Module:
Biological Foundations of Mental Health

Week 4
Biological basis of learning, memory & cognition

Topic 1
Learning, memory and synaptic plasticity – Part 4 of 4

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Lecture transcript

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Welcome to our fourth part on synaptic plasticity and memory. So in this fourth part, we will finally discuss whether LTP in the hippocampus is important for learning and memory. So you will learn about approaches, how LTP was studied in this context, whether LTP is actually induced by training in a memory task. I will explain in a moment. And we will learn about methods-- how LTP has been manipulated, and whether this has impacted on learning and memory.

For LTP-- LTP, as I mentioned in the second-- or first part, in particular-- is an interesting phenomenon, because it's long-lasting synaptic plasticity. It's an enhancement in synaptic plasticity. It has input specificity. That is also a very interesting property. It has associative properties, co-operative properties. So all of these properties make it a very intuitive model for learning and memory. But in addition, it also follows Hebb's postulate.

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In 1949, the Canadian psychologist, Donald Hebb, wrote a book The Organization of Behavior. And in this book, he illustrated a principle in how he thought that neurons should behave when an animal learns new information.

And he basically wrote is when an axon of neuron A excites neuron B, and repeatedly or persistently would do so, when some changes like growth processes, or metabolic changes would take place, in one or both cells, so that A's efficiency to fire B is increased.

So certainly speaking, LTP follows this phenomenon. Because when you have a high frequency stimulation, where basically, you enhance the synoptic transmission between neuron A and neuron B, and so the likelihood that neuron A fires neuron B is increased. It is strictly speaking, however, not the firing of what is enhanced, it's the synaptic transmission that is enhanced. But loosely speaking, LTP follows the Hebb postulate.

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Up till this point, we have always talked about LTP after electrical stimulation. So LTP was induced by electrical stimulation. And the question is, really, does such type of synaptic plasticity really exist in a behaving brain?

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So because when we use electric stimulations, we just assume that these electrical activity patterns really exist. To address this point, researchers have asked the question whether training in a hippocampus dependent memory task can actually induce LTP. So to this end, we have used this passive avoidance task, which I explained in part three.

So the passive avoidance task has restraint. But it's a one trial memory paradigm. It's hippocampus dependent.

So just to briefly repeat, we put an animal into a lid compartment-- a mouse or rat-- in this case, rats were used. And then the rat goes into the dark; door closes. A shock is provided-- a mild foot shock. And so the animal learns that the dark-- it associates with a mild foot shock. So at the time of testing, the animal is put back into a lid compartment. And the animal would avoid to go into it dark.

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Animals have been trained in this task. And before training, multiple electrodes were implanted into area C1 of the hippocampus, as shown in panel A, here. So you see basically eight recording electrodes-- one to eight-- and one stimulation electrode.

The reason for having so many different recording electrodes is that the idea is that behaviour is used to induce LTP. Maybe only a small set of synapses will undergo LTP. And many other synapses will not be affected. Whereas, if all of the synapses would undergo LTP when the animal could only get one memory, and that's the end of it.

So, obviously, the animal should produce many, many different memories. So therefore, only a small set of synapses should undergo LTP. But then you should use multiple recording electrodes to detect the LTP. So they used eight recording electrodes.

And so we trained the animal in the inhibitory avoidance task. And in panel B you can see where the trained animals, in average, basically-- are shown in orange-- show after the training and enhancement of synaptic transmission, and where enhancement seems to be long lasting. So it's measured here up to four hours after the training.

Panels G and I show this in more detail. So in this case, the field, EPSP, is a measure to plot it, versus basically the number of synapses or the percent of the electrodes-- and for four different groups of animals.

So the animals that have been trained, this is the red circles. And the animals that are naive-- let's just focus on these two groups first. The naives are blue. And so what you see here is the population of synapses are such that for blue, that the average is around 100%. And when basically, after the training, the red ones you can see that they have shifted to the right. You see some synapses that show more EPSP slope. So some synapses show 150% EPSP slope, which you never observe for naive animals.

So some synapses are severely potentiated. That is all true for 30 minutes, 1 hour, and 2 hours after conditioning. So these synapses have produced LTP.

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There is also other behavioural control. So for example, animals that have received the shock only-- in other words, the animals are placed immediately into a dark compartment, get a shock, and so it's not-- so the animals really don't know, actually, what the task is about. They just experience a shock, but they cannot really make an association between the shock and something else.

Or alternatively, an animal is just placed into a lit compartment, allowed to go into the dark, but

doesn't receive any shock. So again, there's no association between darkness and shock. And again, these two groups, which are just performance controls-- no LTP was induced. So only LTP was induced when animals were trained in the inhibitory avoidance task.

So this shows that behavioural training can induce LTP. And in this paper-- please, have a read of the paper, if you're interested. This paper will show that it was actually NMDA receptor-dependent. So LTP really occurs during memory formation.

