait sufficiently long enough and then remove it; even though you lose your ability to form any new memories you can still retrieve it. The notion we put forward why we can why it is so is because, in due course of time the cortical modules that actually took part in the formation of the memory initially can wire together by themselves with assistance from some other regions or some other cortical regions.

But, essentially can form an integrated representation outside of a hippocampus and such that if you remove the hippocampus you can still retrieve the aspects of memory, that was that is volt. We also said that such memories are distinct in their characters; we did that through discrimination tasks. Now I want to bring here a important distinction between two processes of consolidation. See the definition of consolidation is about making the memory resistant to external interferences such that they can be robustly

retrieved after certain time. It turns out you can intervene in this process by which the memories become resistant to change in two different timescales.

One timescale we already know that is the protein synthesis dependent pathway that is what we call it as cellular level consolidation alright. So now, that process you can think of that as a represented through here that there is an external input that is coming into the neurons and the neurons respond by changing what is now known as a protein interactome. That is that they are actually you need proteins to be made and the proteins are made and you have, can have regulation of this process at multiple levels at the level of transcription, translation and post translational modification too.

Each one of these processes cell biological processes right. Each one of these processes can modulate the neuronal function thereby modulate the memory the expression of memory. But the key here is that if these processes were to happen this need to be mediated by somebody alright. So, this can happen such that it can happen at overall network level. But eventually the net result is the behavioral output right. But that behavioral output needs to be modulated by somebody. And that is where we are going to introduce a new class of a class of genes called immediate early genes. These are the genes that are first response genes or they start this process of de novo protein synthesis. They help or they assists in this protein interactome that we talked about ok.

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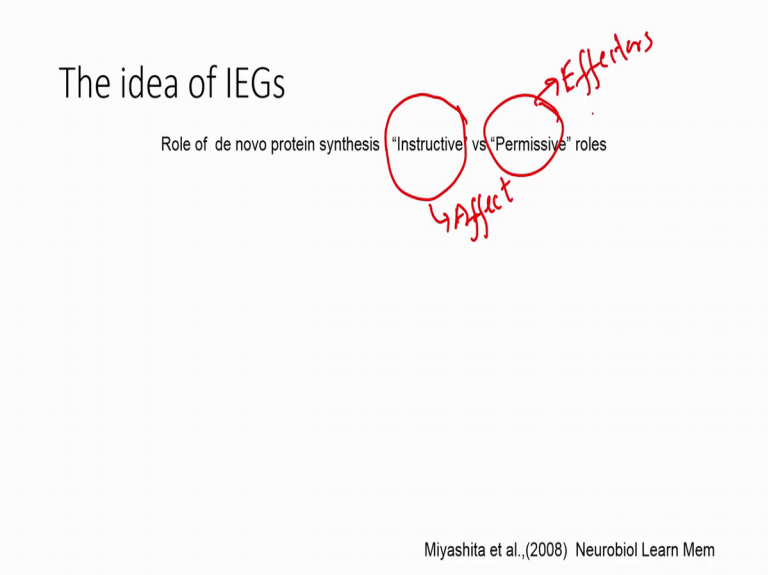

Based on how they actually assist you can call them as you can classify them as two different categories; effectors and affectors. Essentially you say you classify them based on whether they play an instructive role; instructive role is pretty much like a class teacher or a tutor telling you what to do is he is instructing. So, he is the person is going to tell you that do this. So, that these genes send a signal for subsequent cellular processes.

Versus a permissive role where it is an active participation from in a process that, the a process that helps in memory formation. So, you would say these guys are you can call them as affectors because they affects. While these guys can are called as affectors. So, based on these you can classify the IEG' s effectors and affectors.

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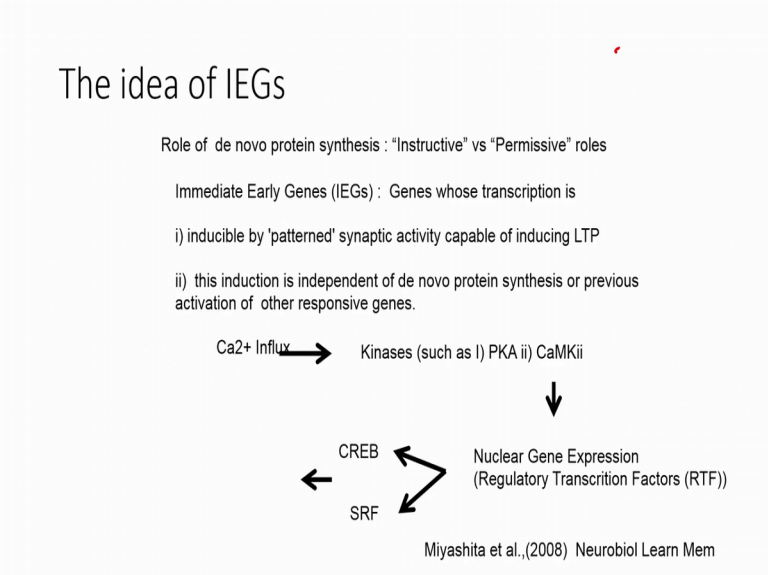

