AMPA Receptor Trafficking and LTP

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Abstract 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. Here I review studies from our laboratory that support a critical role for AMPA receptor trafficking in LTP and experience-dependent plasticity.

INTRODUCTION

There is general belief that a long-lasting change in synaptic function is the cellular basis of learning and memory (Alkon & Nelson 1990; Eccles 1964; Hebb 1949; Kandel 1997). The most thoroughly characterized example of such synaptic plasticity is long-term potentiation (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 & Lomo 1973). Work over the last 25 years that has elucidated many properties of LTP reinforces this view as well as suggests its involvement in various other adult and developmental physiological as well as pathological processes (Cline 2001; Martin et al. 2000; Zoghbi et al. 2000).

Much effort has been directed toward 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 (Isaac et al. 1996; Kullmann & Nicoll 1992; Malinow & Tsien 1990; Stevens & Wang 1994). 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 (Durand et al. 1996; Isaac et al. 1995; Kullmann 1994; Liao et al. 1995). Synapses are postsynaptically silent if they show an NMDA but no AMPA receptor response. Thus, at resting potentials NMDA receptors (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 AMPA receptors (AMPARs).

One potential mechanism envisioned was the rapid delivery of AMPARs from nonsynaptic 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.

MOLECULAR INTERACTIONS OF AMPA RECEPTORS

AMPA receptors (AMPARs) are hetero-oligomeric proteins made of the subunits GluR1 to GluR4 (also known as GluRA-D) (Hollmann & Heinemann 1994; Wisden & Seeburg 1993). 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. 2000b). 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. In contrast, the predominant splice form of GluR2, GluR3, and an alternative splice form of GluR4 that is primarily expressed in 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 proteins (Sheng & Sala 2001). GluR1 forms a group I PDZ ligand while GluR2, GluR3, and GluR4c form group II PDZ ligands. GluR4 and GluR2L have variant C-terminal tails, and if they interact with classical PDZ-domain proteins is unclear. 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 AMPA receptors to actin. GluR2 and GluR3 interact with glutamate receptor-interacting protein (GRIP) (Dong et al. 1997; Dong et al. 1999) and AMPA receptor binding protein (ABP)/GRIP2 (Dong et al. 1999; Srivastava et al. 1998), proteins with six or seven PDZ domains. GluR2 and GluR3 as well as GluR4c also interact with PICK1 (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 interact with other AMPAR subunits as well. 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 (NEM-sensitive-factor) (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 SNAPS (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 behavior are discussed in greater detail in the following sections.

AMPA RECEPTOR DELIVERY TO SYNAPSES AND LTP

Subcellular steady-state distribution of AMPA receptors

A number of 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 nonsynaptic AMPARs near synapses available for delivery. Several studies have used microscopic techniques to examine distribution of glutamate receptors at and near synapses in rat brains (Petralia and 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). While the concentration of AMPARs is normally higher at synapses, these studies generally find ample amounts of nonsynaptic AMPARs on both surfaces and intracellular regions of dendrites. Indeed, given the much larger space occupied by nonsynaptic regions, nonsynaptic AMPARs appear to outnumber synaptic AMPARs by quite a large margin (Shi et al. 1999). The distance between these nonsynaptic receptors and synaptic regions is a few microns, a distance that could be traversed in seconds by membrane trafficking processes. Importantly, recent studies using postembedding immunogold techniques ((Nusser et al. 1998; Petralia et al. 1999; Takumi et al. 1999) found that a sizable fraction of synapses in CA1 hippocampus lacks or has very few AMPARs, while 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; Isaac et al. 1997; Liao & Malinow 1996; Rumpel et al. 1998; Wu et al. 1996). 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. While 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 due to different culture conditions.

