NBL 356-656 Module 8 Review Q&A

1. *How is LTP measured electrophysiologically at the hippocampal CA3-CA1 synapse (Schaffer collateral)? What is a dendritic fEPSP and somatic population spike? Why are these downward responses (more negative) if they are excitatory?*

LTP is studied in the Schaffer collateral using a stimulating electrode to stimulate the CA3 axons to induce them to all fire action potentials at the same time, and uses an extracellular recording electrode to measure the dendritic excitatory post synaptic potentials from the population of dendrites, in response to the stimulated axons. A somatic population spike also occurs at the cell bodies (because there is so much dendritic excitation that action potentials are generated by the CA1 neurons). The extracellular recordings have a downward deflection due to the movement of Na+ ions into the neurons via AMPA receptor (and NMDA receptor) activity. The recording electrode is outside of the dendrites, so it reports the movement of Na+ to the inside of the cell (during the EPSP the outside of the membrane becomes more negative). LTP studies measure the CA1 population dendritic EPSPs or population spikes in response to presynaptic CA3 action potentials.

1. *What are the three types of stimulations (electrophysiological methods) of inducing (triggering) LTP and what do all three share in common?*

-Tetanic stimulation, theta burst stimulation, and pairing postsynaptic depolarization with low frequency presynaptic stimulation.

-All three share: presynaptic stimulation which causes the release of glutamate, and postsynaptic depolarization of the membrane potential, and thus are Hebbian (requires both presynaptic and postsynaptic activity).

1. *Functionally LTP can be divided into three parts: induction, early LTP and late LTP. What are the roles of AMPA and NMDA receptors in LTP induction at the Schaffer Collateral (SC)? Where are the receptors located? How are they activated and what do they do? Why are dendritic spines shown in the diagrams describing LTP?*

AMPA receptors bind glutamate and open to allow Na+ to flow down its electrochemical gradient inside the neuron, producing a Na+ current. In the SC, AMPA receptors participate in LTP induction because when the stimulus is robust (tetanus or theta burst stimulation) then large amounts of glutamate release leads to AMPA receptor activation, that leads to depolarization of the membrane potential, that relieves the Mg2+ block of the NMDA receptor. NMDA receptors must be activated (and allow influx of Ca2+) for LTP induction at the SC synapses. CA1 neurons are spiny pyramidal neurons that receive glutamatergic input from CA3 axons on their dendritic spines. Both AMPA and NMDA receptors are located at the postsynaptic density on the spines of the CA1 neurons. Under physiological conditions, the depolarization (required to unblock the NMDARs) would likely be provided by activity at many active nearby synapses and/or simultaneous excitatory inputs to other dendrites, while glutamate would be provided by the presynaptic CA3 axons. Hence AMPA receptors are not essential for LTP induction, but the membrane depolarization that they mediate is required for LTP. If the dendrite could become depolarized by anther means, such as a multiple other simultaneous synaptic inputs or back propagating action potentials, the AMPARs would not be required for LTP induction at that specific synapse.

1. *LTP expression (during both E-LTP and L-LTP) can be presynaptic or postsynaptic, or most likely, both. List the possible presynaptic and postsynaptic mechanisms involved in LTP expression.*

All the presynaptic mechanisms would involve an increase in glutamate released into the synapse. Some possible mechanisms of presynaptic expression, which would lead to increased glutamate release are: an increase in release probability, which includes: an increase in calcium ion influx to trigger release, increase in sensitivity of calcium ion sensor or threshold for release, or an increase in the number of docked vesicles, more vesicles or more glutamate per vesicle.

Postsynaptic mechanisms: A) Upregulation of AMPA type glutamate receptor levels and activity, involving increases in number of AMPA receptors, increases in AMPA receptor sensitivity to glutamate, and increases in AMPA receptor ion conductance and open time. B) Decreases in glutamate transporter activity. C) Increases in neuronal excitability (through changes in K+ channel expression. And D) Increases in number of active synapses (that respond to glutamate) by activation of silent synapses or formation of new synapses.

1. *Following LTP induction, there are two temporal phases/parts, early (E-LTP) and late (L-LTP). What does E-LTP involve?*

LTP can also be divided into temporal phases of E-LTP and L-LTP. E-LTP involves the biochemical changes that include the activation of protein kinases (CamKII, PKC, PKA and ERK) that occur within about the first 30 min following induction.

Early LTP involves expression, in which the synaptic strength/response is increased. In expression, the elevated postsynaptic Ca2+ levels activate protein kinases. The kinase activity continues even when Ca2+ levels return back to baseline. Early expression involves kinase activity and protein phosphorylation, but doesn't require translation (protein synthesis) or transcription (mRNA synthesis).

