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AMPA receptor trafficking and long-term potentiation

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Activity-dependent changes in synaptic function are believed to underlie the formation of memories. A prominent example is long-term potentiation (LTP), whose mechanisms have been the subject of considerable scrutiny over the past few decades. I review studies from our laboratory that support a critical role for AMPA receptor trafficking in LTP and experience-dependent plasticity.

Keywords: excitatory; transmission; memory; long-term potentiation; experience-dependent plasticity

1. INTRODUCTION

There is general belief that a long-lasting change in synaptic function is the cellular basis of learning and memory (Eccles 1964; Hebb 1949; Alkon & Nelson 1990; Kandel 1997). The most thoroughly characterized example of such synaptic plasticity is LTP. While many neuroscientists like to disparage LTP, and even gain notoriety by their attempts to diminish its importance, this phenomenon continues to hold the interest of most scientists interested in the cellular basis of learning and memory. History will tell who has misspent energies.

A remarkable feature of LTP is that a short period of synaptic activity can trigger persistent changes of synaptic transmission lasting at least several hours and often longer. This property led investigators to suggest that LTP is the cellular correlate of learning (Bliss & Gardner-Medwin 1973; Bliss & Lømo 1973). Work over the past 25 years that has elucidated many properties of LTP reinforces this view and suggests its involvement in various other adult and developmental physiological as well as pathological processes (Martin *et al.* 2000; Zoghbi *et al.* 2000; Cline 2001).

Much effort has been directed towards understanding the detailed molecular mechanisms that account for the change in synaptic efficacy. For many years, studies often yielded conflicting conclusions (Kullmann & Siegelbaum 1995). Although many studies suggested primarily postsynaptic modifications (Davies *et al.* 1989; Kauer *et al.* 1988; Manabe *et al.* 1992; Muller *et al.* 1988), a consistent finding was a change in synaptic failures after LTP (Malinow & Tsien 1990; Kullmann & Nicoll 1992; Stevens & Wang 1994; Isaac *et al.* 1996). Because synaptic failures were assumed to be due to failure to release transmitter (a presynaptic property), these results were in apparent contradiction. A resolution arrived with the identification of postsynaptically 'silent synapses' and the demonstration that they could be converted to active synapses by a postsynaptic modification (Kullmann 1994; Isaac *et al.* 1995; Liao *et al.* 1995; Durand *et al.* 1996). Synapses are postsynaptically silent if they show an NMDA but no AMPA receptor response. Thus, at resting potentials NMDARs are minimally opened, and transmitter release at such a synapse is recorded as a failure. The appearance of an AMPA response at such synapses during LTP, with no change in the NMDA response, suggests a postsynaptic modification consisting of a functional recruitment of AMPARs. One potential mechanism envisioned was the rapid delivery of AMPARs from non-synaptic sites to the synapse. An increase in NMDA responses following some LTP-inducing stimuli (Asztely *et al.* 1992) could represent the formation of new silent synapses (Engert & Bonhoeffer 1999; Maletic-Savatic *et al.* 1999). The role of silent synapses in LTP provided strong motivation for the development of cellular and molecular techniques that could monitor and perturb trafficking of AMPARs to and away from synapses.

2. MOLECULAR INTERACTIONS OF AMPA RECEPTORS

AMPARs are hetero-oligomeric proteins made of the subunits GluR1–GluR4 (also known as GluRA–D) (Wisden & Seeburg 1993; Hollmann & Heinemann 1994). Each receptor complex contains four subunits (Rosenmund *et al.* 1998). In the adult hippocampus two species of AMPAR appear to predominate: receptors made of GluR1 and GluR2 or those composed of GluR3 and GluR2 (Wenthold *et al.* 1996). Immature hippocampus, as well as other mature brain regions, express GluR4, which also complexes with GluR2 to form a receptor (Zhu *et al.* 2000). The intracellular cytoplasmic tails of AMPARs are either long or short. GluR1, GluR4 and an alternative splice form of GluR2 (GluR2L) have longer cytoplasmic tails and are homologous. By contrast, the predominant splice form of GluR2, GluR3 and an alternative splice form of GluR4 that is primarily expressed in the cerebellum (GluR4c) have shorter, homologous cytoplasmic tails. Through their C-terminal tails, each subunit interacts with specific cytoplasmic proteins. Many of these AMPAR-interacting proteins thus far identified have single or multiple PDZ domains, which are well-characterized protein–protein interaction motifs that often interact with the extreme C-terminal tails of target

