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Synaptic Plasticity During Learning

Introduction:

Learning is possibly the single most important ability an animal can possess. How an organism learns and the processes underlying this ability is a complex question. On a neuronal basis, the answer to what learning is lies in the organization of neurons and their interactions. A memory is stored in a neural circuit; the interactions between neurons form memories (Rogerson, et. al., 2014). Synaptic plasticity is the dynamic relationship within neuronal connections that changes the chemical composition of the neuron and its ability to communicate with its neighbors. These changes are broadly split into three categories: short term potentiation, early long-term potentiation, and late long term potentiation. This review will address each of these categories: short term potentiation through dendritic spine morphology; early long term potentiation through AMPA and NMDA receptors, and Hebb's rule for cell assembly; late long term potentiation through astrocyte regulation of synapses and the processes involving local translation in dendrites.

Short Term Potentiation:

While longer lasting synaptic changes may have a stronger effect on learning, the near instant to changes made in less than an hour are important modulatory processes precursing longer lasting changes. Two examples of these processes are dendritic spine dynamics and distributions of post-synaptic density protein-95 (PSD-95).

Dendritic spines (approximately 1 μm across) are not normally easy to visualize with simple light spectroscopy, so Fischer, et al., 1998, used green fluorescing proteins (GFPs) to resolve the dynamic properties of synapses. They attached GFPs to actin, which accumulates within the spines. This method created an outline of the synapse, and allowed an edge finding function to quantitatively analyze changes of volume, shape, and number of dendritic spines. The labeled spines modulated their shape within seconds, but did not change their volume or number within the measured timescale. Some of the synapses changed upwards of 30% in just over two minutes and 10% in 1.5 seconds (Fischer, et al., 1998). This result is important because a change in shape of 10% could result in significant changes within the synaptic connection between dendrite and axon (Fischer, et al., 1998). To investigate whether the changes in shape were based upon actin activity, Fischer, et al., 1998, used cytochalasin D and latrunculin B to prevent actin polymerization. Both treatments stopped further changes in shape, suggesting the mechanism by which the dendrites change their shape is dependent on actin polymerization. Additional drugs acted to slow down polymerization, and the spines reacted accordingly (Fischer, et al., 1998). Therefore, modulation of actin polymerization is an important process for synapses to quickly change their geometry and connectivity with surrounding neurons.

Creating new dendritic spines is a process called spinogenesis (Kwon, Sabatini, 2011). Kwon stimulated new dendritic growth using glutamate and measured their growth with lasers and a specialized GFP. Spinogenesis occurred rapidly, with new spines appearing within 4 milliseconds. These new spines increased their volume from 10% to 90% of maximum within 11.8 cycles of stimulation with glutamate (Kwon, Sabatini, 2011). 70% of the new spines grew 1 μm from the glutamate point of release, and 94% of the new spines grew on the same side of the dendrite as the stimulation. 20% of new spines existed for less than two minutes, but the spines

which existed for five minutes continued to be stable for thirty minutes. Testing using variants of GFP indicate inclusion of newly grown spines into neural circuits after only 30 minutes (Kwon, Sabatini, 2011). Activation of spinogenesis by glutamate implicates NMDAR receptors as important in this process, and inhibition of NMDAR receptors by CPP confirmed this. Additionally, inhibition of AMPA receptors had no effect on spine growth, and thus a post-synaptic potential is not required for this process. Kwon later found that blocking upstream MAPK signaling inhibits spinogenesis. Therefore, glutamate activation of MAPK pathways is important for the rapid creation of new dendritic spines.

PSD-95 is one of the most abundant proteins located within the post-synaptic density (Gray, et al. 2006). The protein is a member of the PDZ scaffolding protein family and interacts with both NMDA and AMPA receptors. PSD-95 creates a section on the plasma membrane where the receptors can bind (Gray, et al. 2006). The size of the dendritic spine and post-synaptic density are directly correlated to the amount of PSD-95 present in the PSD (Gray, et al. 2006). However, PSD-95 has a very short half-life in the dendritic spine. Gray, et al. found the average turnover time for PSD-95 to be between 23 and 63 minutes within the synapse, and determined the protein redistributes itself to other synapses within 50 milliseconds after leaving the synapse (Gray, et al. 2006). The redistribution of the protein slows down in relation to the size and geometry of the synapse. A spine with a narrow neck retains PSD-95 more efficiently, and spines with larger PSDs recruit and retain more PSD-95 (Gray, et al. 2006). As Fischer, et al., 1998, stated, a 10% change in geometry has profound effects on synaptic function. Thus, these smaller changes in the dendritic spines, over time, directly influence the ability of the synapses to activate other longer lasting pathways.

