LECTURE 18: LEARNING AND MEMORY:  
LONG-TERM POTENTIATION IN THE MAMMALIAN HIPPOCAMPUS

PRIMARY MESSAGE: The idea that synaptic long-term potentiation (LTP) is the substrate of learning remains controversial. Nevertheless, several lines of evidence have linked it to memory, particularly spatial memory in rats. Induction of LTP is via a postsynaptic calcium signal generated through glutamate NMDA receptors. Expression is mediated by phosphorylation of pre-existing AMPA receptors and mobilization of new AMPA receptors via a biochemical cascade through calcium/calmodulin-dependent protein kinase II (CaMKII). Stabilization of LTP requires protein synthesis via the cyclic AMP response element binding protein (CREB). Genetic knockout experiments in mice (CaMKII, and NMDAR knockouts) strongly suggest a role for hippocampal LTP in spatial memory as tested using the Morris water maze.

LTP is Hebbian

D.O. Hebb, a famous Canadian psychologist, proposed that the mechanism underlying memory might involve the strengthening of synapses. Hebb's postulate was: "When an axon of cell A is near enough to excite cell B or repeatedly or consistently takes part in firing it, some growth process or metabolic changes take place in one or both cells such that A's efficiency as one of the cells firing B, is increased". Thus if cell A is connected to cell B and contributes to generating the firing of B then the connection between cell A and cell B will be strengthened. Hebbian is a word that has come into the vocabulary to refer to types of synapses and types of plasticity. Note that this differs from much of the synaptic plasticity in the gill withdrawal circuit of *Aplysia* because the latter does not require concurrent activity in pre- and postsynaptic sites.

Hippocampal LTP

Long term potentiation (LTP) in mammalian brains was first described by Bliss and Lømo in 1973. However it was difficult to investigate the phenomenon at the cellular level until the development and improvement of brain slice techniques, particularly the hippocampal slice, in the late 1980's.

The hippocampus is a structure in the medial temporal cortex (phylogenetically old cortex) that to some ancient anatomist's eye looked like a sea-horse - hence hippocampus. It has been pretty well established that it is involved in the acquisition of declarative memories (especially about the spatial environment). Birds that hide food for later consumption have enlarged hippocampi. Unit recording within the hippocampi of intact rats has demonstrated the existence of place cells (cells that fire when the rat is in a particular location in the environment) and head direction cells. Prevention of function in the hippocampi blocks spatial learning such as allows rats to find a hidden platform in a water bath, or hidden food in the Manhattan maze.

The structure of the layers in a hippocampus is relatively complex. The cells that are relevant here are the granule cells of the dentate gyrus and the pyramidal cells of the CA1 and CA3 regions (CA stands for cornu ammonis - Ammon's horn, perhaps the same ancient neuroanatomist...
that saw the sea-horse also named these regions). The important pathways are:

- perforant path from entorhinal cortex to dentate granule cells
- dentate granule cells to CA3 neurons via mossy fibres
- CA3 neurons to CA1 neurons via schaeffer collaterals
- commissural input from opposite hippocampus to CA1 neurons.

**Long-term Potentiation**

LTP as first described by Bliss and Lømo is a long-lasting increase in the strength of perforant path to dentate granule cell synapses after a tetanic stimulus in the pathway. Increase in the strength of the connection is measured as an increase in amplitude or slope of the rising phase of the postsynaptic potential or the postsynaptic current. This is homosynaptic or non-associative LTP.

**Associative LTP**

This has also been described. It can be generated, for example, by pairing weak stimulation of the commissural fibres (CS) with strong stimulation of the schaeffer collaterals (US) to CA1 neurons. Essentially a weak input can only be strengthened if it is concurrently and tetanically active with a strong tetanic input.

**Induction of LTP**

The mechanisms underlying the INDUCTION of LTP are better understood than those underlying its EXPRESSION or maintenance. For the induction of non-associative LTP at CA1 pyramidal neurons the following sequence occurs:

a) The tetanic stimulation depolarizes the postsynaptic neuron sufficiently to relieve the Mg\(^{++}\) block of NMDA receptors.

b) This results in an influx of Ca\(^{++}\) through the NMDA receptor channel.

c) The calcium activates intracellular second messenger pathways (notably calcium calmodulin dependent protein kinase II, CaMKII, and protein kinase C, PKC) to cause expression.

For associative LTP it is the US that depolarizes the postsynaptic element sufficiently to relieve the Mg\(^{++}\) block of the NMDA receptors.