Now, what do they do? First these are the as I was telling these are the genes whose transcription per say is controlled by patterned synaptic activity. That is correlated to cause LTP alright. So, when whenever you are inducing in LTP you are activating these genes alright; they are very very highly correlated. But it is independent of the proteins de novo protein synthesis because, this is the first response elements.

So, they do come in before. So, that the control can happen later on that the prevention of the protein formation can happen later on and, more importantly the mechanisms that you can think of which can trigger this is that the calcium influx which in turn is detected by kinases. We talked about CaM kinase II that is not the only kinase there are many

other kinases that CaM kinase II is the one that we talked about. So, once the kinesis they get activated they trigger nuclear gene expression which in turn triggers the expression of the transcription factors such as CREB and SRF.

So, these members themselves can then trigger subsequent synthesis of proteins the net result being that you end up generating molecules that result in enhanced neurotransmission either across the synapse or in terms of cellular level response for a given input. When that happens you say that the you have a formation of a memory or alteration of a behavior. While a many of the labs are deeply interested in understanding and unraveling the various mechanisms that I have listed out and I am saying what roles each of the members play.

What people in generally in the memory field people do is that they make use of these findings to ask questions to probe the memory encoding and the expression of the memory process in a deeper way. How do we do that? We know that these are the first response elements particularly these are the first response elements when there is a pattern synaptic activity that is highly correlated or highly known to be capable of inducing LTP right.

So, if you want to know which, are the neurons that actually took part in this LTP for example, highly likely right we are talking about correlation. So, we are talking about highly like high likelihood not necessarily direct relationship right. So, in a if you want to ask that you can actually go ahead and find out which of these neurons actually are expressing this IEG's.

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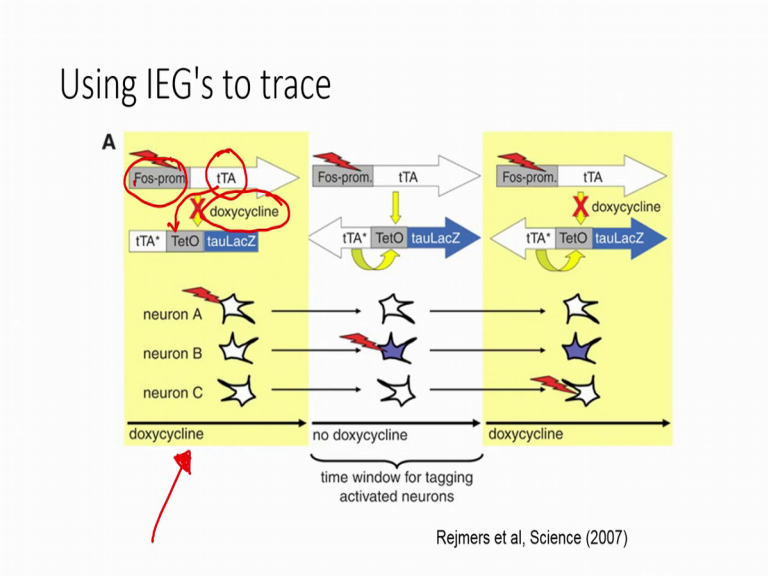

Or in other words you can make use of these phenomena as it was done in the regime acetyl to really mark those neurons which take part in memory. How do they do that, they make use of they made use of a transgenic and these are mice where you have put in an external gene. And, that has become part of it is genome. So, even when the mice is breeding it is passed along and then it exercises the protein depending on during the headed inheritance if it that gene is passed along or not.

Now, what is the gene this has two genes one is fast promoter this is an IEG here there are various different classes of IEG's I told you and there are different members and fast C fast particularly is one of the IEG's. And this we are taken the promoter of that gene and then they have expressed a molecule called tTA ok. Let us not worry about the name, but what is important here is that what it does. This in turn can actually bind to another promoter which is also expressed as a trans gene and only if this guy binds this enables this promoter to get activated and then express a protein called lacZ.