Optical detection of recombinant AMPA receptor trafficking during LTP

To monitor AMPAR trafficking in living tissue, we generated and acutely expressed GFP-tagged GluR1 receptors in organotypic hippocampal slices (Shi et al. 1999). While slices of tissue provide a more challenging experimental preparation to examine receptor trafficking, this tissue was used, rather than dissociated neurons, since 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 dendritic shaft as well as to dendritic spines. These movements of GFP-tagged receptors were detected over the course of about 15-30 min and were prevented by blockade of NMDARs. The tagged receptors remained in at least some 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.

A number of studies have made 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 ~10% of the normal amount of GluR1. This further supports the view that normally there is an overabundance of GluR1 available for generating LTP.

Electrophysiological tagging to monitor synaptic delivery of recombinant AMPA receptors

While optical studies provide important information regarding 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. 2000b). 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. 2000b). 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 post-natal day 10, the LTP observed in CA1 hippocampus of juvenile (~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 (Hollmann & Heinemann 1994; Wisden & Seeburg 1993). Indeed, recent results indicate activity-driven synaptic delivery of recombinant GluR2L (Zhu et al. 2002).

Synaptic delivery of endogenous receptors

While 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 to 3 days, the GluR1 cytoplasmic tail had no effect on the amplitude of AMPA-R 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 following a pairing protocol (Shi et al. 2001a). This construct thus prevents endogenous GluR1 from interacting with critical cytoplasmic proteins required for synaptic incorporation of GluR1.

Another study (Zhu et al. 2000a) 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 hrs 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 NMDA-R 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. 2001a). Transmission was reduced to about 50% of that seen in nearby non-infected neurons, suggesting that about 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 (Kim & Lisman 2001; Luscher et al. 1999; Lüthi et al. 1999; Nishimune et al. 1998; Noel et al. 1999; Shi et al. 2001b), (Ehlers 2000) (Lin et al. 2000; Zhou et al. 2001). A recent report indicates that the critical pore residue, R586Q in GluR2 can affect its exit from ER 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), ~50% synaptic depression), rectification mutant (GluR2(R586Q), ~50% depression at +40 mV) and endogenous GluR2 (depression of ~50% by GluR2 cytoplasmic tail) (Shi et al. 2001a). In addition, an *in vivo* study shows the same synaptic incorporation by GluR2(R586Q) mutant (~50% increased rectification) and endogenous GluR2 (as determined by expression of GluR2 cytoplasmic tail, ~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 AMPA-Rs to synapses (Lu et al. 2001) (Liao et al. 2001).

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 (Bliss & Collingridge 1993; Madison et al. 1991; Malenka & Nicoll 1999). Some kinases [e.g., CaMKII; (Lisman et al. 1997)] are thought to mediate directly the signals leading to LTP, while 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. 1997a; Barria et al. 1997b; 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. 2000b) or by LTP (S-H. Shi & 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; English & Sweatt 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; i.e., 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 & Malinow 2001). 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.

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. 2001b) 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 along 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 transmission constant. 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. 2001b)] must somehow control this. One study provides evidence for replacement of synaptic GluR4-containing receptors by GluR2/GluR3 receptors (Zhu et al. 2000b). This occurs over the course of days after the activity-driven delivery of GluR4-containing receptors.

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, while LTP is a leading contender as a mechanism to encode experience in brain circuits, there are few reports (cf. (Finnerty et al. 1999; Rioult-Pedotti et al. 2000; Rogan et al. 1997)) 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,

unpublished observations). We examined excitatory transmission between layer 4 and layer 2/3 neurons in barrel cortex during a period when considerable experience dependent plasticity occurs (Lendvai et al. 2000; Micheva & Beaulieu 1996; Stern et al. 2001). For instance, between PND12 and PND14 there is a 2-fold increase in the number of synapses in barrel cortex (Micheva & Beaulieu 1996). While synapse numbers appear not affected by sensory deprivation (Vees et al. 1998; Winfield 1981), 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. 2000a), prevents experience-driven synaptic potentiation. These results indicate a large (e.g. ~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 AMPA-Rs. The increase in rectification in neurons expressing homomeric GluR1 is considerably smaller (~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 (Malinow & Malenka 2002; Noel et al. 1999; Scannevin & Huganir 2000; Sheng & Lee 2001; Shi et al. 2001a; Tomita et al. 2001) 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 AMPA-R trafficking identified *in vitro* apply to behaviorally driven plasticity. Thus, the presence of AMPA-Rs with long cytoplasmic tails at a synapse may represent the signature of recent experience-dependent plasticity.