*Support for these interpretations comes from blockage of NMDA receptors with APV and MK801, and from genetic knockout studies. Treatment with APV prevents the acquisition of spatial memories in rats. Most interestingly, mice without the gene for CaMKII have reduced hippocampal LTP and impaired spatial learning. This is also true for mutant mice lacking the NMDA ε1 subunit.*

**The NMDA Receptor and Coincidence Detection**

The unique characteristics of the NMDA receptor suit it particularly well for the
coincidence detection that is the essence of associative synaptic plasticity. Two conditions need to be satisfied before the NMDA receptor opens to generate an intracellular calcium signal: glutamate binding and postsynaptic depolarisation.

In the rat hippocampus associative LTP arises when the postsynaptic NMDA receptor is primed by the conditioned stimulus in the weak pathway AND activated by postsynaptic depolarisation induced by the unconditioned stimulus.

For comparison … in Aplysia associative conditioning arises when presynaptic adenylyl cyclase is primed by the conditioned stimulus causing an increase in calcium and activation of calcium/calmodulin AND subsequently activated by G-protein activation by the unconditioned stimulus. Be aware that Aplysia also has postsynaptic NMDA receptors that have been implicated in associative conditioning.

Expression of LTP

Whereas it is clear the induction is a postsynaptic mechanism there used to be hot debate over the site of expression. Differences in the age, temperature and species providing the slice, as well as stimulus parameters, seem to introduce much variability into the system. In earlier experiments there was strong evidence that expression could be a presynaptic phenomenon. This is interesting because it necessitates a retrograde message passing from the site of induction (postsynaptic) to the presynaptic element. There are two main candidates for the retrograde messenger - arachidonic acid and nitric oxide. These substances diffuse freely through cell membranes (arachidonic acid because it is lipid derived, and NO because it is a gas). Also they have relatively short half-lives (seconds to minutes) which is a requirement for the specificity of the change. It should be noted that just because these substances can diffuse back to the presynaptic element doesn't mean that that is where they have their effect. They could still be having a postsynaptic effect.

The emerging consensus is that the expression of LTP is via CaMKII acting like a switch (autophosphorylation ensures that once turned on the switch remains on). CaMKII then can phosphorylate AMPA receptors in the membrane to increase their single channel conductance and can recruit new AMPA receptors to the postsynaptic membrane.

There is strong evidence from genetic manipulation that CaMKII is necessary both for hippocampal LTP and for spatial memory in rodents, suggesting that the relationship between LTP and memory is causal.

Stabilization of LTP - Memory

Late phase LTP requires protein synthesis and a pathway exists from cAMP through protein kinase A to CREB that regulates gene expression and can induce the transcription of "memory" genes. Protein synthesis would be required for structural alteration of the synaptic site as well as providing new AMPA receptors. In 1999 Engert demonstrated with real-time imaging of living dendritic spines that expression of LTP is correlated with the formation of new spines on the apical dendrites of hippocampal pyramidal neurons - an impressive accomplishment.

Some further points
1. Some forms of LTP do not depend on the activation of NMDA receptors. non-NMDA LTP relies on an increase of Ca\(^{++}\) caused by voltage-dependent Ca\(^{++}\) channels. The time course of NMDA and non-NMDA LTP are different and can be seen at the same synapse.

2. If synaptic weights are to be kept at a dynamic equilibrium dependent upon recent experience then a mechanism to reduce the weights as a result of low levels of activation should exist. Associative and non-associative long-term depression (LTD) has been described in hippocampal slices. It too can be dependent upon NMDA activation and Ca\(^{++}\) influx.

3. LTP and LTD can be induced at the same synapse and which is generated is due to the pattern of stimulation - low frequency tetanus leads to LTD whereas high frequency tetanus leads to LTP. The concentration of intracellular Ca\(^{++}\) could determine whether LTP or LTD occurs.

4. It is possible that the different mechanisms reflect different roles in the establishment of memories. Thus NO could mediate a rapid phase of LTP, taken over by arachidonic acid in a second phase, and for very long term changes gene activation and protein synthesis are required.

5. The common feature in all of these mechanisms is an initial dependence on increases in intracellular Ca\(^{++}\) as a second messenger. But it is increasingly unlikely that Ca\(^{++}\) is acting like a simple switch - graded effects depending upon Ca\(^{++}\) concentration are more likely.

This is an area of intense current research interest. Much is known and much is still confusing. However, it is becoming more obvious that these synaptic changes are the substrates for learning and memory in mammalian brains, ours included.

*Experiments have demonstrated that rapid eye movement (REM) sleep is necessary for consolidation of procedural memory in human subjects. Also, in rats the hippocampal place neurons that are activated by placing a rat in a novel setting are reactivated during slow wave (SW) sleep. The playback is compressed in time and suggests a memory consolidation mechanism. Alternation of REM and SW sleep is thought to be essential for memory consolidation.*