Early Long-Term Potentiation:

The next phase of synaptic plasticity is early long-term potentiation. Early long term potentiation can last for hours and requires the neuron to be strongly stimulated. Such stimulation can be reached by one large stimulus or several stimuli in succession (Levitan, 2015). The effects of this strong stimuli are best demonstrated through the interactions of NMDA and AMPA receptors. These receptors are both glutamate receptors with integral ion channels. When the cell has stimulated, sodium ions enter the cell. When enough sodium enters the cell, the sodium repels a magnesium ion blocking the NMDA receptor. Once this happens, calcium flows into the cell as well as additional sodium ions (Levitan, 2015). The calcium then activates Ca /Cam kinase II (CAMKII) by binding to it. The activated CAMKII then causes more changes within the synapse. The first is the phosphorylation of existing AMPA receptors. This phosphorylation sensitizes the channels so that they open with less bound glutamate (Levitan, 2015). The second important consequence of CAMKII activation is the insertion of AMPA receptors into the cellular membrane. The synapse already has vesicles with pre-existing AMPA receptors waiting to be inserted into the membrane (Levitan, 2015). The insertion process is likely regulated by PSD-95, as mentioned above. Some dendritic spines do not have any AMPA receptors, and only possess NMDA receptors (Levitan, 2015). The existing NMDA receptors are blocked, making the synapse unable to receive signals. When the surrounding synapses have been activated with CAMKII, AMPA receptors are inserted into these synapses, allowing them to become active (Levitan, 2015). This weak synapse and the strong synapse causing its activation demonstrate the result of early long-term potentiation.

Simultaneous firing of two neurons at a synapse can strengthen the connection between the two cells (Levitan, 2015). This is Hebb's rule. When a weak signal enters the post-synaptic

neuron at the same time of a strong signal, then both the weak and strong synapses will undergo long term potentiation (Levitan, 2015), as demonstrated previously by the insertion of AMPA receptors into inactive synapses. The distance between the weak and strong synapse is not necessarily important (if they are on the same dendrite) because dendrites can produce their own action potentials (Levitan, 2015). The timing of the two synaptic signals must be within 5 milliseconds of the action potential produced by the dendrite (Levitan, 2015). If the timing is not the same, then the synapse may undergo depression instead of potentiation.

The process of long term depression is much the same as early LTP. One way to induce long term depression is through repeated low levels of stimuli. This releases low levels of calcium into the synapse. These low levels of calcium will not activate CAMKII, but will instead activate phosphatases which will remove phosphate groups from AMPA receptors, lowering their sensitivity to signaling (Levitan, 2015). When the AMPA receptors are dephosphorylated, they have the potential to move to the side of the post-synaptic density where they will be removed from the membrane and cycled back into vesicles (Levitan, 2015). Both changes resulting in LTD and LTP last upwards of hours within the neuron. For longer changes, more cellular machinery needs to be altered.

Late Long-Term Potentiation

Not all synaptic plasticity is governed by the neurons themselves. Astrocytes can affect synapse association, and maturation. While in pure culture, rodent retinal ganglion cells (RGCs) develop relatively few synapses with one another. However, when grown in culture with astrocytes (or the molecules secreted by astrocytes), the RGCs developed over ten times the number of synapses as the pure RGC culture (Clark, Barres, 2013). These glial cells associate with over 100,000 synapses (in rodent neural tissue), and poses ion channels, receptors, and

surface molecules needed to communicate with surrounding neurons (Clark, Barres, 2013). Some of the signaling molecules possessed by astrocytes include BDNF, cholesterol, thrombospondins, epherins, glypican, and TGFβ-1. Cholesterol was found to induce increased vesicle formation, and thrombospondins (TSPs) were found to promote excitatory synapses. Thrombospondin-4 is found in high levels inside adult human brains (Clark, Barres, 2013). Although not completely known by the authors at the time of publication, Clarke claims TSPs act on molecules responsible for organizing scaffold proteins.