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proteins (Sheng & Sala 2001). GluR1 forms a group I PDZ ligand whereas GluR2, GluR3 and GluR4c form group II PDZ ligands. GluR4 and GluR2L have variant C-terminal tails, and it is unclear if they interact with classical PDZ-domain proteins. In a variety of cell types, proteins containing PDZ-domains have been implicated in playing important roles in the targeting and clustering of membrane proteins to specific subcellular domains (Sheng & Sala 2001).

GluR1 interacts with the PDZ-domain regions of SAP97 (Leonard *et al.* 1998) and RIL (Schulz *et al.* 2001). SAP97 is closely related to a family of proteins (SAP90/PSD95, chapsyn110/PSD93 and SAP102) that interact with NMDAR subunits. RIL, on the other hand, may link AMPARs to actin. GluR2 and GluR3 interact with GRIP (Dong *et al.* 1997, 1999) and AMPAR-binding protein (ABP)/GRIP2 (Srivastava *et al.* 1998; Dong *et al.* 1999), proteins with six or seven PDZ domains. GluR2 and GluR3 as well as GluR4c also interact with protein interacting with C-kinase (Dev *et al.* 1999; Xia *et al.* 1999), which contains a single PDZ domain that interacts with both PKCα and GluR2. Other group II PDZ-domain-containing proteins that interact with GluR2, GluR3 and GluR4c have recently been identified and include rDLG6 (Inagaki *et al.* 1999) and afadin (Rogers *et al.* 2001). No binding partners have yet been reported for GluR4 and GluR2L.

Some additional proteins interact with the cytoplasmic tails of AMPAR subunits at regions that are not at the exact C terminus. GluR1 interacts with band 4.1N and is linked through it to actin (Shen *et al.* 2000). The interaction occurs at a region on GluR1 that is homologous with all other subunits, and thus band 4.1N may also interact with other AMPAR subunits. There are, however, two residues in this region where different subunits contain serines (GluR1) or alanines (GluR2 and GluR4) or one of each (GluR3). This could confer differential binding to proteins such as 4.1, and could be modulated by phosphorylation. A surprising finding is that the cytoplasmic tail of GluR2, in addition to interacting with PDZ proteins, also binds to NSF (Nishimune *et al.* 1998; Osten *et al.* 1998; Song *et al.* 1998), an ATPase known to play an essential role in the membrane fusion processes that underlie intracellular protein trafficking and presynaptic vesicle exocytosis (Rothman 1994). Another key component of membrane fusion machinery, α and β soluble NSF attachment proteins, can also be co-immunoprecipitated with AMPARs containing GluR2 (Osten *et al.* 1998).

Because these AMPAR-interacting proteins contain PDZ domains, are proteins implicated in membrane fusion, or interact with the actin cytoskeleton, they have been suggested to play important roles in controlling the trafficking of AMPARs and/or their stabilization at synapses. The proposed specific functions of each of these proteins in controlling AMPAR behaviour are discussed in greater detail in the following sections.