Now, the beautiful part about this whole process is that this whole binding is can be inhibited by the presence of doxycycline; a drug that can be externally administered. So, now what you have is a possibility wherein you actually can administer the drug and keep this neuron not express this protein at all lacZ at all. So this is very very important because, all if since this is a transgenic mice they are having this from their birth and

throughout it is birth at various different points in time this neuron would have received information would have taken part in memory.

So, if not for this doxycycline drug if you actually go into the brain and then with the hope of actually tracing or identifying the neuron what you will see is that the whole brain almost the whole brain would be labeled with lacZ because at some point or the other this neuron would have received the information and would have taken part in memory. So, now what this doxycycline provides is that an opportunity for you to prevent that.

So, if you feed this mice with the doxycycline. Now that doxycycline goes and then inhibits the binding of tTA to this gene. As a result none of the neurons end up expressing lacZ. So, in this state they took this mice and then train them in fear conditioning. Once they have been trained they removed the mice of the doxycycline the idea here is now they event has happened. I need to mark those neurons that took part in memory that took that actually expressed c-Fos. Because c-Fos would have been expressed because, of the in first as a first information response and the neurons that receive this shock information.

And the context information and the toned information would have probably express the c-Fos I would like to actually label them. And that is why I removed that doxycycline and this helps you to tag those neurons that receive this information and this to take part in plasticity formation. The beauty of this is now you can feed the mice back with the doxycycline. When do we do that we know that the consolidation window the protein synthesis dependent consolidation window is 24 hours remember 6 hours and later to be safe 24 hours and later we know it is cellular consolidation is done.

So, I am going to keep this window open only for 24 hours and after that I start giving doxycycline. Now no more expression of lacZ no more response of the fos promoter activated tTA and then expression of lacZ. What you have now given is that you have labeled the neurons that took part in the memory formation in response to fear conditioning experiment once and for all in the mices life. They went ahead and asked probe this in various different ways insured it is really the memory trace.

But the point of interest why I am mentioning here is that you can use this in along with something else in a more exciting way to probe the behavior of an animal, what that

would be. See if it were to be the neural trace that these animals are the memory trace that these animals are getting labeled. Then I should be able to go and activate those neurons.

And be able to reenact the memory right that is I notion right that is idea of zeroing in on the memory where the substrate of the memory right. So, can I do that you know that we can label this supposedly memory encoding neurons. But how do I go ahead and activate them and them alone this required another technology to. So, to speak that is the optically activatable channels ion channels. So, regular ion channels as we know they actually help in neural conduction right.

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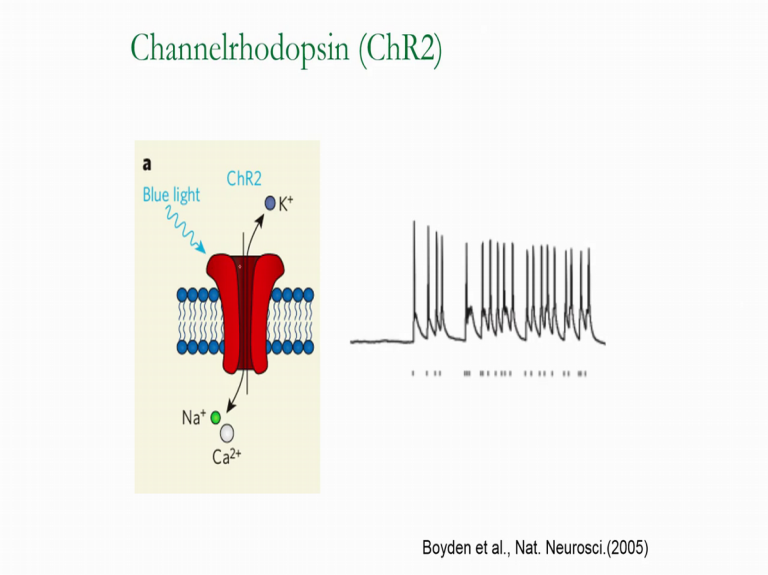

They open and close and then depending on they are open or closed states the lead guy on scene or out depending on the kind of channels they are. So, now the special property of these channels are the called that channelrhodopsins or is that that the they go between the closed and open state depending on the light when you shine a particular wavelength of a light in this case blue light is 488 nanometer light. When you do that when you shine that the ion channel opens letting in sodium along with it calcium to cations come in.

