LTP mechanisms: from silence to four-lane traffic

Roberto Malinow*, Zachary F Mainen† and Yasunori Hayashi‡

Brief periods of strong neuronal activity induce long-lasting changes in synaptic function. This synaptic plasticity is thought to play important roles in learning and memory. One example – long-term potentation in the CA1 region of the hippocampus – has been studied extensively, and conflicting views regarding the underlying mechanisms have emerged. Recent findings, regarding basic properties of synaptic transmission, appear to reconcile these diverging views.

Addresses
Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA
*e-mail: malinow@cshl.org
†e-mail: mainen@cshl.org
‡e-mail: hayashi@cshl.org

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Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole proprionate
DLG	discs large
GFP	green fluorescent protein
GluR1	glutamatergic AMPA-receptor subunit 1
LTD	long-term depression
LTP	long-term potentiation
NMDA	*N*-methyl-D-aspartate
NSF	*N*-ethylmaleimide-sensitive factor
PDZ domain	PSD95, DLG, ZO-1 domain
PSD95	postsynaptic density protein of 95 kDa
ZO-1	zona occludens 1

Introduction

There has been much interest in delineating the mechanisms underlying long-term potentiation (LTP). A detailed understanding of LTP may allow us to determine its roles in learning and memory and developmental plasticity. Mechanistic studies over the last three decades have produced contradictory models regarding the synaptic sites that undergo modification during LTP. However, work over the last few years has led to a model that includes delivery of receptors to functional 'silent' synapses. This model can account for most results in this field. Here, we review the evidence supporting this model and extend the model to include more recent results on receptor trafficking.

Why study long-term potentiation?

For almost 30 years there has been considerable effort by a number of laboratories to determine if the modification underlying long-term potentiation (LTP) takes place pre- or postsynaptically (reviewed in [1–3]). This issue is important for several reasons. First, once the site of the modification that is responsible for LTP is found, it should be easier to identify the relevant cellular and molecular machinery. Such identification could facilitate determining the relationship between LTP and memory formation. For instance, if it becomes possible to detect the molecular signature of LTP, this could reveal which neural circuits in the brain undergo changes during particular experiences in a living animal. Second, once the molecular modifications are elucidated, it will become possible to identify the regulatory molecular pathways that could play modulatory roles in the acquisition or storage of memories. Third, the site of modification may have an impact on how information is transmitted by neural signals. For instance, postsynaptic modifications that merely increase the strength of potential synapses by a fixed scale factor may actually decrease the signal-to-noise ratio of information transfer [4]. On the other hand, presynaptic modifications are likely to interact with short-term forms of presynaptic plasticity, which may be important in dynamic mechanisms such as gain control [5]. Finally, once the signaling pathways that produce activity-induced synaptic plasticity are identified, the relation between diseases that affect cognitive and mnemonic function and the molecular mechanisms of LTP can be investigated on a stronger biological basis. For instance, one may be able to identify, and perhaps correct, aberrations in such signaling once the detailed mechanisms of plasticity are understood. For these (and probably many more) reasons, establishing which parts of synapses undergo modifications during LTP is likely to be an important step toward understanding the neural basis of learning and memory as well as cognitive function in general.