3. AMPAR DELIVERY TO SYNAPSES AND LONG-TERM POTENTIATION

(a) *Subcellular steady-state distribution of AMPARs*

Several studies over the past few years have tested the notion that silent synapses lack AMPARs and that AMPARs are rapidly delivered to synapses during LTP. An important requirement for this model is that there be a pool of non-synaptic AMPARs near synapses available for delivery. Several studies have used microscopic techniques to examine the distribution of glutamate receptors at and near synapses in rat brains (Petralia & Wenthold 1992; Martin *et al.* 1993; Molnar *et al.* 1993; Baude *et al.* 1995; Kharazia *et al.* 1996; Nusser *et al.* 1998; Petralia *et al.* 1999; Takumi *et al.* 1999). Although the concentration of AMPARs is normally higher at synapses, these studies generally find ample amounts of non-synaptic AMPARs on both surfaces and intracellular regions of dendrites. Indeed, given the much larger space occupied by non-synaptic regions, non-synaptic AMPARs appear to outnumber synaptic AMPARs by quite a large margin (Shi *et al.* 1999). The distance between these non-synaptic receptors and synaptic regions is a few microns, a distance that could be traversed in seconds by membrane trafficking processes. Importantly, recent studies using post-embedding immunogold techniques ((Nusser *et al.* 1998; Petralia *et al.* 1999; Takumi *et al.* 1999) found that a sizeable fraction of synapses in CA1 hippocampus lacks or has very few AMPARs, whereas most synapses have NMDARs. The fraction of synapses lacking AMPARs is greater earlier in development, consistent with the electrophysiological observations that silent synapses are more prevalent at these ages (Durand *et al.* 1996; Liao & Malinow 1996; Rumpel *et al.* 1998; Wu *et al.* 1996; Isaac *et al.* 1997). A recent study, employing two-photon uncagion of glutamate (Matsuzaki *et al.* 2001) demonstrated a close correlation between AMPAR responsivity and size of spine. Small spines and filopodia were largely devoid of AMPAR responses. These structures did contain NMDAR responses. Although some studies in dissociated cultured neurons support these views (Gomperts *et al.* 2000; Liao *et al.* 1999) others do not (Renger *et al.* 2001) possibly owing to different culture conditions.

(b) *Optical detection of recombinant AMPAR trafficking during long-term potentiation*

To monitor AMPAR trafficking in living tissue, we generated and acutely expressed GFP-tagged GluR1 receptors in organotypic hippocampal slices (Shi *et al.* 1999). Although slices of tissue provide a more challenging experimental preparation to examine receptor trafficking, this tissue was used, rather than dissociated neurons, because there had been little success in generating LTP using standard electrophysiological protocols in dissociated neurons. These recombinant GluR1-GFP receptors are functional and their cellular distribution can be monitored with two-photon laser scanning microscopy. Upon expression, these receptors distribute diffusely throughout the dendritic tree. Interestingly, they remain in the dendritic shaft regions, with little encroachment into dendritic spines, which are the sites of excitatory contacts. This restriction from synapses is in contrast with what is found in dissociated cultured neurons in which expression of recombinant GluR1 concentrates at synapses (Lissin *et al.* 1998; Shi *et al.* 1999). In slices, little movement of GluR1-GFP was detected in the absence of stimulation. However, high-frequency synaptic activation, which generated LTP, induced movement of GFP-tagged receptors to the surface of the dendritic shaft as well as to dendritic

spines. These movements of GFP-tagged receptors were detected over the course of *ca*. 15–30 min and were prevented by blockade of NMDARs. The tagged receptors remained in at least some of the spines for at least 50 min. This study concluded that GuR1-containing receptors are maintained in reserve at the dendritic shaft and can be delivered to synapses during LTP.

Several studies have produced findings that strengthen these conclusions. Adult knockout mice lacking GluR1 cannot generate LTP, indicating that this subunit plays a critical role (Zamanillo *et al.* 1999). In a follow-up study, GluR1-GFP was genetically inserted into these GluR1 knockout mice and GFP fluorescence was detected in dendritic spines (Mack *et al.* 2001). This distribution differs from what is observed when GluR1-GFP is acutely expressed in hippocampal slices before LTP, but resembles the distribution after LTP. These observations are consistent with the view that an LTP-like process drives the genetically expressed GluR1-GFP into synapses when the animals are alive. This study also found that LTP was rescued by expression of only *ca*. 10% of the normal amount of GluR1. This further supports the view that normally there is an overabundance of GluR1 available for generating LTP.