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The next set of experiments, which were done to probe whether LTP is important for memory, is to manipulate LTP and then to test the impact on water maze behaviour. And so as I explained in part three, with Morris water maze task-- so the water maze task assesses for whether there is spacial memory or not.

In particular, after training, when a memory probe trial is given, during the memory probe trial the platform is removed from the swimming pool. And the animal is allowed to search for the missing platform.

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So here the animals-- some rats were treated with an NMDA receptor blocker, AP5-- and versus controls. And you have seen this graph before. It was a graph for part three.

It indicates, basically, in a memory probe trial, the AP5 treated animals have a random surge. So there is no spatial memory. Whereas, for control animals, which are just treated with some kind of saline, they have a spatial memory, because they surge most of the time in the training quadrant.

Now this experiment then indicates that blocking NMDA receptor impairs spatial learning. So since NMDA receptors are important for the induction of LTP, which suggests that LTP is important for learning. However, as every experiment, there is a catch.

The catch is that the drug dose in these experiments was relatively high. So the animals had some performance abnormalities. They fell off a platform, for example.

Furthermore-- and blocking NMDA receptors does not only block the induction of LTP, it also blocks long-term depression, for example. And so in principle, one cannot exclude the possibility that impairment in long-term depression has resulted in the impaired spatial learning.

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Another way of blocking LTP is to generate mutant mice. So this is because only a limited number of drugs is available to block particular molecules, like the NMDA receptor. Whereas genetics, with mouse genetics, one can manipulate any gene of interest.

The Nobel Prize committee awarded the Nobel Prize for gene targeting to manipulate the mouse genome. So in this case, you basically manipulate any gene at will in mouse embryonic stem cells. And then you generate a mutant mouse, nowadays, where even more quicker methods, like the CRISPR/Cas genome editing system, for example, which can work directly in fertilised embryos in zygotes. So you can manipulate any gene at will in a very quick way.

And so the rationale behind such experiments is that you, basically, impair a molecular process. And that impairs maybe LTP. And then you can ask the question, does it impair learning and memory?

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So one experiment that was done by Susumu Tonegawa, the Nobel laureate, who got actually his Nobel Prize for discovery of antibody diversity and how this is being generated, he's now very interested in learning and memory. So one of his early studies when he turned into the learning/ memory field was to knock out an essential NMDA receptor subunit only in the hippocampus.

And so basically, we sent the NMDA receptor-- consists of the NR1, GluN1 subunit. That is an essential subunit for all of NMDA receptors. So if you knock out the gene that encodes GluN1, you block NMDA receptors.

And you can make a conditional knock out. That's indicated here. So you can basically introduce with LoxP, recombination sequences in the NMDA receptor gene, in the GluN1 gene. And these LoxP sequences are recognised by Cre recombinase, that cuts out when the sequence in between the LoxP sequences and leads to knock out.

And now in that case, you get only a knock out when Cre recombinase is active. And Tonegawa succeeded to have a mutant mouse that has an active Cre recombinase only in hippocampal area CA1.

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So this is indicated here, in the next cartoon. You can see, basically, this is a mouse that provides Cre in area CA1. And in area CA1, there was no expression of the GluN1 subunit anymore.

This is an in situ hybridisation, which shows in control animals-- nice expression of GluN1 in CA1, CA3 and then the gyrus of the hippocampus. But you can see here with the signal in CA1 of the hippocampus is severely reduced. It's actually later confirmed by electrophysiology as completely abolished in the CA1 unit.

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What's the consequence of knocking out NMDA receptors in area CA1? Well, it impairs spatial memory. In the probe trial, after water maze trainings, these mice have been assessed. And you can see here four groups of mice-- the wild-type mice, which basically show in the memory probe trial, that they search most of the time in the training quadrant. And then you have two other mouse lines, which are just controls mouse lines that have a GluN1 flanked by LoxP sites-- so they have also a spatial memory when T29-1, just mice that have Cre recombinase. And they have also a spatial memory.

And then, finally, we see one specific knockout mice for GluN1. They show a random search. So there is no spatial memory.

So this is consistent with the finding where the pharmacological block of the NMDA receptors blocks spatial memory. And it suggests where LTP-- impaired LTP induction impairs spatial memory.

However, the catch is, of course, that blocking NMDA receptors can also block long-term depression. So it's unclear whether these impairments have resulted from impaired long-term depression or impaired long-term potentiation.

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Now an enzyme that has been very much implicated in long-term potentiation is CaM Kinase II, as illustrated in part two. So CaM Kinase II, is the calcium calmodulin-dependent kinase. It has, as almost every kinase, a regulatory domain and a catalytic domain.

