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BRAGging about Mechanisms of Long-Term Depression

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The mechanisms of long-term depression (LTD) underlie various aspects of normal brain function. Therefore, it is important to understand the signaling that underpins LTD. The study by Scholz et al. in this issue of *Neuron* describes how BRAG2, mGluRs, and AMPARs come together to produce LTD through AMPAR internalization.

Picture the scene: the London underground—one of the world's busiest subway systems—at rush hour on a Friday evening. As is probably the case in most similar transport systems, entry into or out of the underground is controlled by electronic gates. At rush hour it takes a LONG time to get the thousands and thousands of commuters through the limited numbers of gates. Wouldn't it be great if it were possible to increase rapidly the number of gates when needed and reduce the number of gates when not needed? However, one cannot even begin to imagine the mechanical, electrical, and computer engineering and design needed to make this possible. How would the gates be brought into the station lobby and how would they be removed? Where would they be stored? Would the station lobby have to increase and decrease in size to accommodate these changes? What multitude of different control mechanisms would need to be in place to ensure that everything happened in a controlled and regulated manner?

Remarkably, however, the central nervous system deals with a similar problem at synaptic junctions. At most synapses fast chemical transmission is mediated by release of glutamate acting on AMPA receptors. One of the most impressive things about synapses is that their strength can be increased and decreased very rapidly, a property known as synaptic plasticity. These changes can last a long time, if required (LTP, long-term potentiation; LTD, long-term depression). One of the most well-studied mechanisms responsible for synaptic plasticity is alterations in the numbers of AMPA receptors on the receiving neuron. In many ways, this is akin to the problem of the underground—in response to particular demands the synapse increases or decreases the number of AMPA receptors, thus providing almost instantaneously greater or reduced capacity to cope with the demands thrown at the synapse. The mechanisms that control these changes in synaptic strength are turning out to be hugely complex and are subject to a level of fine tuning that could not have been imagined even a few short years ago.

Currently, there is pretty good consensus concerning the processes that initiate or trigger synaptic plasticity, generally a rise in intracellular calcium resulting from activation of particular classes of receptors (e.g., NMDA or mGluRs). We are also fairly confident that insertion or removal of AMPARs at the synapse is one of the key final steps that bring about the change in synaptic strength. However, the details of the precise mechanisms between the initial trigger and the final insertion or removal of AMPARs is still the subject of intense investigation and evidence exists for a variety of different intracellular processes that are likely to be involved in some way. Every so often in the investigation of such mechanisms, an exciting, new, and controversial discovery is put forward, such as in this issue of *Neuron*, in which Scholz et al. (2010) describe a novel signaling mechanism that controls the removal of synaptic AMPARs, thereby controlling LTD.

There is a great deal of evidence that LTD induced by NMDAR activation requires serine/threonine phosphatases that dephosphorylate certain target proteins. The removal of AMPARs is brought about by dynamin- and clathrin-mediated endocytosis which in turn relies on interactions between intracellular proteins (e.g., AP2, PICK1, GRIP) and specific regions of the C terminus of the GluA2 subunit of AMPARs. How dephosphorylation regulates the interaction between the GluA2 C terminus and intracellular proteins and how this regulates endocytosis is still very uncertain.

In addition to the well-established role of serine/threonine phosphatases there has been increasing evidence that LTD may rely on a protein tyrosine phosphatase (PTP). Pharmacological activation of group I mGluRs by the agonist DHPG results in the induction of robust LTD that is blocked by PTP inhibitors but not by serine/threonine phosphatase inhibitors (for review see Gladding et al., 2009; Lüscher and Huber, 2010). In addition, LTD is associated with tyrosine dephosphorylation of the GluA2 subunit and the trafficking of AMPARs that underlies LTD is blocked by PTP inhibitors (Huang and Hsu, 2006; Moult et al., 2006). Activity-dependent LTD that is mGluR-dependent is also shown to rely on PTP activity (Moult et al., 2008). Thus, evidence is accumulating that tyrosine dephosphorylation is important in mGluR-LTD and other mGluR functions (e.g., regulation of the slow afterhyperpolarization) may also be PTP dependent (Ireland et al., 2004).

In the paper by Scholz et al. (2010), new evidence is provided that shows how tyrosine dephosphorylation may trigger LTD. This requires a signaling cascade based around a protein called BRAG2, a guanine nucleotide exchange factor for the plasma membrane GTPase Arf6. Arf6 can recruit AP2 to synaptic membranes, but no role for BRAG2 has been identified in the brain. In the current work, Scholz et al. (2010) carry out a very comprehensive and detailed study of the interactions between BRAG2 and AMPARs. They show that BRAG2 interacts with the tyrosine-rich sequence in the C-terminal of GluA2 and that the tyrosine 876 residue is critical for this interaction. They further demonstrate that BRAG2 colocalizes with synaptophysin, suggesting its presence at the synapse, and they show that it is highly concentrated in the PSD. So, what is the consequence of the interaction between BRAG2 and GluA2? Scholz et al. (2010) show that binding of GluA2-BRAG2 causes an increase in BRAG2 catalytic activity on Arf6. Next, they demonstrate that the increased BRAG2 catalytic activity upon binding GluA2 relies on the phosphorylation state of Y876; when dephosphorylated there was an increase in BRAG2 activity, but when phosphorylated there was no such increase. Thus, the phosphorylation state of Y876 controls BRAG2-mediated GDP/GTP exchange on Arf6 (see Figure 1).