(c) *Electrophysiological tagging to monitor synaptic delivery of recombinant AMPARs*

Although optical studies provide important information about receptor distribution, the location of a receptor (even with electron microscopic resolution) cannot unambiguously reveal its contribution to synaptic transmission. To address this issue we developed electrophysiologically tagged recombinant AMPARs. Such receptors differ in their rectification from endogenous receptors. Rectification is an intrinsic biophysical property of a receptor that can be detected as the ratio of the response observed at −60 mV to that at +40 mV. Most endogenous AMPARs contain the GluR2 subunit and can pass current equally well in both inward and outward directions. In contrast, AMPARs lacking GluR2 (or containing GluR2 that is genetically modified) exhibit profound inward rectification such that they can pass minimal current in the outward direction when the cell is depolarized to +40 mV. Thus, incorporation of recombinant AMPARs into synapses and their contribution to synaptic transmission can be monitored functionally. With this assay for AMPAR delivery, it has been possible to show that LTP and overexpression of active CaMKII induce delivery of GluR1-containing receptors into synapses (Hayashi *et al.* 2000). An interaction between GluR1 and a PDZ-domain protein is necessary for LTP or CaMKII to drive synaptic delivery of GluR1, as point mutations in the PDZ-binding region of GluR1 prevent its synaptic delivery. The identity of the GluR1-interacting PDZ-domain protein(s) responsible for LTP is not known. It appears, however, that an interaction between GluR1 and a PDZ-domain protein is required for GluR1 to reach dendritic spines (Piccini & Malinow 2002).

An important role for GluR1 in LTP is supported by studies with mice lacking GluR1, which show no LTP in adults (Zamanillo *et al.* 1999). Interestingly, LTP is neither absent in all brain regions (e.g. LTP in dentate gyrus is present; Zamanillo *et al.* (1999)) nor in all ages (e.g. LTP in CA1 is present in juvenile animals; Mack *et al.* (2001)). This suggests that AMPAR subunits other than GluR1 may play critical roles in activity-dependent synaptic plasticity. Indeed, the CA1 hippocampal region in immature animals, as well as the dentate gyrus in older animals, contain GluR4, a subunit with considerable homology to GluR1. Studies using electrophysiological assays to monitor the synaptic delivery of recombinant GluR4 indicate that this subunit mediates activity-dependent AMPAR delivery in immature hippocampus (Zhu *et al.* 2000). Interestingly, this delivery of recombinant GluR4 to synapses required NMDAR activity (i.e. delivery was blocked by APV) but not CaMKII activity.

As expression of GluR4 in hippocampus decreases to near undetectable levels by postnatal day 10, the LTP observed in CA1 hippocampus of juvenile (approximately postnatal day 28) animals that lack GluR1 may be mediated by other AMPAR subunits. It is possible that this role is played by GluR2L, the alternative splice form of GluR2 with a cytoplasmic tail that resembles GluR1 and GluR4 (Wisden & Seeburg 1993; Hollmann & Heinemann 1994). Indeed, recent results indicate activity-driven synaptic delivery of recombinant GluR2L (Zhu *et al.* 2002).

(d) *Synaptic delivery of endogenous receptors*

Although the studies described above monitored synaptic delivery of recombinant AMPARs, other studies have tested if such a process occurs for endogenous receptors. One study expressed the cytoplasmic tail of GluR1 to block the trafficking of GluR1. This construct is known to bind to cytoplasmic proteins that interact with GluR1, and thus it should compete with endogenous GluR1 with such binding. As such, interactions are important for LTP (for instance, mutations of GluR1 at its PDZ interaction site, or PKA phosphorylation site, see below, can block LTP). When expressed in organotypic slices for 2–3 days, the GluR1 cytoplasmic tail had no effect on the amplitude of AMPAR-mediated transmission. This supports the view that GluR1-containing receptors are not constitutively delivered to synapses in the absence of strong (LTP-like) stimuli. This construct also had no effect on the amplitude of NMDA-mediated responses. These results indicate that this construct is not generally perturbing protein trafficking; even those mediated by type I PDZ interactions (which are important for NMDA-R trafficking; Barria & Malinow (2002)). However, cells expressing this construct showed no LTP after a pairing protocol (Shi *et al.* 2001). This construct thus prevents endogenous GluR1 from interacting with critical cytoplasmic proteins required for synaptic incorporation of GluR1.