Normally, the regulatory domain inhibits the catalytic domain. So the enzyme is not active. However, when calcium levels rise in the post-synapse, calcium binds to calmodulin, and the calcium calmodulin complex binds to a regulatory domain, inducing a conformational change. So now the enzyme can phosphorylate substrates.

This configuration of the enzyme is dependent on the presence of high levels of calcium calmodulin. So when calcium calmodulin levels drop back to baseline, the enzyme is inactive. However, the enzyme can undergo an autophosphorylation, it can phosphorylate itself at threonine-286.

Threonine-286 is within the regulatory domain. And when you get phosphorylation of threonine-286, you get a further conformational change that entraps the bound calcium calmodulin. So we're now at baseline levels of calcium calmodulin. There is still bound calcium calmodulin, and the enzyme remains active.

This autophosphorylation event has been thought to be an important event for maintaining long- term potentiation. And therefore, there was interest to make a mutation that blocks for the autophosphorylation. And actually, I myself did this work when I was in New York. As a post-doc, I made a mutant mouse that has threonine-286 change to alanine.

So if you change the amino acid to alanine, the alanine cannot be phosphorylated. And it doesn't have a hydroxyl group. So you basically block the autophosphorylation at threonine-286. And you end up with an enzyme that is normally active with a presence of calcium calmodulin.

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So what happens to these animals? So they have severely impaired LTP in CA1 in the hippocampus. So this is indicated here. So in panel A you see the open circles is LTP in normal wild-type mice. So you have first STP and then followed by LTP.

In the mutants we have only some kind of PTP and maybe STP briefly, but mostly PTP, and then no LTP. So LTP is completely abolished. Well, it was also shown in vivo, so in the alive animal where LTP was completely abolished.

So the autophosphorylation of CaMKII at threonine-286 is fundamentally important for induction of LTP. It's not so clear whether it's important for LTD. It seems to be rather important for LTP.

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What happens to animals in the water maze? Well, you saw this graph also before, where basically there is no improvement when the animals were trained in the water maze. And at the memory probe trial, you can see on the right where mutants are shown in black, there is an equal surge in all four quadrants, indicating a random surge. Whereas wild-type animals have more surge in TQ.

In this case, please note that the proximity is plotted, which is inversely correlated to the time spent in the area-- so if you have less proximity, when you spent more time basically, in the target quadrant. And so in wild-type animals, they have selective surging in the target quadrant, while various mutants have a random surge, indicating they have no spatial memory.

So these mutants were in lack of LTP induction and were lack spatial memory, further strengthening a correlation between LTP and memory.

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Finally, we can also manipulate the maintenance of LTP and ask the question whether this impairs an

established memory, which is work that has been done by Todd Sacktor, who is based in New York. And so what he has found, he has identified a peptide that blocks protein kinase M zeta, where it's important for the maintenance of LTP. And this experiment shows where the peptide, ZIP, can block the maintenance of LTP.

In panel D you see that LTP was induced and then recorded for many hours. So this is a late-phase LTP. After a few hours the LTP consolidates into late-phase LTP when it's being maintained.

And now ZIP is provided to a hippocampus slice or recorded in the hippocampus slice. And when the ZIP treatment wipes out the LTP, the ZIP is therefore blocking the LTP maintenance mechanism. And it was suggested to work via PKMzeta.

However, nowadays, we are not so certain. Because when PKMzeta is knocked out in mice, ZIP is still able to block LTP maintenance. And therefore, it might act also on our molecules and not only on PKMzeta.

In panel E is another behavioural task that requires the hippocampus-- and to ask the question, whether blocking of a ZIP erases hippocampus dependent memory. So the animals, in this case, were placed with the rats. They're placed on a rotating platform.

When the platform rotated into a particular area, the animal would receive a mild electrical foot shock. And so what the animal learns from this task is to avoid to go into the area. It's an active avoidance task.

Basically, so then he has to move away from this area. It has to identify where the area is using these cues in the box. So you can see that it requires a few training trials. And then the animal can avoid to go into the danger zone.

So initially, it goes very quickly into a danger zone-- within just maybe 10 seconds or so. And after training, it needs more than 200 seconds to be in the danger zone.

So well, this memory can last a long time. So for example, it can be tested at 24 hours after the training-- so one day after the training. So when the animals are treated with saline, you can see that they are still avoiding to go into the danger zone.

However, when our animals are injected with ZIP, well, there's no memory, because now we the animal is caught almost immediately back into the danger zone. So ZIP has erased the memory here.

And it has also erased LTP. So it has blocked the LTP maintenance. And so that's a very strong correlation between LTP maintenance and memory maintenance.

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Taking these things together, there's very strong evidence that LTP is a memory mechanism. So there are theoretical grounds, loosely speaking. LTP follows Hebb's postulate.

We have seen that LTP can be induced by behavioural training. Furthermore, blocking LTP induction seems to block spatial memory formation. And further, blocking LTP maintenance seems to erase a spatial memory.