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AMPA Receptors Unbound: Membrane Cycling and Synaptic Plasticity

Minireview

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How does one modify the strength of a synapse? On first inspection, this is a deceptively straightforward question. After all, the strength of a synaptic connection between two neurons is simply a function of how many synaptic contacts there are, the probability of transmitter release at each site, and the number of receptors clustered in the postsynaptic membrane. Ergo, changing any of these parameters will modify synaptic strength. But simple questions have a habit of multiplying upon close inspection, and the neuroscientist studying synaptic plasticity may come to feel like Hercules fighting the many-headed Hydra: for each question dispatched, two more spring up to take its place. Several decades of work has made it abundantly clear that synaptic transmission is a highly dynamic process that can be modified on many different time scales ranging from seconds to days, and different forms of plasticity may target different aspects of the same pre- or postsynaptic process (Malenka and Nicoll, 1999; Turrigiano and Nelson, 2000). Disentangling these (often interacting) mechanisms has necessitated ever-deeper forays into the minutiae of the synaptic machinery, as seemingly pedestrian aspects of synaptic function are identified as crucial players in the mechanics of synaptic plasticity. So it is perhaps not surprising to find that the latest obsessions of the long-term potentiation/depression (LTP/LTD) community are those most basic of cell biological processes—exocytosis and endocytosis (Lin and Sheng, 1998; Lledo et al., 1998; Lüscher et al., 1999; Morales and Goda, 1999; Man et al., 2000; Wang and Linden, 2000).

Why Exo- and Endocytosis?

Recent work has suggested that changes in the number of glutamate receptors clustered at the postsynaptic density (PSD) play an important role in several forms of long-lasting activity-dependent synaptic plasticity, including LTP, LTD, and synaptic scaling (O'Brien et al., 1998; Turrigiano et al., 1998; Malenka and Nicoll, 1999; Shi et al., 1999). These observations have launched a wave of studies on the mechanisms by which glutamate receptors—in particular, ionotropic AMPA receptors (AMPARs)—can move into and out of the postsynaptic membrane, and how this process can be regulated by activity. Like all integral membrane proteins, glutamate receptors turn over in the membrane. Currently the only known mechanism for inserting integral membrane proteins into the plasma membrane, or for removing them again, is via vesicular exocytosis and endocytosis. The relative rates of exocytosis and endocytosis will therefore influence the size of the receptor pool that accumulates in the membrane (Figure 1). The sites of exo- and endocytosis in the postsynaptic membrane are unknown but are likely to be lateral to the PSD, and possibly in dendritic shafts rather than in spines themselves. Once inserted into the membrane glutamate receptors will diffuse freely until they are trapped and stabilized at synaptic sites through association with multiprotein complexes that anchor them to the cytoskeleton. These anchoring proteins include a number of PDZ domain–containing scaffolding proteins such as GRIP (AMPARs) and PSD-95 (NMDA receptors) (Kornau et al., 1997; O'Brien et al., 1998). Activity could therefore influence receptor accumulation at synaptic sites in two fundamentally different ways. First, activity could influence the size of the receptor pool in the plasma membrane, either by triggering a regulated endocytotic or exocytotic pathway or through a change in the relative rates of constitutive endocytosis and exocytosis. The speed with which receptor number could be regulated by changing the rate of constitutive receptor cycling depends critically on the turnover rate of AMPARs, a matter currently under hot debate (see below). Second, if glutamate receptors are resistant to endocytosis when firmly anchored to the PSD, activity could modulate the number of receptors at the synapse by regulating the binding of glutamate receptors to their anchoring proteins.