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 favor 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 represent significant advances in the field. They provide 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. A number of 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 receptors with long cytoplasmic tail, driven by specific types of experiences? What signaling 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.

Literature Cited

Alkon, D. L. & Nelson, T. J. 1990 Specificity of molecular changes in neurons involved in memory storage. *Faseb Journal* **4**, 1567-76.

Asztely, F., Wigstrom, H. & Gustafsson, B. 1992 The Relative Contribution of NMDA Receptor Channels in the Expression of Long-term Potentiation in the Hippocampal CA1 Region. *Eur J Neurosci* **4**, 681-690.

Barria, A., Derkach, V. & Soderling, T. 1997a Identification of the Ca2+/calmodulin-dependent protein kinase II regulatory phosphorylation site in the alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate-type glutamate receptor. *Journal of Biological Chemistry* **272**, 32727-30.

Barria, A. & Malinow, R. 2002 Subunit-specific NMDA receptor trafficking to synapses. *Neuron* **35**, 345-53.

Barria, A., Muller, D., Derkach, V., Griffith, L. C. & Soderling, T. R. 1997b Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation [see comments]. *Science* **276**, 2042-5.

Benke, T. A., Luthi, A., Isaac, J. T. & Collingridge, G. L. 1998 Modulation of AMPA receptor unitary conductance by synaptic activity. *Nature* **393**, 793-7.

Bliss, T. V. & Collingridge, G. L. 1993 A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**, 31-9.

Bliss, T. V. & Gardner-Medwin, A. R. 1973 Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. *Journal of Physiology* **232**, 357-74.

Bliss, T. V. & Lomo, T. 1973 Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol (Lond)* **232**, 331-56.

Blitzer, R. D., Wong, T., Nouranifar, R., Iyengar, R. & Landau, E. M. 1995 Postsynaptic cAMP pathway gates early LTP in hippocampal CA1 region. *Neuron* **15**, 1403-14.

Chen, H. J., Rojas-Soto, M., Oguni, A. & Kennedy, M. B. 1998 A synaptic Ras-GTPase activating protein (p135 SynGAP) inhibited by CaM kinase II. *Neuron* **20**, 895-904.

Cline, H. T. 2001 Dendritic arbor development and synaptogenesis. *Curr Opin Neurobiol* **11**, 118-26.

Colledge, M., Dean, R. A., Scott, G. K., Langeberg, L. K., Huganir, R. L. & Scott, J. D. 2000 Targeting of PKA to glutamate receptors through a MAGUK-AKAP complex. *Neuron* **27**, 107-19.

Davies, S. N., Lester, R. A., Reymann, K. G. & Collingridge, G. L. 1989 Temporally distinct pre- and post-synaptic mechanisms maintain long- term potentiation. *Nature* **338**, 500-3.

Derkach, V., Barria, A. & Soderling, T. R. 1999 Ca2+/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 3269-74.

Dev, K. K., Nishimune, A., Henley, J. M. & Nakanishi, S. 1999 The protein kinase C alpha binding protein PICK1 interacts with short but not long form alternative splice variants of AMPA receptor subunits. *Neuropharmacology* **38**, 635-44.

Dong, H., O'Brien, R. J., Fung, E. T., Lanahan, A. A., Worley, P. F. & Huganir, R. L. 1997 GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors [see comments]. *Nature* **386**, 279-84.