Another study (Zhu *et al.* 2000) tested the endogenous synaptic delivery of GluR4 during early postnatal hippocampal development. Again, GluR4 cytoplasmic tail was expressed in neurons. Expression of this construct in neurons of age postnatal day 11 or older had no effect on transmission. Expression of this construct in neurons at postnatal day 6 for 24 h led to a large decrease in synaptic transmission relative to nearby non-infected neurons. However, this depression was not observed if spontaneous activity was blocked in the slices during the expression period. This indicates that spontaneous activity drives GluR4-containing receptors into synapses during early

postnatal development, and the GluR4 cytoplasmic tail can block this. In these experiments, the GluR4 cytoplasmic tail had no effect on the NMDAR responses, supporting the specific actions of cytoplasmic tail constructs.

In contrast to the expression of cytoplasmic tails from long-tailed receptors, expression of the GluR2 cytoplasmic tail depressed transmission, even when slices were incubated in conditions that blocked spontaneous activity (Shi *et al.* 2001). Transmission was reduced to *ca*. 50% of that seen in nearby non-infected neurons, suggesting that *ca*. 50% of receptors are continually undergoing replacement. This is consistent with numerous reports indicating that GluR2-containing receptors are continually cycling into and out of the synapse (Nishimune *et al.* 1998; Luscher *et al.* 1999; Lüthi *et al.* 1999; Noel *et al.* 1999; Ehlers 2000; Lin *et al.* 2000; Kim & Lisman 2001; Shi *et al.* 2001; Zhou *et al.* 2001). A recent report indicates that the critical pore residue, R586Q in GluR2 can affect its exit from the endoplasmic reticulum and surface expression in dissociated cultured neurons (Greger *et al.* 2002). However, in cultured slices and in *in vivo* systems (see below), the synaptic incorporation of GluR2 appears not to be affected by this residue. For instance, in slices, the same synaptic incorporation is seen by a pore-dead mutant (GluR2(R586E), *ca*. 50% synaptic depression), rectification mutant (GluR2(R586Q), *ca*. 50% depression at +40 mV) and endogenous GluR2 (depression of *ca*. 50% by GluR2 cytoplasmic tail) (Shi *et al.* 2001). In addition, an *in vivo* study shows the same synaptic incorporation by GluR2(R586Q) mutant (*ca*. 50% increased rectification) and endogenous GluR2 (as determined by expression of GluR2 cytoplasmic tail, *ca*. 50% depression) *in vivo*.

LTP in cells expressing the GluR2 cytoplasmic tail was not reduced, supporting the view that interactions by GluR2 are not critical for the generation of LTP. This is supportive of earlier findings with mice lacking GluR2 that showed LTP (Jia *et al.* 1996). Indeed, LTP was observed to be quite large, although this may simply be due to the fact that transmission began at a depressed level, and a normal level of GluR1 delivery would produce potentiation that appears large.

Some studies in dissociated cultured neurons have supported the view that LTP produces delivery of AMPARs to synapses (Liao *et al.* 2001; Lu *et al.* 2001).

(e) *Role of AMPA receptor phosphorylation in synaptic delivery*

There has been considerable evidence indicating that protein kinases play critical roles in the generation of LTP (Madison *et al.* 1991; Bliss & Collingridge 1993; Malenka & Nicoll 1999). Some kinases (e.g. CaMKII; Lisman *et al.* (1997)) are thought to mediate directly the signals leading to LTP, whereas others (e.g. PKA; Blitzer *et al.* (1995)) may 'gate' (i.e. modulate) its generation. The targets of these kinases responsible for mediating or gating LTP have been the source of considerable investigation. During LTP the CaMKII-phosphorylation site on GluR1, Ser831, is phosphorylated (Barria *et al.* 1997*a*,*b*; Mammen *et al.* 1997). Such phosphorylation can increase conductance through GluR1 receptors (Derkach *et al.* 1999), and AMPARS show increased conductance during LTP (Benke *et al.* 1998) and following expression of constitutively active CaMKII (Poncer *et al.* 2002). Thus, it was of considerable interest to determine if phosphorylation of Ser831 is required for synaptic delivery of GluR1-containing receptors. However, mutations on GluR1-Ser831 that prevent its phosphorylation by CaMKII do not prevent delivery of the receptor to synapses by active CaMKII (Hayashi *et al.* 2000) or by LTP (S.-H. Shi and R. Malinow, unpublished observations). Thus, CaMKII must be acting on a different target to effect synaptic delivery of GluR1. Recent studies indicate that CaMKII can phosphorylate a synaptic rasGAP (Chen *et al.* 1998; Kim *et al.* 1998) and potentially control levels of ras activity. Ras activity appears to be necessary to generate LTP and is the downstream effector of CaMKII that drives synaptic delivery of AMPARs (Zhu *et al.* 2002). This conforms with results indicating a critical role for MAP kinase, a downstream effector for ras, in LTP (English & Sweatt 1996, 1997).