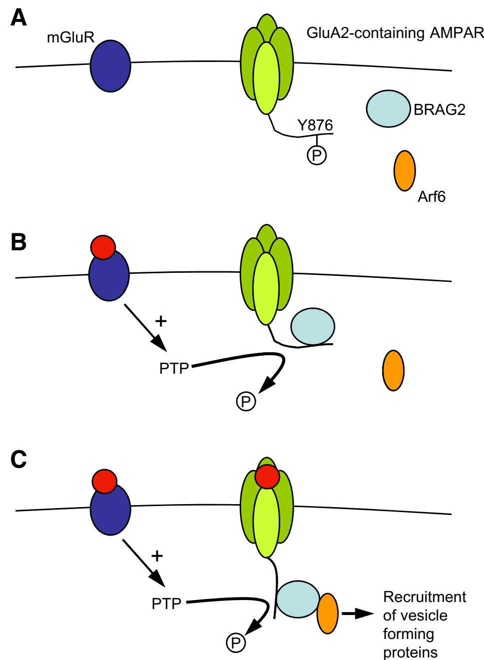

Figure 1. Mechanisms of mGluR-LTD
(A) Y876 of the GluA2 subunit is basally phosphorylated, preventing activation of BRAG2.
(B) Activation alone of group I mGluRs will stimulate protein tyrosine phosphatase (PTP) to dephosphorylate Y876. However, (C) AMAPR activation is also required for BRAG2 to activate Arf6 which subsequently recruits vesicle-forming proteins to the complex and promotes internalization of AMPARs.

As mentioned above, mGluR-LTD has been shown consistently to rely on tyrosine dephosphorylation, and Scholz et al. (2010) demonstrate that Y876 is the specific residue on GluA2 that is dephosphorylated. In addition, mGluR-LTD was associated with increased activity of Arf6, and this increase in Arf6 activity was prevented by PTP inhibitors that also prevent mGluR-LTD. This suggests a mechanism by which mGluR stimulation triggers a PTP that dephosphorylates Y876 on GluA2; this allows an increase in BRAG2 activation of Arf6, which in turn regulates endocytosis. If this were the case, then the decrease in surface AMPARs and mGluR-LTD should be blocked by preventing BRAG2 function. Indeed, RNAi knockdown of BRAG2 prevented DHPG-induced reduction in surface GluA2, but also importantly this knockdown in hippocampal slices resulted in a block of DHPG LTD in RNAi-infected neurons. Furthermore, DHPG-LTD was absent in neurons with Cre-mediated deletion of BRAG2 but was normal in noninfected neurons.

The data in this manuscript up to this point are interesting since they provide a link between tyrosine dephosphorylation, BRAG2—a protein that previously had no known role in brain—and a reduction of surface AMPARs. Significantly, however, this work goes much further and suggests that activation of the AMPAR itself is critical in controlling these signaling cascades and therefore controlling the induction of LTD (see Figure 1). First, the authors demonstrate that glutamate stimulation of HEK cells coexpressing GluA2, BRAG2, and Arf6 results in increased Arf6 activity, leading to the suggestion that glutamate binding to AMPARs may be critical for LTD. To examine this further, two independent pathways were stimulated in hippocampal slices and DHPG was applied to induce LTD. However, stimulation in one pathway was stopped during DHPG application. In this pathway, no LTD was observed whereas normal LTD was observed in the other pathway. Furthermore, blocking AMPARs with kynurenic acid prevented induction of DHPG-LTD. Therefore, under these conditions ligand binding to AMPARs is critical for mGluR-LTD.

This finding is likely to be somewhat controversial given that there is published

work that directly contradicts these results. Two studies have previously shown that DHPG-LTD is induced in hippocampus in the absence of synaptic stimulation (Fitzjohn et al., 1999; Huber et al., 2001), and Fitzjohn et al. (2001) showed that DHPG-LTD is induced in zero external calcium, i.e., in the absence of synaptic transmission. Both of these findings clearly show that AMPAR activation is not required for LTD. In addition, other forms of LTD (e.g., induced by carbachol) also do not require synaptic stimulation (Massey et al., 2001). This raises the question: under what conditions is there a requirement for AMPAR stimulation in mGluR-LTD? Some work that may provide a clue was published by Kemp and Bashir (1999), which indicated that synaptically induced LTD required activation of AMPA and mGluRs. Another question that remains unanswered is "how exactly does AMPAR activation contribute to this form of LTD?" It is possible that ligand binding causes a conformational change to the GluA2 subunit that allows better access for Y876 dephosphorylation and BRAG2 binding, but this remains to be proved.