L-LTP begins by about 30 min (after induction) and lasts for many hours to days to weeks. L-LTP involves (and requires) new mRNA transcription and new protein translation. In addition to changes in AMPA receptor number and activity, L-LTP also involves morphological and other biochemical changes in synapses.

1. *What is the role of AMPA receptors in E-LTP expression? What are three aspects of AMPA receptor regulation that are affected by kinases and how? What are the roles of TARPs and scaffolding proteins?*

After Ca2+ increases in the postsynaptic spine, Ca2+ binds to calmodulin and activates Ca2+ calmodulin dependent kinase II (CaMKII). In addition, Ca2+ activates PKC, PKA and Erk (through a number of pathways). These kinases phosphorylate AMPA receptors, and/or AMPA receptor trafficking proteins and/or transmembrane AMPA receptor regulatory proteins (TARPS), and lother scaffolding proteins. Together these activities lead to more AMPA receptors at the postsynaptic density, and enhanced channel activity of the AMPA receptors that are located there.

Another explanation: In LTP expression, it is the increased synaptic AMPA receptor location, density and activity that mediates the majority of increased EPSP responses to glutamate at the synapse. Several Ca2+ activated protein kinases directly phosphorylate AMPARs, enhancing their affinity for glutamate, and enhancing their open time, so more Na+ can flow into the postsynaptic neuron. Ca2+ activated protein kinases can also phosphorylate trafficking and scaffolding proteins, which bring/incorporate more AMPARs to the postsynaptic membrane, tether them there and reduce their removal/endocytosis. Phosphorylation of TARPs and other scaffolding proteins anchor the AMPARs and lead to increased tethering of AMPARs to the postsynaptic density (PSD). AMPARs in the PSD region become more concentrated there, and can’t diffuse to perisynaptic or extrasynaptic regions or be internalized/endocytosed as easily.

1. *What is LTD, what causes it and what does LTD involve? Why is LTD necessary and important?*

From Wikipedia: “Long-term depression (LTD), in neurophysiology, is an activity-dependent reduction in the efficacy of neuronal synapses lasting hours or longer following a long weak patterned stimulus. The most common neurotransmitter involved in LTD is glutamate. LTD can result from persistent weak synaptic stimulation in the hippocampus. LTD is thought to result mainly from a decrease in postsynaptic AMPA receptor density, although a decrease in presynaptic neurotransmitter release may also contribute. Cerebellar LTD has been hypothesized to be important for motor learning. Hippocampal LTD may be important for the clearing of old memory traces. LTD is one of several processes that serves to selectively weaken specific synapses in order to make constructive use of future synaptic strengthening caused by LTP. This is necessary because, if allowed to continue increasing in strength, synapses would ultimately reach a ceiling level of efficiency, which would inhibit the encoding of new information.”

1. *What is a silent synapse? Describe the mechanism involved in activation/ unsilencing of silent synapses.*

Silent synapses are synapses characterized by their lack of AMPA receptors, but presence of NMDA receptors in the post-synaptic membrane. Under basal transmission, they would not produce an EPSP response since they lack AMPA receptors and even though they have presynaptic release of glutamate, the NMDARs would be blocked by Mg2+. These synapses are un-silenced through the coincident depolarization of the membrane potential (by activity of nearby synapses and the depolarizations they produce, which can spread to the nearby membranes), and release of glutamate from the presynaptic terminus. If the depolarization is large enough in the previously silent synaptic membrane, NMDA Mg2+ block will be removed, glutamate will activate the NMDA receptors, and Ca2+ will then flow into the post-synaptic membrane, causing LTP induction through the activation of Ca2+ dependent kinase pathways and the resulting up-regulation of AMPA receptors and activity. These would contribute to LTP because they would show an increase in synaptic transmission in a synapse that previously showed no response.

1. *Describe the main principles/features/characteristics of LTP?*

The features of LTP: long term/persistent, cooperative and associative, input (synapse) specific (also called Hebbian), reversible, and saturable.

Input specific: Only pathways (synapses) that receive input (axonal stimulation) are strengthened. For the SC, glutamate must bind and activate both the AMPARs (which cause depolarization) and activation of NMDARs to allow Ca2+ influx. If no glutamate or no activation of NMDARs and Ca2+ influx, no LTP occurs.