That is sufficient enough to trigger an action potential it is as if that the neuron is has received and information it is responding to that right you. So, the bottom bars here are the instances where the light is on you can clearly see the light the neural response. Now,

let us put this together with the fact that we can actually label the neurons that could have taken part in memory formation.

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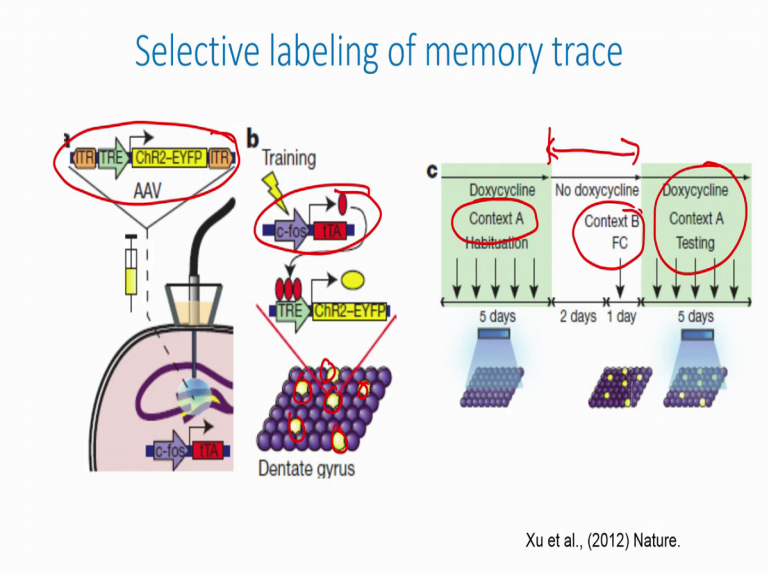

That is what Susumu Tonegawa's group at MIT did they took this mice where they mice were expressing c-Fos in response I mean expressing tTA in response to c-Fos very much like the previous paper. But they also injected they coupled with a different probe here and like them they were not in they were not using a lacZ as a indicator they were actually using an AAV virus a virus which can actually deliver a particular kind of a gene.

The gene is that of the channelrhodopsin that would express only if the tTA comes and binds to the promoter all right. So, this helps to mark and this helps to activate, but then this will get expressed only if the tTA gets and comes. And binds to this that is what this whole picture is all about now you can see they did that at dentate gyrus. And they what you can see is that there are bunch of neurons here both of them expressing both the genes.

However, only a fraction of them marked in yellow like this where both these things go inside that is the expression of I mean the receiving of the information as a result what you see is the expression of the c-Fos which is triggering tTA and tTA goes and binds to the promoter TRE which is starting the expression of channelrhodopsin. The idea here is now all this can be controlled with doxycycline remember that now the idea here is that I

am going to take make use of the system and then trying the mice such that I label those neurons that are actually encoding ok.

So, what we had what they are done is that they have used the doxycycline to prevent the formation of the memory right. To prevent the labeling of I mean not formation of the memory to prevent the labeling of the neurons when the animal is exposed to context a habituation context. Then they open the window of doxycycline no doxycycline. Here now, the tTA can go here and trigger the expression of channelrhodopsin there by labeling the neurons.

And then they did fear conditioning for context b they put back in doxycycline in context a and they are going to test it. Now, since it is context a different from context b and it is recent time point the animals should not express any fear.

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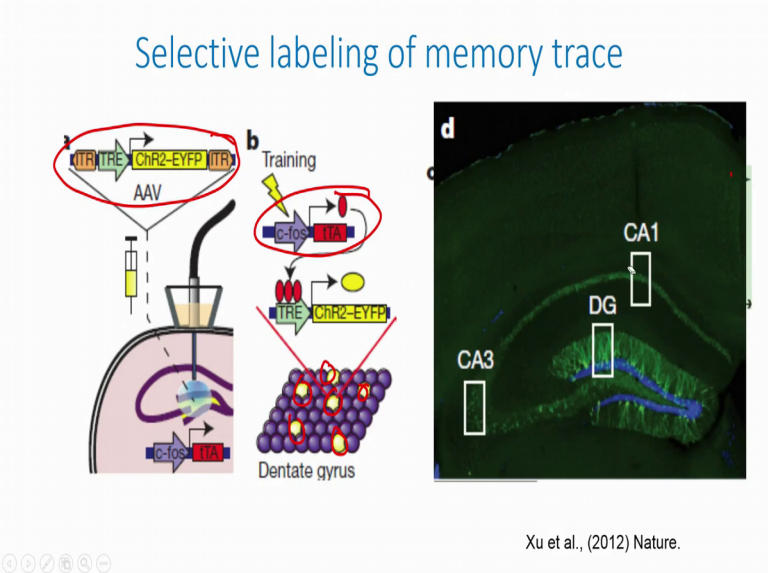

And what do they see I mean you can actually go ahead and see that they are labeling a subpopulation of the DG neurons that is what is shown in this picture. But more importantly when you actually go ahead and ask in the behavior right.