A Brief Overview of the Exocytotic and Endocytotic Machinery

To follow the logic of experiments designed to test these different models for how AMPAR number at central synapses could be rapidly increased or decreased, it is useful to review what is known about the exocytotic and endocytotic machinery (for a more complete review, see Rothman and Wieland, 1996; Lin and Sheng, 1998). Vesicles are thought to be targeted to fusion sites on the plasma membrane by interactions between SNARE complexes—a v-SNARE on the vesicle will bind selectively to a t-SNARE on the target membrane. The best-characterized v- and t-SNARE complexes are those which mediate synaptic vesicle exocytosis: the v-SNARE synaptobrevin, which interacts with the t-SNARE complex syntaxin/SNAP-25 through coiled-coil protein interactions. The formation of this "core complex" allows vesicles to dock, but the events underlying fusion are less clear. Fusion is dependent on the ATPase N-ethylmaleimide-sensitive fusion protein (NSF). NSF can be recruited to the core complex through binding to SNAP (soluble NSF attachment protein, unrelated to SNAP-25), which in turn binds to the SNARE proteins. When ATP is hydrolyzed by NSF, the core complex is dissociated. This dissociation is critical for multiple cycles of vesicle fusion, and NSF is thought to interact with a number of different v- and t-SNARE complexes throughout the cell to regulated fusion of many different vesicle membrane partners (Rothman and Wieland, 1996; Lin and Sheng, 1998).

Vesicles are reclaimed from the plasma membrane by endocytosis, which is dependent upon cytoplasmic coat

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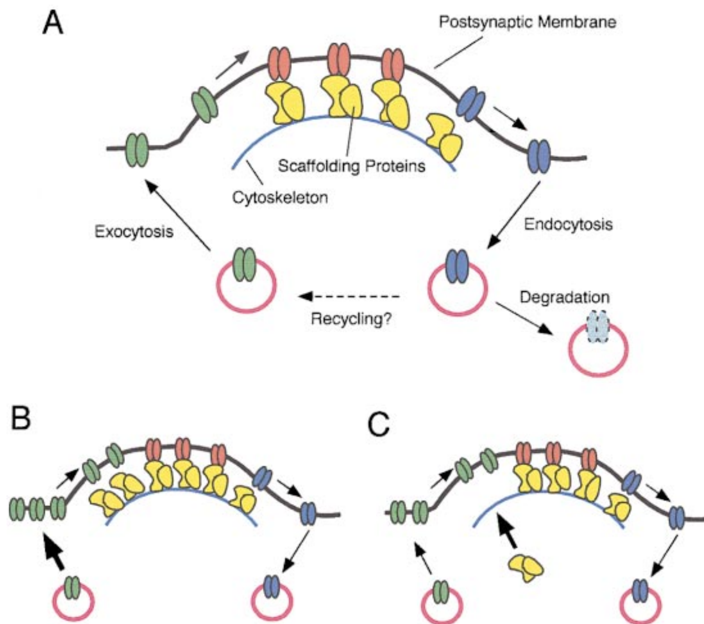

Figure 1. Postsynaptic AMPA Receptor Cycling

(A) AMPARs cycle into and out of the membrane via exocytosis and endocytosis. Once in the membrane, receptors are trapped and stabilized by scaffolding proteins that bind receptors to the cytoskeleton. Two possible mechanisms for clustering more receptors at a synaptic site are illustrated in (B) and (C).

(B) If the rate of constitutive exocytosis were selectively increased, more receptors would accumulate in the membrane. This model assumes there are enough sites available to stabilize these excess receptors at the synapse. Similarly, if the rate of endocytosis were selectively increased, the number of receptors would decrease.

(C) If receptor binding sites are limiting, then increasing exocytosis would have little effect on receptor number. To increase the number of receptors, the number of available sites must be increased (either by increasing the number of binding proteins or by phosphorylating existing proteins to allow them to associate with receptors).

proteins such as clathrin that assemble into a spherical shell and slowly pinch the vesicle off from the surrounding membrane. This process is dependent on the GTPase dynamin, which forms rings around the base of the endocytotic pit and through a conformational change following GTP hydrolysis aids in the fission event. Dynamin binding to an SH3 domain in the protein amphiphysin is also necessary for dynamin function; this interaction may localize dynamin to the appropriate site on the vesicle membrane (Cremona and De Camilli, 1997). It should be stressed that while the cellular and molecular events underlying vesicle fusion and fission have been well characterized for neurotransmitter release and for vesicle budding from intracellular organelles, very little is known about the molecular basis of receptor cycling in neuronal dendrites.