Interestingly, Scholz et al. (2010) show that inhibitors of src family tyrosine kinases can by themselves lead to dephosphorylation of Y876 and activation of Arf6. If AMPAR activation itself causes increased Arf6 activity, and if inhibition of src family tyrosine kinases can also lead to Arf6 activation, one question is "why do simple AMPAR activation or tyrosine kinase inhibition alone not lead to AMPAR internalization and LTD?" The study by Moult et al. (2008) provides an answer to this question. They showed that mGluR-LTD required not just activation of a PTP, but also activation of another parallel pathway involving p38 MAPK. Activation of just one of these pathways is not sufficient to induce LTD. Hence, although AMPAR activity or tyrosine kinase inhibition will lead to GluA2 Y876 dephosphorylation, LTD does not occur as the MAPK is not also activated.

Another issue that needs to be resolved regarding tyrosine phosphorylation state and LTD is that AMPAR internalization by insulin relies on tyrosine phosphorylation (Ahmadian et al., 2004) rather than dephosphorylation of the C terminus of GluA2.

This study also raises questions about NMDA-LTD, as it is shown that NMDA-LTD is also dependent on BRAG2. However, given that NMDA-LTD does not rely on PTP activity and given that the present study shows that PTP activity is essential for the increase in BRAG2 activation of Arf2, the relationship between BRAG2 and NMDA-LTD still remains to be determined.

The results of this study raise some intriguing new concepts related to potential mechanisms of LTD. Why would a system develop whereby cooperative activation of both mGluRs and AMPARs is required to produce AMPAR internalization and LTD? There may be many different possible explanations, but some possible scenarios are described here and illustrated in Figure 2:

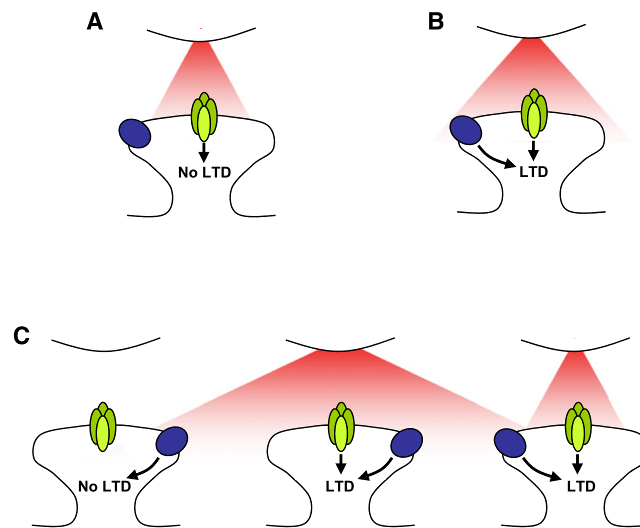

Figure 2. Consequences of the Requirement of Both AMPAR and mGluR Activation for LTD Induction

(A) At a weakly active synapse AMPARs, but not perisynaptic group I mGluRs, are stimulated by glutamate and LTD is not induced.

(B) When a synapse is strongly activated both AMPARs and mGluRs are stimulated and LTD occurs.

(C) Glutamate spillover can activate perisynaptic mGluRs. If glutamate spills over from a strongly activated synapse (middle), then LTD will occur at this synapse. However, LTD will also occur at a neighboring synapse that is concurrently only weakly active since both mGluR and AMPARs are stimulated (right). LTD will not be induced at a neighboring inactive synapse as only mGluR but not AMPAR stimulation will occur at this synapse (left).

1. A weakly active synapse will only stimulate AMPARs but not group I mGluRs, which are located perisynaptically, which will prevent LTD from occurring under basal conditions (Figure 2A).
2. When a synapse is strongly active, glutamate release will stimulate both synaptic AMPARs and perisynaptic group I mGluRs. Therefore, under these conditions LTD can occur (Figure 2B). This is consistent with the observations that stimulus-induced mGluR-LTD relies on strong induction protocols (Kemp and Bashir 1999; Huber et al., 2000).

Since group I mGluRs are perisynaptic, they are more likely to be activated by glutamate spillover from neighboring strongly active synapses. This has two further possible consequences:

3. Activation of perisynaptic group I mGluRs by glutamate spillover at an otherwise inactive synapse will not result in LTD since there is no activation of AMPARs (Figure 2C). So a mechanism whereby both receptors need to be activated may prevent the LTD mechanism from being inadvertently stimulated by spillover, and thus LTD is kept synapse specific; only those synapses where group I mGluRs and AMPARs are activated will undergo LTD.
4. In contrast, synaptic glutamate release at a weakly active synapse may result in activation of AMPARs. In this case, spillover of glutamate from strongly active neighboring synapses may activate group I mGluRs at the weakly active synapse and result in LTD (Figure 2C). This induction of LTD brought about by glutamate release from neighboring synapses provides a mechanism of cooperative plasticity.

The study of both LTP and LTD has uncovered a plethora of different signaling cascades that regulate AMPAR surface expression. The work of Scholz et al. (2010) describes some exciting, novel and controversial findings that provide a greater understanding of the regulation of synaptic AMPARs and LTD—now that's something to BRAG about.

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