Astrocytes affect synaptic maturation through the actions of glypican and their effects on dendritic spines. When glypican is released into the cell, AMPA receptors increase their numbers on the cell membrane. Mouse neurons deficient in glypican exhibit phenotypes associated with decreased post-synaptic potentials (Clark, Barres, 2013). There are three proposed mechanisms by which astrocytes strengthen dendritic spines: a response to calcium, localizing neuro-transmitters, and through ephrin. Astrocytes possess thin protrusions capable of sensing changes in calcium concentrations in the dendrite. This allows the astrocyte to respond to changes in the synapse previously thought to be too fast for the astrocyte to respond by other means. This process may be responsible for the ability of astrocytes to quickly modulate their proximity to dendritic spines (Clark, Barres, 2013). The proximity of astrocytes to the synapse allows them to regulate secreted neurotransmitters like glutamate (responsible for dendritic formation). Glutamate is absorbed by transporters possessed by the astrocytes, which in turn regulates the concentrations of the glutamate in the synaptic cleft. Also, the astrocytes can guide the glutamate concentrations to cause new spine growth towards active pre-synapses (Clark, Barres, 2013). Dendritic spines contain EPH receptors on their surface, and astrocytes contain ephrin (the ligand to EPH receptors) on their surfaces. Stimulation of EPH receptors results in the retracting of the

spine. When either molecule is knocked out in rodent hippocampal tissue, similar phenotypes of malformed spines appear and the surrounding astrocytes increase their glutamate uptake. On a behavioral level, the mice exhibit learning deficits (Clark, Barres, 2013). These phenotypic changes suggest the EPH/ ephrin system provides bidirectional communication between the astrocyte and the synapse.

For late long term potentiation to occur, transcription in the nucleus and translation of proteins is required. The genes which first respond in a stimulated cell are called early-response genes: fos and jun (Levitan, 2015). These two genes are transcription factors that form dimers with one another. These then bind to ap-1 sites on the DNA, and allow certain transcripts of neuronal genes to be produced (Levitan, 2015). In response to depolarization, fos can be upregulated by over 100 times its basal level (Levitan, 2015). Another set of regulatory processes is the rearrangement of chromatin in response to neuronal activation. DNA is organized by nucleosomes which consist of an octamer of histone proteins wrapped in DNA. When the DNA is wrapped tightly around the histone cores, it becomes inaccessible to transcription (Cox, et al., 2015). The mechanism by which chromatin is changed is thought to be through histone modifying enzymes and chromatin remodeling complexes. Chromatin remodeling complexes move entire nucleosomes to various parts of the DNA, remove them, or replace them. Histone modifying enzymes modify the histone octamer tails. Histone acetylation usually loosens the DNA coiling, making it more accessible to transcription. Histone deacetylation (HDAC) represses genes (Cox, 2015). Guan (2009) studied the effects of various HDAC proteins on memory formation in mice by creating neurons exhibiting over expression of HDAC1 and 2. These mice demonstrated decreased levels of acetalization in homogenized neural tissue samples (Guan, et. al., 2009). The morphology of the mice brains appeared normal

and distributions of cells within their brains were also normal. HDAC 1/2 over expression did not interrupt development of the mouse brains, but the ability to form memories within the organisms was compromised (Guan, et. al., 2009). Mice exhibiting over-expression of HDAC 2 performed below wild type mice in tests involving Pavlovian responses, swimming memory tests, and maze navigation. Short term memory between the groups was not affected; only long term memory deficits appeared in HDAC 2 over expression mice (Guan, et. al., 2009). Guan found that HDAC 2 interacts with histones regulating BDNF, FOS, CAMKII, and NMDA subunits. Early response genes like fos and jun must therefore undergo histone relaxation to allow their transcription, likely by histone acetyl transferase directly antagonistic to HDAC 2.

Once the transcripts of genes have been made, the cell can translate them. However, these processes require a large amount of regulation, and neurons can be very long. If the proteins were translated immediately after transcription, they would not arrive to their destination until much later. Conditions could have changed, and by the time the proteins arrived at their proper synapse, it could be too late for the neuron to effectively respond. How neurons provide control in these situations is a unique solution: local protein translation at the synapse. The clear majority of proteins synthesized within the cell are created in the cell body, with only about 400 being locally translated (Dahm, et. al., 2008). The first step in this process is packaging the mRNA transcripts into their transport molecules. This early stage is thought to occur in parallel to the normal processing of mRNA transcripts (Dahm, et. al., 2008). Other factors are added into the mixture of mRNA before nuclear export, and the transcripts are assembled by interactions of their cis-elements with trans-elements (proteins-RNA). The trans elements interact with one another and the mRNA is placed inside of vesicles. The vesicles are transported by motor molecules on the cytoskeleton (Dahm, et. al., 2008). At the time of publication, not all the

proteins involved in the process were known, especially the proteins anchoring these vesicles at their destination.