Associative: Weak stimulation of an axon and low levels of glutamate release at synapse 1 cannot induce LTP (and may induce LTD). However, if there is strong stimulation to a nearby synapse 2 that occurs at the same time as the weak input to synapse 1, then LTP can occur at both synapses. Mechanism: the strong stimulation at synapse 2 produces a large depolarization which will spread/move along the membrane (as the Na+ ions diffuse) and this will depolarize the membrane at synapse 1, and relieve the NMDAR Mg2+ block from synapse 1. Since synapse 1 also has input/glutamate, the NMDARs will be activated, Ca2+ will flow in and LTP will be induced. From Wiki: “Associativity refers to the observation that when weak stimulation of a single pathway is insufficient for the induction of LTP, simultaneous strong stimulation of another pathway will induce LTP at both pathways.

Cooperative: LTP requires that presynaptic stimulation be linked temporally with postsynaptic depolarization. In the non-physiological experimental paradigm, this involves prolonged high frequency stimulation (tetanic); repeated bursts of 4 pulses (theta); or, a pairing between induced postsynaptic depolarization and low frequency presynaptic stimulation.

Under physiological situations, induction can be cooperative, since many weak signals to a neuron can cooperatively cause sufficient postsynaptic depolarization (even if on its own, each would be insufficient to produce LTP by itself.) From Wiki “LTP can be induced either by strong tetanic stimulation of a single pathway to a synapse, or cooperatively via the concomitant weaker stimulation of many pathways. When one pathway into a synapse is stimulated weakly, it produces insufficient postsynaptic depolarization to induce LTP. In contrast, when weak stimuli are applied to many pathways that converge on a single patch of postsynaptic membrane, the individual postsynaptic depolarizations generated may collectively depolarize the postsynaptic cell enough to induce LTP cooperatively.

Associative and Cooperative May be Similar. Associativity refers to the requirement for coincident timing of presynaptic glutamate release and postsynaptic depolarization. Cooperativity refers to the requirement for enough coincident activity to produce enough depolarization to relieve the Mg2+ block of the NMDA receptors. Some neuroscientists argue that any difference between associativity and cooperativity is strictly semantic. Synaptic tagging, discussed later, may be a common mechanism underlying associativity and cooperativity.

Reversible: Stimulation of the SC with a tetanus or theta burst stimulation (TBS) will induce LTP. After LTP has been induced, stimulation of that same SC with a low frequency stimulation will reverse the LTP and the response will return to its pre-stimulation level, and even undergo LTD. Likewise, if LTD is induced first with low frequency stimulation of the SC, if this is then followed by a tetanus or TBS, the synaptic response will return to the pre-stimulation level, and even undergo LTP. This is an important feature of LTP/LTD since it is consistent with reversible biochemical pathways such as protein phosphorylation and dephosphorylation, membrane trafficking in exocytosis and endocytosis, and mRNA and protein turnover (synthesis and degradation).

Saturable: There is a maximal level of increase in LTP or decrease in LTD that can be achieved. So even if one stimulated with a longer time with a higher frequency tetanus for LTP, or lower frequency stimulation for LTD, the magnitude of LTP or LTD cannot be increased beyond a certain level. Presumably this means that there is a maximum and minimum to the volume of the spine and size of the synapse. This is also consistent with biochemical mechanisms underlying long term plasticity.

Persistent: From Wiki: “LTP is persistent, lasting from several minutes to many months, and it is this persistence that separates LTP from other forms of synaptic plasticity (such as post-tetanic potentiation, a type of short term plasticity).”

Input specific/Hebbian: LTP is input specific and requires coincident presynaptic and postsynaptic stimulation. For the SC, both the presynaptic stimulation and postsynaptic depolarization must be temporally linked to ensure NMDAR activation (since the NMDAR requires both glutamate binding and depolarization, which removes the Mg2+ block, to be activated).

1. *Following LTP induction, there are two temporal phases, early (E-LTP) and late (L-LTP). What does L-LTP involve?*

Late LTP involves expression as well as maintenance, and long term mechanisms allows the synaptic strength to last much longer than the stimulus that triggers it. Late LTP lasts longer than an hour and requires the kinases activated during E-LTP and transcription (new mRNA synthesis) and translation (new protein synthesis). Late LTP expression is maintained by a persistent enhancement of AMPA receptors at the synapse. Late LTP maintenance involves changes in gene expression and synaptic and spine morphology, the formation of new spines/synapses, and epigenetic modifications.