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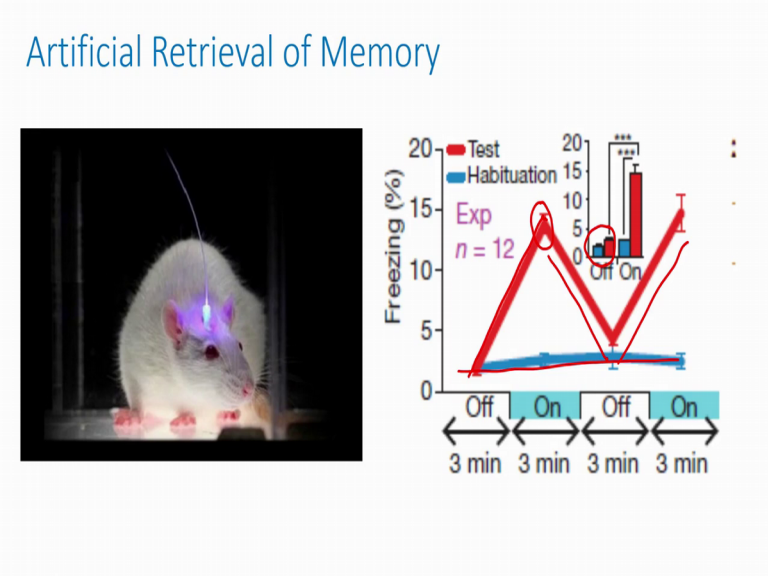

So, what do they see is that the following. So, they take this animals and put in an optical fiber right because you need to deliver the blue light into it is head. So, they put in an optical fiber through which they can actually deliver the blue light at will. So, what they are what you are going to do is that it is, since it is in a new context what you see is that when you do not deliver the light the animal do not freeze that is almost the same as that of the habituation group right.

And on the other hand the moment you turn on the light right these are the off on cycles here right. So, the moment you turn on the light you can see the freezing goes up. Only in those animals that are trained in the context b. And labeled in the context b not in the control you turn off the light comes back again, and turn on it goes back again. All this is happening in a context that is completely neutral context a non-training context. That is why it can go back to the 0 stage.

Now you are making the animal think even though it is in the different context not in the training context you are making the animal think it is the training context there by eliciting freezing thereby retrieving it is memory and making sure I mean and the expression of that is freezing. Now, this is the stage where we have reached now and from the starting point of asking the question of where exactly the memory is located I hope through these lectures.

I was able to convince you that learning is a behavior that is developed in response to in the evolutionary response to adapt ourselves to changing stimuli. And there are rules that one can elucidate; under what circumstances we will be able to change and what a circumstances we will not be able to. And the beauty of this whole process is that you can trace all the way down to individual molecules small molecules.

And thereby, showing that the complex behaviors do have representations at this very simple levels. Not to say that this is the only I mean these are the only factors that matter that is not true, but what I am saying is without them you cannot have those complex behaviors and you can go down all the way to this individual molecules it is very very fascinating to know that these individual molecules can control that is such complex behaviors.

In doing so we have overlooked few of the phenomenons because, it is not an all encompassing lecture. And so, what I will be doing is that in the next lecture I will be specifically focusing on those phenomenons that we have not spent time and understanding it right with to list out. We have not talked about various different behaviors that people use to study various different behavioral experiments or a behavioral protocols that people used to study learning and memory in animals.

We have not talked about some of the key learning mechanisms, we talked about associative memory formation we talked and then particularly the classical conditioning and operant conditioning there is a huge set of literature about model based learning. And we have not touched upon that at all. And even in the class of operant conditioning we have not talked about the, we talked only about two kinds of reinforces primary and secondary we did not talk about social reinforces.

And, these are the few of the phenomenas that we will be covering in the next lecture. And, hopefully I will also show you some of the videos of how these behaviors are carried out and when you carry out what do you expect to see.

Thank you.