Interestingly, mutations at Ser845, the PKA phosphorylation site of GluR1 (Roche *et al.* 1996), do prevent delivery of GluR1 to synapses by active CaMKII or LTP (Shi & Malinow 2001). Phosphorylation at this site of GluR1 also accompanies surface reinsertion of receptors (Ehlers 2000) and LTP induction after prior LTD (Lee *et al.* 2000). Phosphorylation at this site by exogenous application of drugs that raise cAMP does not induce delivery of recombinant GluR1 (Shi & Malinow 2001). Thus, PKA phosphorylation of GluR1 is necessary, but not sufficient, for its synaptic delivery; that is, phosphorylation of Ser845 acts as a gate. Of note, the PKA-scaffolding molecule, AKAP, binds to SAP97 and thereby effectively brings PKA to GluR1 (Colledge *et al.* 2000). Thus, it is possible that the PDZ mutation on GluR1 blocks its synaptic delivery, at least in part, because it prevents PKA phosphorylation at Ser845. Of note, SAP97 associates with GluR1 primarily in intracellular sites (Sans *et al.* 2001), consistent with its playing a role in making GluR1 competent for synaptic delivery.

Recent studies indicate that activity-driven phosphorylation of GluR4 by PKA is necessary and sufficient for delivery of these recombinant AMPARs to synapses during early development (Esteban *et al.* 2003). Such phosphorylation relieves a retention interaction that, in the absence of synaptic activity, maintains GluR4-containing receptors away from the synapse. Thus, a mechanism (PKA phosphorylation of AMPARs) that mediates plasticity early in development (with GluR4) becomes a gate for plasticity (with GluR1) later in development. Increasing requirements over development may be one way that plasticity becomes more specific and also recalcitrant with age.

4. GENERAL TRAFFICKING MECHANISMS

A key question has been if plasticity acts by directly modulating a process that is responsible for turning over receptors at synapses (e.g. increasing rate of delivery or decreasing rate of removal) or if there are distinct processes responsible for plasticity and receptor turnover. One recent study (Shi *et al.* 2001) examined this question and argues for distinct AMPARs responsible for LTP and receptor turnover. AMPARs composed of GluR1 and GluR2 (or any receptor with a long cytoplasmic tail together with GluR2) participates in regulated delivery.

In the absence of electrical activity, these receptors are restricted from accessing synapses. LTP (for GluR1-containing receptors) or spontaneous activity (for GluR4-containing receptors) drives these receptors (along with associated scaffolding) into synapses. The long cytoplasmic tails, and not the short cytoplasmic tails, of GluR1/GluR2 heteromers are critical for this activity-dependent synaptic delivery. Receptors composed of GluR2 and GluR3 continuously replace synaptic GluR2/GluR3 receptors in a manner that maintains constant transmission. How can this model explain long-term changes in synaptic receptor number following plasticity that enhances transmission? At some point after their synaptic delivery, receptors containing GluR1 or GluR4 become replaceable by GluR2/GluR3 receptors. The scaffolding associated with GluR1 or GluR4 (called 'slot' complexes; Shi *et al.* (2001)) must somehow control this. One study provides evidence for replacement of synaptic GluR4-containing receptors by GluR2/GluR3 receptors (Zhu *et al.* 2000). This occurs over the course of days after the activity-driven delivery of GluR4-containing receptors.