An important molecule for guiding the mRNA transcripts to the correct location within the dendrite has been hypothesized as a "synaptic tag" (Levitan, Kaczmarek, 2015). The exact molecular identity of this tag is unknown, but CAMKII, protein kinase A, CREB, and actin polymerization are all important molecules in setting this tag (Rogerson, et al., 2014). The tag is produced locally at the activated dendrite and is present for about three hours (Levitan, Kaczmarek, 2015). The transport of the mRNA stops at this tag until their translation (Levitan, Kaczmarek, 2015).

One important trait of these mRNAs is that they are kept translationally inactive until they are needed. The molecule responsible for the silencing of the mRNA is fragile-x mental retardation protein (FMRP). FMRP is likely one of the additional factors added to the mRNA transcripts before nuclear export. The protein binds to the mRNA transcripts until the local synapse has been activated (Levitan, Kaczmarek, 2015). When metabotropic glutamate receptors are activated, FMRP is dephosphorylated and disassociates from the transcript bound to it (Levitan, Kaczmarek, 2015). The transcript is then processed by ribosomes which are located at specific sites and may be attached to the post-synaptic density These proteins are specific to the synapse where they are created. (Levitan, Kaczmarek, 2015). As mentioned earlier, there are over 400 mRNA transcripts translated locally, but Levitan focused on the importance of three: protein kinase C, BDNF, and ARC/PSD-95. Post synaptic density -95/ARC's function and implications as a major component in the post-synaptic density were described earlier.

Brain derived netrin factor (BDNF) is both an autocrine and retrograde messenger. As an autocrine signal, it has two functions: stimulation of new protein synthesis and synapse

elimination. When acting on TrkB receptors in the dendrite it was released from, BDNF stimulates scaffolding proteins and new receptors (Levitan, Kaczmarek, 2015). Whether this signaling cascade interacts with FMRP was not mentioned, but it is likely the case as FMRP is the main regulator of local translation. When acting (as an autocrine signal) on the p75NTR receptor, BDNF causes inactive synapse elimination (Levitan, Kaczmarek, 2015). The mechanism underlying this change is not fully understood, but it may follow similar pathways as long-term depression. As a retrograde messenger, BDNF acts on the TrkB receptors located in the pre-synaptic terminal and causes the active post-synaptic terminals to quickly undergo morphological changes (Levitan, Kaczmarek, 2015).

Protein kinase C (PKC) is a member of the protein kinase family, which regulate protein function by adding phosphate groups onto either serine, tyrosine, or threonine amino acid residues. The proteins antagonizing this function are phosphatases (Lim, et. al., 2015). PKC has two lobes that surround a binding pocket for ATP. When ATP is bound, the protein is activated and can transfer phosphate groups onto other proteins. When a phosphate is bound, it changes the structure of these proteins, and therefore alters their ability to function (Lim, et. al., 2015). Although the targets for PKC after local translation were not fully specified by Levitan, the upregulation of a regulatory protein in the post-synapse will have profound consequences for the neuron. An example of this PKC's effects would be how the phosphorylation of AMPA receptors was an integral part of early-LTP for the neuron.

Conclusions:

The ability for neurons to change their chemical properties is a highly complex and overlapping series of events. Dendrites can change their spine shape, volume, and number within seconds through the MAPK pathway and actin polymerization. Early long-term potentiation

requires the modulation of protein function and is demonstrated by NMDA and AMPA receptors. Their interaction strengthens synapses and allows for neuronal circuits to mature. Astrocytes do more than support neuronal function; they actively shape the landscape surrounding neurons and regulating synapses in the process. Late long-term potentiation requires transcriptional factors to activate in the nucleus, RNA to travel through the dendrite to its proper location, and for proteins to be translated locally. These processes are only a portion of the complex regulatory system governing inter-neuronal communication underlying the ability of an organism to learn. As these and other pathways are elucidated more fully, the knowledge of synaptic plasticity will hopefully identify new targets for the study of how the brain functions as a larger system and new targets for drug therapies.

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