1. *What are CREB and CBP and how do CREB and CBP function?*

CREB is a transcription factor that binds to the DNA sequence called CRE which is a DNA element/sequence in a gene promoter found in hundreds of genes. CREB has to be phosphorylated to activate transcription, where it binds DNA and recruits CREB Binding Protein (CBP/p300). CBP is a histone acetyltransferase (HAT) that acetylates histones, which helps open up the DNA so the transcriptional machinery can bind and activate transcription. CREB binds CRE, which is the cAMP Response Element (a promotor in DNA). After its initial discovery downstream in cAMP signaling through activation of cAMP dependent protein kinase (PKA), it was determined that CREB can be phosphorylated by many different protein kinases, not just PKA. In LTP there is a central role for the kinases MSK and RSK in CREB phosphorylation. MSK and RSK are activated by the kinase ERK. ERK also phosphorylates the transcription factor Elk.

1. *How can ERK be activated in the postsynaptic spine? How do its major substrates MSK, RSK and Elk1 function in L-LTP?*

ERK is also called MAP Kinase, and was originally identified as a kinase activated by growth factors and mitogens (that stimulate cell proliferation) that were first identified in non-neuronal systems, in cancer and cell growth research. ERK is activated downstream of numerous Ca2+ pathways. In L-LTP ERK phosphorylates RSK2 (ribosomal S6 kinase 2) and MSK1 (Mitogen and stress activated kinase 1). RSK2 and MSK1 are protein kinases that phosphorylate CREB and thus stimulate CREB dependent transcription; ERK also directly phosphorylates and regulates the transcription factor Elk-1. Elk-1 binds to a region in DNA, the DNA element called serum response element (SRE), and activates transcription.

1. *Which genes show increased transcription in L-LTP? In addition to their transcription being rapidly activated during E-LTP, what do IEG/ARGs share in common? What are memory suppressor genes?*

The genes that are increased during LTP are called immediate early genes (IEGs) or activity related genes (ARGs). These genes are also called “memory genes” and “plasticity-related proteins/products (PRPs”. The two categories of IEG/ARGs are 1) transcription factors (such as cFos, Zif-268, and cJun), which regulate the expression of other genes involved in synaptic plasticity, and 2) effector IEGs (AMPA receptors, neurotrophic factors such as BDNF, Homer (a scaffold), and Arc (a cytoskeletal protein) etc.) which have protein products that probably function at postsynaptic sites of excitatory synapses. IEGs/ARGs are activated at the transcriptional level and have either CRE (which binds CREB) or SRE (serum response element, which binds Elk-1) in their promotor regions. Some genes also show decreased transcription in LTP, such as the protein phosphatase calcineurin PP2B, and these are called memory suppressor genes. Together, the genes that show changes in expression in LTP are also called “plasticity related proteins/products, or PRPs.”

1. *What is local protein synthesis and how may it be important in LTP? Why would an increase in spine volume be important in L- LTP?*

The majority of neuronal protein synthesis occurs in the cell body/soma. Local protein synthesis is protein synthesis by ribosomes that occurs in dendrites and near dendritic spines. Local synaptic protein synthesis would involve the transport of mRNA to the dendrites and allows for rapid localized changes in the levels and types of proteins in the dendrite/ spine/ synapse. For transmembrane proteins, local protein synthesis would require membrane bound ribosomes (RER) and Golgi, which would synthesize and sort proteins into secretory/transport vesicles. Erk and other kinases are involved in regulation of translation. An increase in spine volume would accommodate more AMPA receptors, and captured PRPs.  Newly synthesized proteins during L-LTP (probably including IEG/ARG/PRPs) likely contribute to the increase in dendritic spine number, surface area, and post synaptic sensitivity associated with LTP. Cell adhesion molecules at the synapse also increase in LTP.

1. *What is synaptic tagging and capture? What are two proposed tags? What needs to be captured? How would this be involved in the input specificity and associative/ cooperative properties of LTP?*

Synaptic tagging and capture is a hypothesis that attempts to explain the synapse specificity and Hebbian nature of LTP. It suggests that LTP induction and Ca2+ signaling at a particular activated synapse leaves behind a type of permanent tag, such that during L-LTP, after transcription and translation, the newly synthesized plasticity proteins will be shipped back to and captured by that tag, so that only the previously stimulated synapses will be strengthened. It is important in the specificity and also persistence of LTP. Two proposed tags are CaMKII and PKM zeta. The ability of the NMDA receptor and/or actin cytoskeleton to bind to activated CaMKII in a complex in the activated spine could be important in synaptic tagging, because activated CaMKII (the tag) would be tethered at the active synapse, so it would remain there, waiting to capture a newly produced PRP.