(a) *Role of trafficking in experience-dependent plasticity*

Considerable progress has been made in uncovering the cellular and molecular mechanisms underlying activity-dependent synaptic plasticity *in vitro*. However, although LTP is a leading contender as a mechanism to encode experience in brain circuits, there are few reports (cf. Finnerty *et al.* 1999; Rogan *et al.* 1997; Rioult-Pedotti *et al.* 2000) suggesting that LTP occurs *in vivo* in response to natural stimuli. We have recently tested if synaptic modifications identified to occur during LTP *in vitro* are also driven by experience in the intact brain (Takahashi *et al.* 2003). We examined excitatory transmission between layer 4 and layer 2/3 neurons in barrel cortex during a period when considerable experience-dependent plasticity occurs (Micheva & Beaulieu 1996; Lendvai *et al.* 2000; Stern *et al.* 2001). For instance, between PND12 and PND14 there is a twofold increase in the number of synapses in barrel cortex (Micheva & Beaulieu 1996). While synapse numbers appear not affected by sensory deprivation (Winfield 1981; Vees *et al.* 1998), other aspects of synaptic function, such as receptor content, could be dependent on experience.

In agreement with *in vitro* models of AMPAR trafficking, we find that recombinant GluR1 is driven into synapses by experience. Furthermore, GluR1-ct, which can block LTP *in vitro* (Hayashi *et al.* 2000), prevents experience-driven synaptic potentiation. These results indicate a large (e.g. *ca*. 2.5-fold) increase in transmission at synapses between layer 4 and layer 2/3 neurons between PND 12 and PND 14 that is driven by experience and mediated by synaptic delivery of GluR1-containing AMPARs. The increase in rectification in neurons expressing homomeric GluR1 is considerably smaller (*ca*. 1.3-fold). This is consistent with transient delivery of GluR1-containing receptors with subsequent replacement by GluR2-containing receptors. In accordance with *in vitro* studies (Noel *et al.* 1999; Scannevin & Huganir 2000; Sheng & Lee 2001; Shi *et al.* 2001; Tomita *et al.* 2001; Malinow & Malenka 2002), we find that replacement of synaptic receptors depends on interactions by the GluR2 cytoplasmic tail and that it can occur in the absence of experience. Our results indicate that the rules of AMPAR trafficking identified *in vitro* apply to behaviourally driven plasticity. Thus, the presence of AMPARs with long cytoplasmic tails at a synapse may represent the signature of recent experience-dependent plasticity.

5. CONCLUSIONS

Lynch & Baudry (1984) proposed almost two decades ago that LTP is due to an increase in the number of synaptic glutamate receptors. However, the idea did not gain universal favour and a vigorous exchange over the ensuing decades debated the pre- and postsynaptic contributions to the expression of LTP. Thus, the general acceptance of postsynaptic silent synapses and AMPAR trafficking as playing important roles in synaptic plasticity represents a significant advance in the field. It provides a clear conceptual framework that should facilitate studies aimed at determining which molecules play critical roles in LTP and exactly what role they play.

A molecular blueprint of LTP should allow us to begin probing experience-driven plasticity. Several issues should be experimentally approachable. What brain regions show experience-dependent receptor trafficking, and what experiences drive this? Does experience-dependent trafficking show a 'critical period'? Are there specific patterns of activity at different ages that drive experience-dependent trafficking for each age? Is the trafficking of each glutamate receptor with a long cytoplasmic tail, driven by specific types of experiences? What signalling pathways are activated and required for plasticity *in vivo*? One can hope that gains from *in vitro* studies will aid in elucidating the nature of synaptic modifications driven by experience.

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GLOSSARY

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazole propionate
AMPAR: AMPA receptor
GFP: green fluorescent protein
GRIP: glutamate receptor-interacting protein
LTP: long-term potentiation
NSF: N-ethylmaleimide-sensitive-factor
NMDA: *N*-methyl-D-aspartate
NMDAR: *N*-methyl-D-aspartate receptor