Dong, H., Zhang, P., Song, I., Petralia, R. S., Liao, D. & Huganir, R. L. 1999 Characterization of the glutamate receptor-interacting proteins GRIP1 and GRIP2. *Journal of Neuroscience* **19**, 6930-41.

Durand, G. M., Kovalchuk, Y. & Konnerth, A. 1996 Long-term potentiation and functional synapse induction in developing hippocampus. *Nature* **381**, 71-5.

Eccles, J. C. 1964 *The physiology of synapses*. New York,: Academic Press.

Ehlers, M. D. 2000 Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. *Neuron* **28**, 511-525.

Engert, F. & Bonhoeffer, T. 1999 Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature* **399**, 66-70.

English, J. D. & Sweatt, J. D. 1996 Activation of p42 mitogen-activated protein kinase in hippocampal long term potentiation. *Journal of Biological Chemistry* **271**, 24329-32.

English, J. D. & Sweatt, J. D. 1997 A requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. *J Biol Chem* **272**, 19103-6.

Esteban, J. A. & Malinow, R. 2001 A molecular mechanism for the regulated synaptic delivery of GluR4-containing AMPA receptors. In *Society for Neuroscience Annual Meeting*. San Diego.

Finnerty, G. T., Roberts, L. S. & Connors, B. W. 1999 Sensory experience modifies the short-term dynamics of neocortical synapses. *Nature* **400**, 367-71.

Greger, I. H., Khatri, L. & Ziff, E. B. 2002 RNA editing at arg607 controls AMPA receptor exit from the endoplasmic reticulum. *Neuron* **34**, 759-72.

Hayashi, Y., Shi, S. H., Esteban, J. A., Piccini, A., Poncer, J. C. & Malinow, R. 2000a Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science* **287**, 2262-7.

Hayashi, Y., Shi, S.-H., Esteban, J. A., Piccini, A., Poncer, J. C. & Malinow, R. 2000b Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science* **287**, 2262-7.

Hebb, D. 1949 *The Organization of Behavior*. New York: Wiley.

Hollmann, M. & Heinemann, S. 1994 Cloned glutamate receptors. *Annual Review of Neuroscience* **17**, 31-108.

Inagaki, H., Maeda, S., Lin, K. H., Shimizu, N. & Saito, T. 1999 rDLG6: a novel homolog of Drosophila DLG expressed in rat brain. *Biochem Biophys Res Commun* **265**, 462-8.

Isaac, J. T., Crair, M. C., Nicoll, R. A. & Malenka, R. C. 1997 Silent synapses during development of thalamocortical inputs. *Neuron* **18**, 269-80.

Isaac, J. T., Hjelmstad, G. O., Nicoll, R. A. & Malenka, R. C. 1996 Long-term potentiation at single fiber inputs to hippocampal CA1 pyramidal cells. *Proc Natl Acad Sci U S A* **93**, 8710-5.

Isaac, J. T., Nicoll, R. A. & Malenka, R. C. 1995 Evidence for silent synapses: implications for the expression of LTP. *Neuron* **15**, 427-34.

Jia, Z., Agopyan, N., Miu, P., Xiong, Z., Henderson, J., Gerlai, R., Taverna, F. A., Velumian, A., MacDonald, J., Carlen, P., Abramow-Newerly, W. & Roder, J. 1996 Enhanced LTP in mice deficient in the AMPA receptor GluR2. *Neuron* **17**, 945-56.

Kandel, E. R. 1997 Genes, synapses, and long-term memory. *Journal of Cellular Physiology* **173**, 124-5.

Kauer, J. A., Malenka, R. C. & Nicoll, R. A. 1988 A persistent postsynaptic modification mediates long-term potentiation in the hippocampus. *Neuron* **1**, 911-7.

Kim, C. H. & Lisman, J. E. 2001 A labile component of AMPA receptor-mediated synaptic transmission is dependent on microtubule motors, actin, and N-ethylmaleimide-sensitive factor. *Journal of Neuroscience* **21**, 4188-94.

