Learning about Learning A Course on Neurobiology of Learning and Memory
Prof. Balaji Jayaprakash
Centre for Neuroscience
Indian Institute of Science, Bangalore

Lecture – 16
Memory in Molecular Terms - I: Protein synthesis in memory consolidation

Hello and welcome to the lecture 16 of the Learning about Learning lecture series. And, till now what we have seen are at most specifically, in the last lecture what we have seen is that, how molecules as small as dopamine, a very small molecule can actually control; the levels of dopamine can modulate and control the expression of a behavior. And, then switch an animal from a sign tracking verses to a goal tracking and so on and so forth is just to illustrate a point of how a complex behavior can be controlled by single individual molecules.

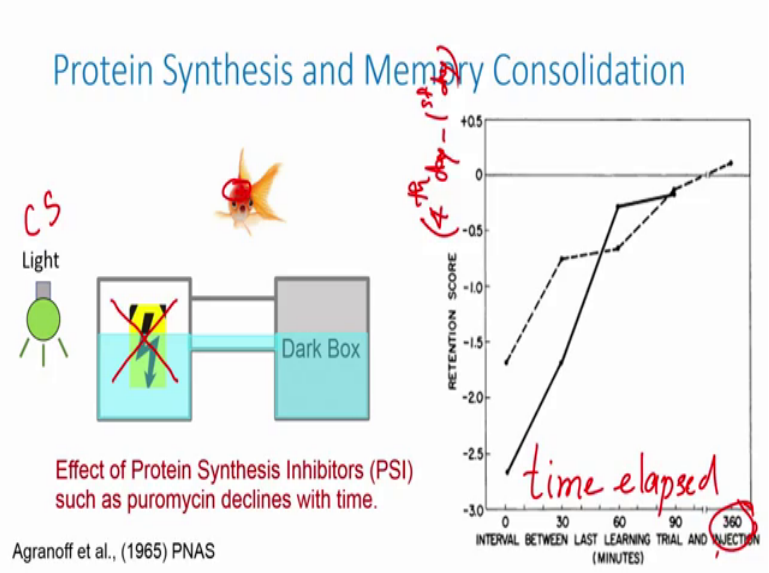
In line with that what we are going to see in the coming lectures are that, we are going to take this idea of complex behavior tracking down at multiple scales. And, while we are doing that we will see that it is not just a molecule see the behavior can be at multiple levels. It can be at the level of neuronal networks, it can be at the level of synapses that the junctions through which the neurons communicate at, it can be at the levels of a molecules as we have seen already. In doing so, for us to do so, requires few preliminary, you know I mean few preliminary information and a knowledge and we would we would gather them as we go along.

Now, remember our first very first lecture. In the very first lecture when we talked about memory. When, we talked about memory we said memory has different phases and these phases can be separated by using selective drugs right, by in infusing drugs at several different points in time. And, one of them was protein synthesis inhibitors anisomycin. Now, let us take I promise to you at that point in time we will look at it in a deep little bit more deeper manner later in the course and that is the time now, just to rehash at that time what we said was that if you train the mice a group of mice in a task, and then infuse protein synthesis inhibitors in the specific case of that study we said anisomycin.

And, then infuse this drug, test them like half an hour or one hour later you would see that their memory is pretty good compared to the control they are indistinguishable.

However, if you were to do that test 24 hours later; 24 hours after the training and right after the training you are infusing anisomycin of the training, but either way we were talking about in infusing them right after the training. And, we said we will not see this memory expressed at all; how did that result come by? Originally that result that experiment was done by Agranoff et al shown in the slide here.

(Refer Slide Time: 03:58)



And, they used goldfish to study this process. To study the effect of protein synthesis are the usefulness of the protein synthesis in memory consolidation. So, what did they do? They took a behavioral paradigm wherein they will take the goldfish, they will use the goldfish and then the fish; obviously, swim in water. So, there are 2 chambers in this fish tank. And, one chamber is well lit nice and other chamber is dark. There is a connecting path alright, there is a there is a connecting path between these 2 chambers. And, the as you can see the way I have indicated the water level in blue.