Mechanistic studies

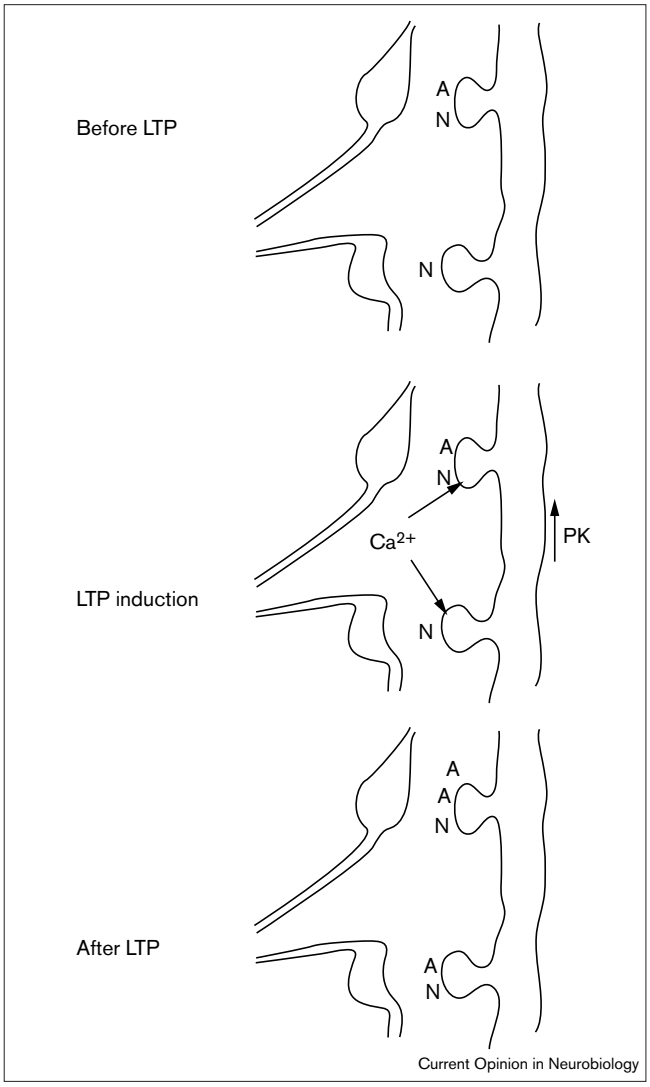
The initial steps that trigger LTP have long been agreed on. Evoking synaptic transmission at a low frequency activates primarily AMPA-type glutamate receptors and leads to a small rise in intracellular calcium concentration. Intense synaptic activity that triggers LTP activates NMDA receptors and produces a significant rise in the postsynaptic calcium concentration. Events downstream of this are progressively less well understood. The activation of the calcium/calmodulin-dependent protein kinase II (and probably other kinases) is known to be important, but the critical substrates of these kinases are still unknown. Eventually, the initial induction cascade must be transformed into a persistent molecular modification of the synapse. This forms the basis for the most problematic issues regarding LTP: first, the localization of the ultimate synaptic modifications that support enhanced transmission; and second, the understanding of the mechanisms by which these modifications are made persistent. To address these issues, investigators have used increasingly sophisticated methodologies. These have led to a greater understanding of synaptic transmission in the central nervous system (CNS) and to converging views on the locus of modification during LTP.

Silent synapses: biophysical basis

A postsynaptic mechanism for LTP was proposed a decade ago, based on two observations: first, LTP selectively

increases the postsynaptic current that is attributable to AMPA receptor channels [6,7]; second, LTP can increase the sensitivity to exogenously delivered AMPA [8]. This view was seriously challenged a few years later by studies showing that the frequency of failures in synaptic transmission (a classic measure of presynaptic function) changes during LTP [9–15]. This presynaptic challenge, however, depended critically on a long-standing assumption regarding synaptic transmission: that the failure to record a postsynaptic response to presynaptic stimulation is attributable to a failure in the release of neurotransmitter [16,17]. At glutamatergic synapses, this important assumption appears not to be the case. Several laboratories have provided evidence that synaptic failures can be caused by postsynaptic mechanisms [18–25]. Specifically, transmitter release that acts only on NMDA receptors will be recorded as a failure if the postsynaptic membrane is at resting levels. This is because the NMDA receptor channel is blocked at resting potentials by extracellular Mg^{2+} [26]. Thus, synaptic failures are not necessarily due to a failure of transmitter release. Post synaptic responses that are mediated solely by activation of NMDA receptors (that do not produce a response when the cell is at resting potential) have been termed 'silent synapses'. Silent synapses have now been documented in virtually every glutamatergic synapse where NMDA-receptor dependent LTP occurs, including hippocampal area CA1 [18,21,22], the hippocampal dentate gyrus [25], the somatosensory cortex [27], the visual cortex [24], and the spinal cord [23], as well as in frog optic tectum [19]. The biophysical basis of pure NMDA responses appears to be primarily due to the existence of synapses with NMDA but not AMPA receptors, as indicated by immuno-gold electron microscopic studies from intact rat hippocampus [28•–30•], as well as immuno-fluorescent studies from dissociated neurons [31•–33•]. Other mechanisms, such as the delivery into the synaptic cleft of low concentrations of transmitter, may also produce pure NMDA responses [34,35], although the role of this process in LTP is not established.

Figure 1



LTP model that accounts for physiological observations. Before LTP, some synapses contain AMPA (A) and NMDA (N) receptors. During LTP induction, a Ca^{2+} influx leads to activation of protein kinase(s) (PK). This leads to the delivery of AMPA receptors to both synapses.