Kim, J. H., Liao, D., Lau, L. F. & Huganir, R. L. 1998 SynGAP: a synaptic RasGAP that associates with the PSD-95/SAP90 protein family. *Neuron* **20**, 683-91.

Kullmann, D. M. 1994 Amplitude fluctuations of dual-component EPSCs in hippocampal pyramidal cells: implications for long-term potentiation. *Neuron* **12**, 1111-20.

Kullmann, D. M. & Nicoll, R. A. 1992 Long-term potentiation is associated with increases in quantal content and quantal amplitude. *Nature* **357**, 240-4.

Kullmann, D. M. & Siegelbaum, S. A. 1995 The site of expression of NMDA receptor-dependent LTP: new fuel for an old fire. *Neuron* **15**, 997-1002.

Lee, H.-K., Barbarosie, M., Kameyama, K., Bear, M. F. & Huganir, R. L. 2000 Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* **405**, 955-959.

Lendvai, B., Stern, E. A., Chen, B. & Svoboda, K. 2000 Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex in vivo. *Nature* **404**, 876-81.

Leonard, A. S., Davare, M. A., Horne, M. C., Garner, C. C. & Hell, J. W. 1998 SAP97 is associated with the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit. *Journal of Biological Chemistry* **273**, 19518-24.

Liao, D., Hessler, N. A. & Malinow, R. 1995 Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature* **375**, 400-4.

Liao, D. & Malinow, R. 1996 Deficiency in induction but not expression of LTP in hippocampal slices from young rats. *Learn Mem* **3**, 138-49.

Liao, D., Scannevin, R. H. & Huganir, R. 2001 Activation of silent synapses by rapid activity-dependent synaptic recruitment of AMPA receptors. *Journal of Neuroscience* **21**, 6008-17.

Lin, J. W., Ju, W., Foster, K., Lee, S. H., Ahmadian, G., Wyszynski, M., Wang, Y. T. & Sheng, M. 2000 Distinct molecular mechanisms and divergent endocytotic pathways of AMPA receptor internalization. *Nature Neuroscience* **3**, 1282-90.

Lisman, J., Malenka, R. C., Nicoll, R. A. & Malinow, R. 1997 Learning mechanisms: the case for CaM-KII [see comments]. *Science* **276**, 2001-2.

Lissin, D. V., Gomperts, S. N., Carroll, R. C., Christine, C. W., Kalman, D., Kitamura, M., Hardy, S., Nicoll, R. A., Malenka, R. C. & von Zastrow, M. 1998 Activity differentially regulates the surface expression of synaptic AMPA and NMDA glutamate receptors. *Proc Natl Acad Sci U S A* **95**, 7097-102.

Lu, W., Man, H., Ju, W., Trimble, W. S., MacDonald, J. F. & Wang, Y. T. 2001 Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. *Neuron* **29**, 243-54.

Luscher, C., Xia, H., Beattie, E. C., Carroll, R. C., von Zastrow, M., Malenka, R. C. & Nicoll, R. A. 1999 Role of AMPA receptor cycling in synaptic transmission and plasticity. *Neuron* **24**, 649-58.

Lüthi, A., Chittajallu, R., Duprat, F., Palmer, M. J., Benke, T. A., Kidd, F. L., Henley, J. M., Isaac, J. T. & Collingridge, G. L. 1999 Hippocampal LTD expression involves a pool of AMPARs regulated by the NSF-GluR2 interaction [see comments]. *Neuron* **24**, 389-99.

Mack, V., Burnashev, N., Kaiser, K. M., Rozov, A., Jensen, V., Hvalby, O., Seeburg, P. H., Sakmann, B. & Sprengel, R. 2001 Conditional restoration of hippocampal synaptic potentiation in Glur-A-deficient mice. *Science* **292**, 2501-4.