The water levels in the tanks are pretty good and they can the fish can actually swim all around. However, right at this point where there is a bridge that is connecting these 2 chambers, the water level is just about bad minimum, basically this possesses a hurdle for a barrier. So, to speak for the goldfish to go from one place together you can actually put some physical barriers to, but the idea is essentially the same.

You want to make this crossing the bridge as slightly difficult task it is a possible task, but it is a difficult task. Once you have done that, then what they are doing, what they are

going to do is that they are going to do the C S you are sparing here. So, what is the C S? The C S is a light coupled with electric shock ok. Right before, the electric shock has given the light goes off that is the C S, the C S comes on and then it goes off and then the electric shock is given. Nobody likes to receive the electric shock showed us the fish, then in response to that it and it needs to take an evasive action it has to escape from this chamber all the way into the bridge to the dark box.

In this place, if they go in there is no electric shock. Clearly the animal does not learn it and to very in first instance, they had to do this you know block of like 10 or 20 trials. And, every time when they are scoring when they are giving the training they are going to score, whether the fish successfully went from the shock box to the or the light box to the dark box. If it did then you would say that it successfully exhibited the learning, if it did not then you say it does not exhibit the learning, but that is about learning.

Here, we are talking about memory retention or consolidation to speak. What is distinction here? The distinction is we have to test, if the animal remembers something and when we are testing if the animal is remembering something, typically we do not give the US, we just test the animal's response to the C S alone.

So, on the test day you are not going to give this electric shock, but you are going to expose the animal to the C S Q this is the C S remember. And, we are going to test the animal to the C S Q and then ask, can the animal remember the association that, it developed during the learning between the light and the shock. If, it did it is going to go into the dark box. Again, we do it in my since it is a 0 or 1 response you do it in multiple trials. And, then get an average score of how many times actually the goldfish went from the light to the dark box.

Now, you compare this with the trials that are done during the training. And, they get a score something called a retention score, there is basically the difference. If, the difference is low then what you say is that the animal is not retaining anything at all if, it is high negative and low in total value. And, if on the other hand if the animal really retains well then you would see that they remember this. So, they would elicit a response predict nicely and go to the dark box, not very different from what they have initially started with.

So, we remember this is equivalent of our comparison of the 30 minutes or short term memory with the long term memory in the mice experiment. Here, they are comparing after the training trials I mean in the later training trials with that of the retrieval trials that are presented 4 days later ok. What they, but in between what they are going to do is that they are going to divide the animals in 2 groups; control those guys receive just the vehicle or the ceiling. And, then the other guys receive puromycin another drug another protein synthesis inhibitor and that protein synthesis inhibitor is injected into this animal.

The reason why they were actually doing this in the goldfish is because it is easier to access the cranial cavity in the goldfish. Actually, they have all that they have to do is they have to take that a needle just syringe and a needle just like, what do we do, what we get, what we use for giving a shot to ourselves or at a doctor uses to give a shot. And, then go and then directly in inject at the cranium. Alright, at this in this place and when you do that fish really require very fast, I mean the in few minutes they do not exhibit any signs of having been injected and then you can subject them to the test.

So, they did that right after the training is over and then waited for 4 days and asked how well are they doing it? They did even slightly better, what they did was they did they measure the dose response curve and they clearly show as a function of different dose. The ability to retain or ability to lose changes and in this particular case 2 of such doses has been shown one with the dotted line, other with the solid line.

What you are actually looking at is the graph of when they injected as a function of time. In this axis this is the time elapsed since the trainee it is not coming out now. So, I will write it here. So, it is the time elapsed since the training. And, then you are measuring the animals ability recollect on the fourth day. Now, what you can see from here is that if the injection were to be given right then and there versus 360 minutes later right. Then and there you inject clearly, they are performing less compared to a test that was done right after the training ok. That is what the retention score is measuring. If it is more negative, it means on the fourth day they are performing less that is why it is negative. So, it is a fourth day performance minus the first day performance.