Plasticity through Receptor Stabilization/ Destabilization at the Neuromuscular Junction

Much more is known about receptor cycling at the neuromuscular junction (NMJ) than at central synapses. At the NMJ, acetylcholine receptors (AChRs) are clustered at extremely high concentrations that are maintained by a dense network of scaffolding proteins (Sanes and Lichtman, 1999). Analysis of receptor turnover at the NMJ has been aided by this high concentration of receptors, as well as the availability of α-bungarotoxin, which binds irreversibly and with very high affinity to AChRs. At the NMJ, receptor insertion and internalization appears to occur in the perijunctional region rather than within the junctional region itself. This suggests that receptors are internalized only after they dissociate from scaffolding proteins and diffuse into the perijunctional membrane. While AChR internalization at the mature NMJ is very slow under normal conditions (with a receptor half-life of 14 days), it has recently been demonstrated that the rate of perijunctional diffusion and internalization can be dramatically accelerated by activity blockade (to a half-life of less than a day) (Akaaboune et al., 1999). Because AChRs dwell a long time in the perijunctional membrane before internalization, reversing the activity blockade allows receptors to diffuse back and be trapped once again. This suggests that at the NMJ, binding of receptors to scaffolding proteins, rather than the rate of internalization, is the limiting step determining receptor number (Akaaboune et al., 1999).

Binding or Turnover at Central Synapses?

Whether this model also holds for central synapses is not clear. Given the small number of receptors present at central synapses and the lack of a high-affinity toxin such as α-bungarotoxin, it has not been possible to detect a lateral dispersion of receptors in the membrane, or reclustering of perisynaptic receptors, following LTD or LTP protocols. Interestingly, a number of recent studies have suggested that the GluR2 subunit of the AMPAR binds directly to NSF (to a site distinct from the GRIP binding site, also on the GluR2 subunit), and that disrupting this binding causes a rapid reduction in the amplitude of AMPA synaptic currents. This "run-down" stabilizes at a 30%–40% reduction and has been shown to occlude LTD, suggesting that LTD operates on a pool of receptors that are influenced by NSF binding (for review, see Lin and Sheng, 1998; Morales and Goda, 1999). While interfering with NSF–AMPA binding reduces excitatory postsynaptic current (EPSC) amplitude, staining studies using cultured hippocampal neurons have demonstrated that AMPARs are lost completely from some synaptic sites, while other sites are unaffected, and in keeping with this result miniature EPSC (mEPSC) frequency decreases but amplitude is either unaffected (Noel et al., 1999) or is only reduced by about 10% (Lüscher et al., 1999). These data suggest that disrupting the NSF–AMPA interaction causes a loss of receptors from particular synapses in an all-or-none manner, while other synapses are unaffected. In contrast, LTD protocols that reduce surface expression of AMPARs result in a large reduction in mEPSC amplitude, suggesting that a percentage of the AMPARs at each synapse is lost (Carroll et al., 1999). Thus, while occlusion studies suggest that LTD targets the same pool of

receptors that interact with NSF, other data argue that these two processes may be mechanistically distinct.

It is currently unclear whether disrupting NSF–AMPA binding is causing synaptic rundown by hindering the insertion of AMPARs into synaptic sites, or whether NSF plays a role in AMPAR stabilization at the synapse that is independent of its role in exocytosis. For example, NSF could act as a chaperone protein that regulates the interaction of AMPARs with other proteins such as GRIP (Lin and Sheng, 1998). In this model, blocking NSF binding to AMPARs would cause diffusion away from synaptic sites and subsequent internalization. Why some synapses should be more sensitive to disruption of the NSF–AMPA interaction than others is unclear. It may be that due to heterogeneity in the composition of the PSD, or in the subunit composition of AMPARs, NSF only interacts with AMPARs at a subset of excitatory synapses. Another rather speculative possibility is that when AMPARs and NSF are already associated it takes some time for their interaction to be disrupted, but that once a few receptors at a synapse become unbound, some protein–protein interactions in the PSD are destabilized and the majority of AMPARs at that synapse are rapidly untethered. Such a process could result in the complete and rapid loss of AMPARs from some synaptic sites while others are unaffected.