Madison, D. V., Malenka, R. C. & Nicoll, R. A. 1991 Mechanisms underlying long-term potentiation of synaptic transmission. *Annu Rev Neurosci* **14**, 379-97.

Malenka, R. C. & Nicoll, R. A. 1999 Long-term potentiation--a decade of progress? *Science* **285**, 1870-4.

Maletic-Savatic, M., Malinow, R. & Svoboda, K. 1999 Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science* **283**, 1923-7.

Malinow, R. & Malenka, R. C. 2002 AMPA receptor trafficking and synaptic plasticity. *Annu Rev Neurosci* **25**.

Malinow, R. & Tsien, R. W. 1990 Presynaptic enhancement shown by whole-cell recordings of long-term potentiation in hippocampal slices [see comments]. *Nature* **346**, 177-80.

Mammen, A. L., Kameyama, K., Roche, K. W. & Huganir, R. L. 1997 Phosphorylation of the alpha-amino-3-hydroxy-5-methylisoxazole4-propionic acid receptor GluR1 subunit by calcium/calmodulin-dependent kinase II. *Journal of Biological Chemistry* **272**, 32528-33.

Manabe, T., Renner, P. & Nicoll, R. A. 1992 Postsynaptic contribution to long-term potentiation revealed by the analysis of miniature synaptic currents. *Nature* **355**, 50-5.

Martin, S. J., Grimwood, P. D. & Morris, R. G. 2000 Synaptic plasticity and memory: an evaluation of the hypothesis. *Annual Review of Neuroscience* **23**, 649-711.

Matsuzaki, M., Ellis-Davies, G. C., Nemoto, T., Miyashita, Y., Iino, M. & Kasai, H. 2001 Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nat Neurosci* **4**, 1086-92.

Micheva, K. D. & Beaulieu, C. 1996 Quantitative aspects of synaptogenesis in the rat barrel field cortex with special reference to GABA circuitry. *J Comp Neurol* **373**, 340-54.

Muller, D., Joly, M. & Lynch, G. 1988 Contributions of quisqualate and NMDA receptors to the induction and expression of LTP. *Science* **242**, 1694-7.

Nishimune, A., Isaac, J. T., Molnar, E., Noel, J., Nash, S. R., Tagaya, M., Collingridge, G. L., Nakanishi, S. & Henley, J. M. 1998 NSF binding to GluR2 regulates synaptic transmission. *Neuron* **21**, 87-97.

Noel, J., Ralph, G. S., Pickard, L., Williams, J., Molnar, E., Uney, J. B., Collingridge, G. L. & Henley, J. M. 1999 Surface expression of AMPA receptors in hippocampal neurons is regulated by an NSF-dependent mechanism. *Neuron* **23**, 365-76.

Nusser, Z., Lujan, R., Laube, G., Roberts, J. D., Molnar, E. & Somogyi, P. 1998 Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. *Neuron* **21**, 545-59.

Osten, P., Srivastava, S., Inman, G. J., Vilim, F. S., Khatri, L., Lee, L. M., States, B. A., Einheber, S., Milner, T. A., Hanson, P. I. & Ziff, E. B. 1998 The AMPA receptor GluR2 C terminus

can mediate a reversible, ATP-dependent interaction with NSF and alpha- and beta-SNAPs. *Neuron* **21**, 99-110.

Petralia, R. S., Esteban, J. A., Wang, Y. X., Partridge, J. G., Zhao, H. M., Wenthold, R. J. & Malinow, R. 1999 Selective acquisition of AMPA receptors over postnatal development suggests a molecular basis for silent synapses. *Nat Neurosci* **2**, 31-6.

Piccini, A. & Malinow, R. 2002 Critical postsynaptic density 95/disc large/zonula occludens-1 interactions by glutamate receptor 1 (GluR1) and GluR2 required at different subcellular sites. *J Neurosci* **22**, 5387-92.