So, the retention score you can think of that as fourth day minus the first day alright. So, you can see that more negative here means that the first day performance was better, they are the performance has declined their ability to retain has come down, that is why it is

negative. And, that is to that even that is dose dependent the amount of funny summer puromycin in that you inject actually pushes down there are performance levels. As you increase the time until about 90 minutes or so, there is some effect, but anything after 90 minutes particularly at 360 minutes. It is about 6 hours or so, you do not see any difference it is 0, it is the performance is as good as the first day alright.

So, the consolidation has taken place are at least seem to have taken place at 6 hours yeah in any time afterwards, if you try and infuse this proteins in the inhibitor, it is not of much use. Since, then lot other more experiments people have done and what we know is that, if you were to be safely out of the consolidation domain, then it is good to wait for about overnight or 24 hours to be safe. Anything greater than 24 hours you can be you can safely assume we are out of the protein synthesis a dependent consolidation phase right. So, that is all happened.

And, if you want to intervene before that it is best to intervene right then and there, but if you cannot at least half an hour before or 1 hour before not any time later it becomes the gray zone of whether it has happened or not happened.

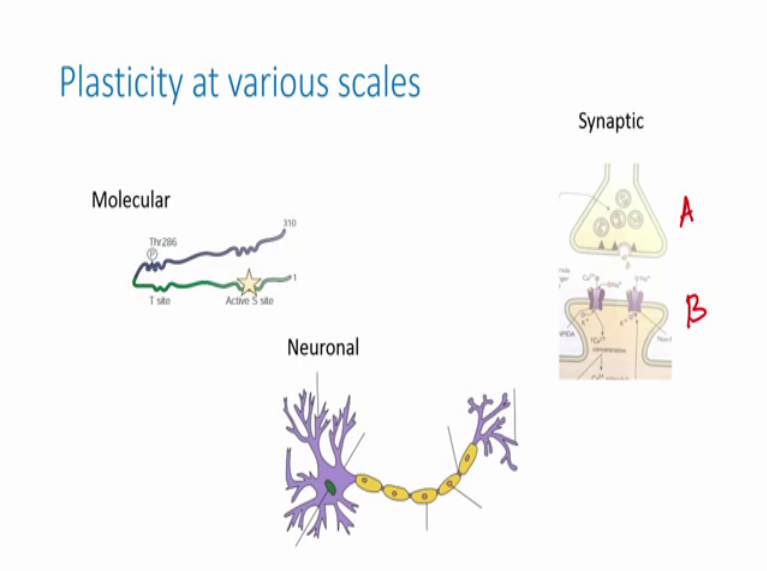
So, clearly synthesis of new proteins synthesis of proteins (Refer Time: 14:16) is very very very very important here, and you are stopping as when you stop that the memories does not seem to be getting consolidated. This is yet another proof that, how a behaviors like memory retention can be traced down to the levels of a molecule right proteins are molecules. And, we have not zeroed it on one particular protein here or, but that we have then a blanket blocking of the new proteins, but the point is you need to be able to synthesize new proteins to be able to store this memory for a long time.

When say this and then when I give these lectures, you people typically ask that. So, can we test down all this behavior, all this memories to molecules and if it is the root of all this behavior. I mean it is hard for us to realize the fact that if things as complex as such behaviors can be traced down to individual molecules are there not any other explanations, or are we missing something here.

Clearly the answer is that yes you can trace down to individual molecules, no doubt about it, but these behaviors have multiple scales they are operating at multiple scales, while my point here is to stress on the fact that, you do need these molecules to be able

to express this behavior, they by themselves are not just sufficient and this kind of plastic. So, called the plasticity occurs at multiple scales.

(Refer Slide Time: 16:10)



The scales can range from molecular scales that as we have seen, at the individual proteins or even down to dopamine levels, or neurotransmitter levels, or it could be at the level of synapses; where the efficacy of the through put like. And, your synapses are the places where the neuron A communicates with neuron B, in the brain and then the efficacy of this communication could be a way of storing the plasticity. And, at pan neuronal level, that the response of a neuron for a given stimuli could be altered right. The general excitability of the neuron through expression of different ion channels and so on and so forth.