A Role for AMPAR Cycling in Synaptic Plasticity

Two recent studies highlight the current uncertainty about the relationship between constitutive receptor cycling and the rapid changes in AMPAR number thought to underlie some forms of LTP and LTD. If AMPARs cycle rapidly in the membrane, then interfering with endo- or exocytosis should cause rapid synaptic runup or rundown, and speeding up or slowing down the rate of constitutive internalization would provide a viable mechanism for LTD and LTP. In support of this model, a recent report used whole-cell recording techniques to infuse agents that interfere with exocytosis or endocytosis into postsynaptic neurons, and found that baseline AMPA-mediated synaptic transmission was rapidly altered. For example, infusion of botulinum toxin (botox, which prevents exocytosis by cleaving the v-SNARE synaptobrevin) reduced AMPA amplitudes by about 30%, and this reduction occluded LTD. Furthermore, infusion of a peptide that prevents amphiphysin from binding to dynamin (an interaction thought to be crucial for endocytosis) rapidly enhanced AMPA synaptic transmission (Lüscher et al., 1999). However, a very different result was obtained in an earlier study (including many of the same authors) (Lledo et al., 1998). In this study, intracellular infusions through sharp electrodes of NEM (which blocks NSF function), of a peptide that prevents the interaction between NSF and SNAP (an interaction that is essential for many membrane fusion events), or of botox all blocked tetanus-induced LTP in hippocampal slices but had no effect on baseline transmission (Lledo et al., 1998). These data suggest, in contrast to the data of Lüscher et al. (1999), that AMPAR turnover is slow enough that blocking constitutive receptor turnover for an hour or two does not influence the number of AMPARs clustered at synaptic sites.

An explanation for these conflicting results suggested by Lüscher et al. (1999) is that the intracellular concentration of botox (and other pharmacological agents) achieved with sharp electrodes (Lledo et al., 1998) was too low to block constitutive exocytosis. Since LTP was blocked by the concentration of botox achieved with sharp electrodes (Lledo et al., 1998), this scenario implies that the mechanisms by which botox interferes with LTP and with baseline transmission are not the same. It is possible, for example, that LTP involves the insertion of receptors by a regulated exocytotic mechanism that is more sensitive to botox than is constitutive exocytosis. An alternative possibility is that the agents used to disrupt exocytosis have some as yet unidentified (and possibly indirect) role in receptor stabilization, so that when infused at a high concentration they destabilize AMPAR binding at synaptic sites and cause synaptic rundown. As there was no verification in these studies that the agents used were blocking (or failing to block) dendritic exocytosis, these issues remain unresolved.

AMPAR Half-Life and Rapid Receptor Cycling

The half-life of AMPARs in cultured spinal neurons measured by pulse chase receptor labeling or by surface biotinylation is about 30 hr (Mammen et al., 1997). In contrast, a recent report using a pulse of antibody to label surface receptors in living human embryonic kidney (HEK) cells and cultured hippocampal neurons found that labeled receptors were internalized quite rapidly, with a time constant of about 40 min, and these internalized receptors were colocalized with proteins associated with clathrin-coated pits (Man et al., 2000). This suggests that receptor endocytosis is much faster than receptor breakdown, so that the majority of internalized AMPARs remain intact (and possibly functional). This in turn raises the possibility that internalized AMPARs could be recycled back into the synaptic membrane. While models of constitutive receptor cycling at the NMJ have stressed a slow and stately replacement of receptors over a time scale of days (Sanes and Lichtman, 1999), these recent studies suggest that central AMPARs may be constantly shuttling between extracellular and intracellular compartments, although a direct test of this would require the demonstration that internalized receptors reappear at the synaptic membrane. Other grounds for caution in interpreting the discrepancy between half-life and internalization measures is the possibility that the method used to measure internalization (antibody binding to AMPARs in living cells) itself influences the rate of receptor internalization (Man et al., 2000). It would be nice to know, for example, whether receptor half-life is decreased by antibody treatment.