Poncer, J. C., Esteban, J. A. & Malinow, R. 2002 Multiple mechanisms for the potentiation of AMPA receptor-mediated transmission by alpha-Ca2+/calmodulin-dependent protein kinase II. *J Neurosci* **22**, 4406-11.

Rioult-Pedotti, M. S., Friedman, D. & Donoghue, J. P. 2000 Learning-induced LTP in neocortex. *Science* **290**, 533-6.

Roche, K. W., O'Brien, R. J., Mammen, A. L., Bernhardt, J. & Huganir, R. L. 1996 Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. *Neuron* **16**, 1179-88.

Rogan, M. T., Staubli, U. V. & LeDoux, J. E. 1997 Fear conditioning induces associative long-term potentiation in the amygdala [published erratum appears in Nature 1998 Feb 19;391(6669):818]. *Nature* **390**, 604-7.

Rogers, C. A., Maron, C., Schulteis, C., Allen, W.-R.-. & Heinemann, S.-F. 2001 Afadin, a link between AMPA receptors and the actin cytoskeleton. In *Society for Neuroscience Annual Meeting*. San Diego.

Rosenmund, C., Stern-Bach, Y. & Stevens, C. F. 1998 The tetrameric structure of a glutamate receptor channel [see comments]. *Science* **280**, 1596-9.

Rothman, J. E. 1994 Mechanisms of intracellular protein transport. *Nature* **372**, 55-63.

Rumpel, S., Hatt, H. & Gottmann, K. 1998 Silent synapses in the developing rat visual cortex: evidence for postsynaptic expression of synaptic plasticity. *Journal of Neuroscience* **18**, 8863-74.

Sans, N., Racca, C., Petralia, R. S., Wang, Y. X., McCallum, J. & Wenthold, R. J. 2001 Synapse-associated protein 97 selectively associates with a subset of AMPA receptors early in their biosynthetic pathway. *J Neurosci* **21**, 7506-16.

Scannevin, R. H. & Huganir, R. L. 2000 Postsynaptic organization and regulation of excitatory synapses. *Nat Rev Neurosci* **1**, 133-41.

Schulz, W., Nakagawa, T., Kim, J.-H., Sheng, M., Seeburg, P. H. & Osten, P. 2001 Novel interaction of the GluR-A AMPA receptor subuint with the PDZ-LIM domain protein RIL. In *Society for Neuroscience Annual Meeting*. San Diego.

Shen, L., Liang, F., Walensky, L. D. & Huganir, R. L. 2000 Regulation of AMPA receptor GluR1 subunit surface expression by a 4. 1N- linked actin cytoskeletal association. *J Neurosci* **20**, 7932-40.

Sheng, M. & Lee, S. H. 2001 AMPA receptor trafficking and the control of synaptic transmission. *Cell* **105**, 825-8.

Sheng, M. & Sala, C. 2001 PDZ domains and the organization of supramolecular complexes. *Annu Rev Neurosci* **24**, 1-29.

Shi, S., Hayashi, Y., Esteban, J. A. & Malinow, R. 2001a Subunit-specific rules governing ampa receptor trafficking to synapses in hippocampal pyramidal neurons. *Cell* **105**, 331-43.

Shi, S.-H., Hayashi, Y., Esteban, J. A. & Malinow, R. 2001b Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. *Cell* **105**, 331-43.

Shi, S.-H., Hayashi, Y., Petralia, R. S., Zaman, S. H., Wenthold, R. J., Svoboda, K. & Malinow, R. 1999 Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation [see comments]. *Science* **284**, 1811-6.

Shi, S.-H. & Malinow, R. 2001 Synaptic trafficking of AMPA-Rs containing GluR1 is gated by PKA phosphorylation at Ser845. In *Society for Neuroscience Annual Meeting*. San Diego.

Song, I., Kamboj, S., Xia, J., Dong, H., Liao, D. & Huganir, R. L. 1998 Interaction of the N-ethylmaleimide-sensitive factor with AMPA receptors [see comments]. *Neuron* **21**, 393-400.