Apart from this it could also be and a network level phenomena. However, what we will see is that we from the vast literatures we had in this field so far we could say much of this could be traced back to 1 of these 3 and not to say that the network do not play a role, but then even those network level phenomena's could in some way be directed to one of these 3 levels, neuronal synaptic molecular levels. And, that is the line that we are going to do in this lecture at least well.

If they are at the operating at multiple levels, then what evidence do we have to say that it is at the synaptic or even at the neuronal levels? So, while behaviorists and

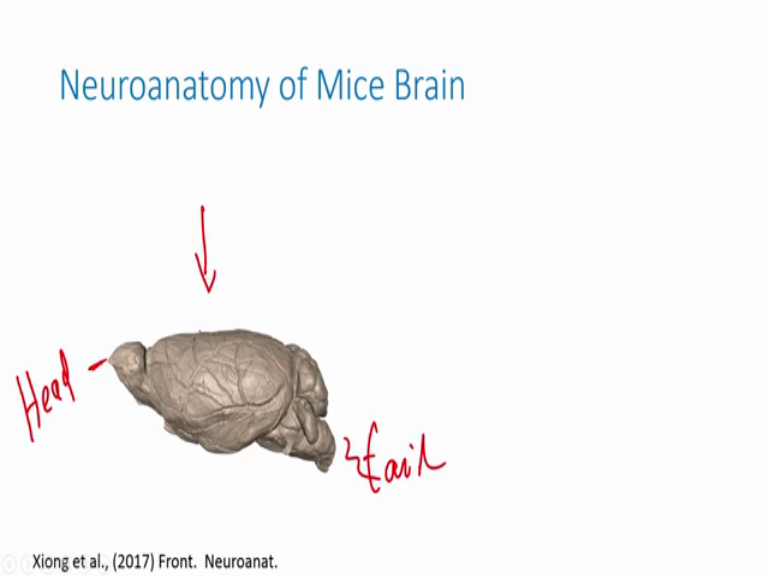
psychologists were very busy figuring out, what are all the rules that govern the learning in human beings and mammals are in animals in general.

Neuroscientists trying to understand the functions of the brain and how it relates to the behavior were basically trying to establish the physical correlates or the physical relationship between the function and the organ itself the brain itself. In one of such experiments bliss and Lomo they were trying to understand the role of neuronal circuits at the hippocampus itself. Remember hippocampus, the removal of the hippocampus is in HM resulted in loss of declarative memory.

Now, using that as a cue they really wanted to probe on to this effect of onto this hippocampal region and then see if they can actually 0 in on some correlates of plasticity. So, now so, here when you are looking at that kind of a region I mean that at that scale, then we are specifically looking at changes in the input output characteristics of a region, or input output characteristics of a neuronal circuitry that you are looking at, or even a pair of neurons that you are looking at.

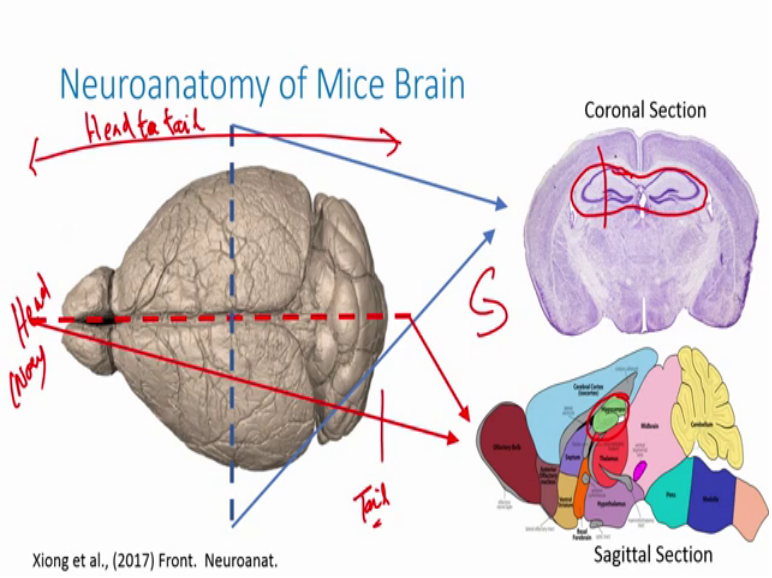
So, in order to understand their experiment and then what their question really is we need to understand a little bit about the hippocampus region itself specifically, the neuro anatomy of the hippocampus itself.