An intriguing observation of Man et al. (2000) is that in cells treated with hypertonic sucrose or transfected with a dominant-negative mutant dynamin (both manipulations that should inhibit endocytosis), constitutive internalization of AMPARs was greatly reduced, but the percentage of total surface AMPARs did not increase. This observation led the authors to suggest that the rates of constitutive receptor internalization and insertion are coupled in some way, so that altering one produces a coordinated change in the other, leaving total surface receptor number constant. This interpretation could explain the lack of effect on baseline transmission observed by Lledo et al. (1998) following blockade of exocytosis but is in direct conflict with the results of Lüscher et al. (1999), who found that agents that block

exocytosis had a profound effect on baseline transmission.

If the rates of endocytosis and exocytosis are coupled in the way suggested by Man et al., how does surface receptor number change following specific stimuli? Man et al. (2000) showed that insulin treatment reduced the number of surface receptors on HEK or cultured hippocampal neurons, and that this reduction was sensitive to agents that disrupt endocytosis. In addition, insulin treatment and LTD mutually occluded each other in hippocampal slices. This suggests that some agents (such as insulin and activity) are able to transiently uncouple endocytosis and exocytosis and generate a net gain or loss of receptors at the cell surface. These conclusions are strengthened by the accompanying report that cerebellar LTD (and an insulin-mediated reduction in synaptic transmission) was strongly attenuated by inhibitors of clathrin-mediated endocytosis, while baseline transmission was unaffected (Wang and Linden, 2000). These reports suggest that synaptic plasticity mechanisms from distinct brain regions (hippocampus and cerebellum) that utilize different transduction mechanisms may ultimately converge on the same cellular machinery to control the number of AMPARs expressed at synaptic sites. Whether this reduction in the total number of surface receptors is a result of speeding up a constitutive endocytotic pathway, or engaging a distinct regulated endocytotic pathway, remains to be seen. The later model is suggested by recent data showing that overexpressed GFP-labeled AMPARs (composed of the GluR1 subunit) are not constitutively inserted into the membrane but can be recruited into spines following tetanic stimulation (Shi et al., 1999).

As Always, More Work Is Needed

Postsynaptic receptor cycling is a complicated cell biological process that is poorly understood. While it is clear that interfering with interactions between many of the dozens of proteins mediating exo- and endocytosis can disrupt synaptic function and plasticity, interpreting these results unambiguously will require a much more complete understanding of the role these proteins play in postsynaptic function. While proteins like NSF, synaptobrevin, and amphiphysin serve well-characterized roles in presynaptic vesicle cycling, little is currently known about the postsynaptic localization or function of these proteins. The unexpected finding that NSF interacts directly with AMPARs raises the possibility that other proteins involved in vesicle fusion or endocytosis may also serve dual functions as receptor chaperones, or may have other vital roles in maintaining the integrity of the PSD.

While there is as yet no consensus on the rate of AMPAR cycling and the direct role of constitutive turnover in rapid forms of synaptic plasticity, it seems likely that regulated endo- and exocytosis will emerge as an important mechanism for rapidly influencing synaptic strength. It may turn out that constitutive AMPAR cycling is too slow to play a role in LTP and LTD (as suggested by half-life studies). On the other hand, long-term modulation of the relative rates of exo- and endocytosis could play an important role in homeostatic forms of plasticity such as synaptic scaling (O′Brien et al., 1998; Turrigiano et al., 1998; Turrigiano and Nelson, 2000), or activity-dependent or developmental changes in receptor localization (Craig, 1998), that operate over a time scale of hours to days. Finally, it remains to be seen what role regulation of AMPAR binding proteins plays in both rapid and slow forms of central synaptic plasticity. It is not clear that inserting more receptors into the membrane is useful without the resources to trap those receptors at the synapse. It may very well turn out that producing a long-lasting change in receptor number at a synapse requires both delivery of more receptors to the membrane and an increased capacity to bind and immobilize those receptors.

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