Srivastava, S., Osten, P., Vilim, F. S., Khatri, L., Inman, G., States, B., Daly, C., DeSouza, S., Abagyan, R., Valtschanoff, J. G., Weinberg, R. J. & Ziff, E. B. 1998 Novel anchorage of GluR2/3 to the postsynaptic density by the AMPA receptor-binding protein ABP. *Neuron* **21**, 581-91.

Stern, E. A., Maravall, M. & Svoboda, K. 2001 Rapid development and plasticity of layer 2/3 maps in rat barrel cortex in vivo. *Neuron* **31**, 305-15.

Stevens, C. F. & Wang, Y. 1994 Changes in reliability of synaptic function as a mechanism for plasticity [see comments]. *Nature* **371**, 704-7.

Takumi, Y., Ramírez-León, V., Laake, P., Rinvik, E. & Ottersen, O. P. 1999 Different modes of expression of AMPA and NMDA receptors in hippocampal synapses. *Nat Neurosci* **2**, 618-24.

Tomita, S., Nicoll, R. A. & Bredt, D. S. 2001 PDZ protein interactions regulating glutamate receptor function and plasticity. *J Cell Biol* **153**, F19-24.

Vees, A. M., Micheva, K. D., Beaulieu, C. & Descarries, L. 1998 Increased number and size of dendritic spines in ipsilateral barrel field cortex following unilateral whisker trimming in postnatal rat. *J Comp Neurol* **400**, 110-24.

Wenthold, R. J., Petralia, R. S., Blahos, J., II & Niedzielski, A. S. 1996 Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. *Journal of Neuroscience* **16**, 1982-9.

Winfield, D. A. 1981 The postnatal development of synapses in the visual cortex of the cat and the effects of eyelid closure. *Brain Res* **206**, 166-71.

Wisden, W. & Seeburg, P. H. 1993 Mammalian ionotropic glutamate receptors. *Current Opinion in Neurobiology* **3**, 291-8.

Wu, G., Malinow, R. & Cline, H. T. 1996 Maturation of a central glutamatergic synapse. *Science* **274**, 972-6.

Xia, J., Zhang, X., Staudinger, J. & Huganir, R. L. 1999 Clustering of AMPA receptors by the synaptic PDZ domain-containing protein PICK1. *Neuron* **22**, 179-87.

Zamanillo, D., Sprengel, R., Hvalby, O., Jensen, V., Burnashev, N., Rozov, A., Kaiser, K. M., Köster, H. J., Borchardt, T., Worley, P., Lübke, J., Frotscher, M., Kelly, P. H., Sommer, B., Andersen, P., Seeburg, P. H. & Sakmann, B. 1999 Importance of AMPA receptors for hippocampal synaptic plasticity but not for spatial learning [see comments]. *Science* **284**, 1805-11.

Zhou, Q., Xiao, M. & Nicoll, R. A. 2001 Contribution of cytoskeleton to the internalization of AMPA receptors. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 1261-6.

Zhu, J. J., Esteban, J. A., Hayashi, Y. & Malinow, R. 2000a Postnatal synaptic potentiation: delivery of GluR4-containing AMPA receptors by spontaneous activity. *Nat Neurosci* **3**, 1098-106.

Zhu, J. J., Esteban, J. A., Hayashi, Y. & Malinow, R. 2000b Synaptic potentiation during early development: Delivery of GluR4-containing AMPA receptors by spontaneous activity. *Nature Neuroscience* **3**.

Zhu, J. J., Qin, Y., Zhao, M., Van Aelst, L. & Malinow, R. 2002 Ras and Rap control AMPA receptor trafficking during synaptic plasticity. *Cell* **110**, 443-55.

Zoghbi, H. Y., Gage, F. H. & Choi, D. W. 2000 Neurobiology of disease. *Curr Opin Neurobiol* **10**, 655-60.