(Refer Slide Time: 19:55)



So, now let us take a look at the mice brain and the mice brain when we look at it and that is the Rostrocaudal axis. So, the nose of the head is in this direction, and the tail is in this direction, of the mice you can think of that mice being here and then you are placing the nostrils of the mice, where to be here and then the tail where to be here, and you extract at the brain that is how it looks like.

(Refer Slide Time: 20:28)



Now, you can look at this brain from the top, when you look at it that is how it looks? That is right. So, same again the regions the directions are the same. So, that is the head, there is a nose and this is where the tail of the mice is situated. In this and this axis is called a rostrocaudal axis.

Now, when you are looking at the anatomy of the brain; anatomy we are going to look at the multiple sections, and then say and then look at how the neuronal circuits are arranged? And, neurons are arranged on how what are the kind of connections that you are making. Especially, we are going to look at hippocampus alright. So, when we look at the hippocampus. So, when you are talking about sections, then and this 3 time dimension any tree dimensional structure for that matter particularly this 3 dimensional structure. Can be taken sections at various different ways, and 2 routine ways in which people take sections are along 2 mutually perpendicular axis.

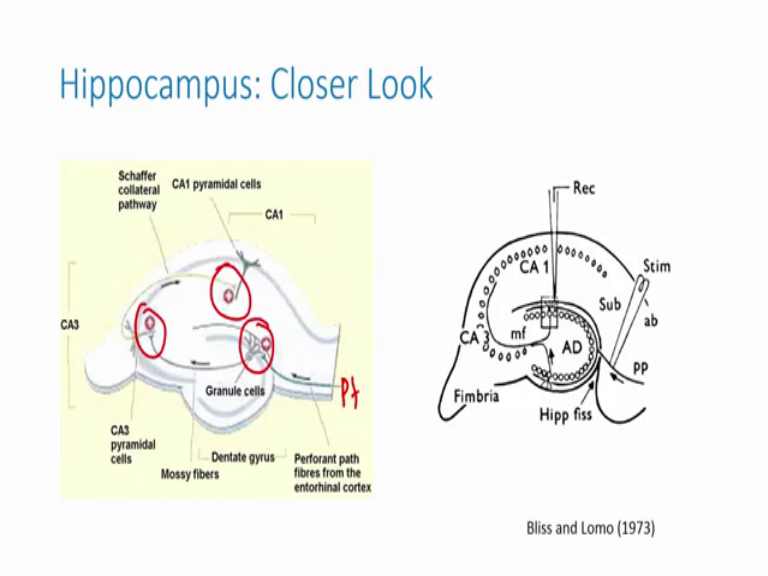
So, when you play some Mice Brain, Orion Mice main in this way and if you take a plane that is perpendicular to the rostrocaudal axis see, that is this is our rostrocaudal axis right head to tail axis. Let us call it as head to tail axis.

So, if you take a section that is perpendicular to the head to tail axis and then flip it around so, that you and then the clearly the other axis would be parallel to the head to tail axis. So, let us say if you take a section perpendicular to that the blue line and then take a section and flip it around so, that we can actually see it in a plane, and that is how it looks this coronal section? I am pretty sure we if you have been watching these lectures. This section is very familiar to us when we looked at where the picture next to him was a mice brain. And, I wanted to illustrate why Karl Lashley failed in his attempts to localize memory right.

And, to reject your memory, even said that is the region called hippocampus and that is the region that equivalently in H M that is been removed it is Mice. So, in H M's case it is human brain and the medial temporal lobe was removed which included the hippocampus.

On the other hand, if you had to take a section along the red line, along the head to tail axis and then look at it and that is how it will look like ok. There are various different regions which are marked here and the sections that you obtained on an axis perpendicular on a plane perpendicular to the head to tail axis we call it as coronal section. And, while the other section which is perpendicular to the coronal section we call it as sagittal sections. And, the region that we are particularly interested is in Hippocamp this region hippocampus. So, for various different reasons, it is easy to look at the circuitry how the hippocampus in these sagittal sections.

(Refer Slide Time: 24:05)



So, when you look at it the sagittal section of a hippocampus. What you are seeing are subdivisions, sub regions of the hippocampus, now just go going back here. So, whether you look at it in this direction or take a section this way that that would be the sagittal section, because of the 2 inter or overlapping C sections right; 2 C S you can think of that as this going up and down and then if you take a section here on this way they look very similar alright.

So, and that is how it looks in the sagittal sections. And, this hippocampus then is divided into 4 main regions in this section; region of which 3 of the regions are called as CA regions a Corona Aminars regions and they are called CA 1 marked here, CA 2 not marked here, CA 3 again marked here and dentate gyrus is also called a CA 4 or the dentate gyrus regions. Each of these regions are have a marked difference in their cell types and they have a very characteristic input output relationships.

So, the input to this hippocampal region comes from entorhinal cortex through pathway called perforant path ok. So, they saw the perforant path and the action the actional fibers that coming in they are called as the perforant fibers or perforant fibers. And, then they come and then make synapse into the dentate gyrus cells and the dental gyrus cells gyrus cells make synapses send their inputs to the CA 3 neurons, and the output of the CA 3 then goes into CA 1 pyramidal cells..

So, since it is a very fixed neuronal circuitry involving 3 synapses; synapse number 1, 2 and 3. It is also called as a tri synaptic circuit and it is quite extensively studied. It is very uniform and they have very remarkable characteristics number 1. Because, it is very uniform you can actually structurally say where exactly the fiber bundles are like; they are very distinct very very easy to visualize, and then thereby you can actually if you want to stimulate you can electrically put in an electrode in a region, and be sure about that you are actually stimulating the fibers and not in the inputs are not anything else.

And, you can also be because the cell body layer and so on are separate, you can also be sure about placing an electron in the right electrode in the right place, near the cell body and be sure enough that you are recording at the output end of the or the output end of the neuron. Thus having the pretty regular anatomy helps to be able to dissect out the circuit behavior.

And, it helped bliss and lomo to perform the experiment they much needed wanted, what is it experiment? They said hey, look if the hippocampus is important to store memories and we think I mean memories, we think are something that we form in response to events that are happening around us. There has to be some plastic change, some change that has happened because of an event when that stays just the way it is only the even there by the event has an ability to leave it is mark in the brain, even after the event has happened.

So, that is that ability to retain to leave a mark in the brain you we call it as plasticity, it is it is plastic like, a plastic you can mold it and then that stays there unlike an elastic where you release the force and it collapses back into it is original state. Here it is plastic. So, it is stays there.

So, that you can go back and then retrieve that memory. If, that were to happen in hippocampus and particularly if it were to happen through the synapses, then why do not we make use of this architecture internal architecture of the hippocampus. And, then architecture I mean the neural anatomy of the hippocampus and then probe does it really happen?

So, how do you probe it I am what they said this I am going to implant 2 electrodes; one a stimulating electrode and recording electrode. So, the idea is that we are going to stimulate the neuron and then record the electrical activity ok. And, see what happens to

the electrical activity in response to the stimulation. That is the brain the hippocampal region has an ability to store this fact that there is a stimulation that has happened in time. And, if it stores what is the expression of this storage, how do you see that, on what form does it store? Now, these were the questions that they asked by Bliss and Lomo and we will in the next lecture see the exact experiment how they did it and what did this what do they see.

Thank you.