Silent synapses: role in LTP

The discovery and general acceptance of silent synapses has had two important consequences for LTP research. The first of these consequences is that their presence provides critical evidence for a purely postsynaptic mechanism for LTP that can explain a wide range of previous physiological observations [3,36]. In this model (see Figure 1), LTP results from the delivery of functional AMPA receptors to synapses from non-synaptic sites. These sites could be either intracellular, or on nearby extrasynaptic membrane. Receptor addition at synapses already containing AMPA receptors can explain increases in quantal size [10,12,15,37]. AMPA receptor addition at previously silent synapses after LTP can explain decreases in the frequency of transmission failures: silent synapses are now functional at resting potential because they now possess AMPA receptors. Indeed, an increase in AMPA-receptor function at silent synapses has been seen following LTP-inducing stimuli in area CA1, as well as in other excitatory synapses [18,19,21–25,27]. Direct visualization of this process has been achieved by investigation of green fluorescent protein (GFP)-tagged AMPA receptors [38•]. Receptor addition is also compatible with independent lines of evidence for postsynaptic modifications. For example, synaptic AMPA receptors show an increased conductance [39] and increased phosphorylation [40] at a site that can increase their conductance [41]. These effects could be seen if new receptors with increased conductances are added to synapses during LTP. Thus, AMPA receptor addition stands as a simple account of a large and diverse set of physiological data concerning the locus of LTP expression. Furthermore, several independent groups, using synaptic [42,43] or perisynaptic [44,45] detectors of synaptic transmitter release,

have found no increase in transmitter release following LTP. This argues against presynaptic modifications, at least during the early stages following LTP induction in CA1 hippocampus. Some observations in dissociated cell culture [46,47] are not consistent with a purely postsynaptic mechanism; this may reflect the possibility that potentiation in such preparations differs from LTP in more intact systems. Some results in slice preparations are also not supportive of postsynaptic modifications [13,14]; however, these results are not universally obtained [10,12,15,18,21,22,48].

Focus on AMPA receptors

The second, and arguably more significant, fallout of the exciting discovery of silent synapses is that it has moved the field of LTP research beyond the general pre- versus postsynaptic dichotomy and focused attention on a much more specific molecular question: how are functional AMPA receptors added to synapses? Our understanding of the cellular and molecular mechanisms controlling the organization of these receptors is rapidly expanding. The AMPA receptor is a heteromeric complex made up of variable combinations of four subunits, GluR1 to GluR4. Each complex contains four or five such subunits (reviewed in [49]). In adult hippocampal excitatory neurons, most AMPA receptors are composed of GluR1–GluR2 complexes, or GluR3–GluR2 complexes [50]. The trafficking and stabilization of AMPA receptors in synapses seems to be controlled, at least in part, through interactions between the AMPA receptors' intracellular carboxy (C)-tails and a variety of cytosolic proteins. Many of these proteins contain PDZ-domains — structures known to interact with the carboxyl-terminus of various transmembrane proteins — and probably form a functional scaffolding complex. Some of the sites on AMPA receptor subunits that are responsible for interactions with specific PDZ-proteins have been characterized [51,52], but many potential sites remain to be identified. The interactions that have been characterized are subunit specific: GluR2 interacts with the proteins GRIP (glutamate-receptor-interacting protein), NSF (*N*-ethylmaleimide-sensitive factor), PICK1 (protein interacting with C kinase 1), and ABP (AMPA-receptor-binding protein) [53,54•,55•,56,57•,58,59], while GluR1 is, as yet, only known to interact with SAP97 (synapse-associated protein 97) [60]. It is possible that the associations between GluR subunits and the various GluR-interacting proteins will be largely subunit-specific, since GluR1 and GluR2 have very different carboxy-tails. The GluR1 carboxy-tail is long and similar to that of GluR4, whereas the GluR2 carboxy-tail is short and resembles that of GluR3. Future work will probably expand the catalog of GluR-interacting molecules and determine which interactions are shared and which are unique to particular subunits.

AMPA receptor traffic

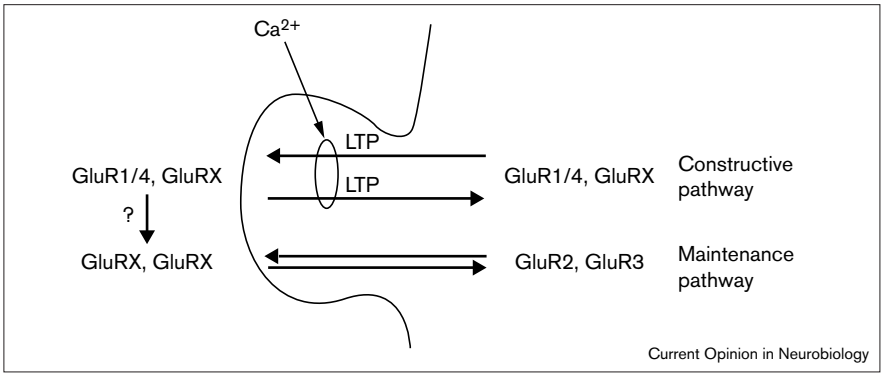
How do the molecular interactions between AMPA receptors and their associated proteins regulate synaptic plasticity and stability? Our current understanding is still murky, but a number of recent studies provide interesting clues. NSF is a protein involved in the presynaptic fusion machinery that is also present in postsynaptic sites and interacts with GluR2 [54•,55•,57•,61•]. Expression of a peptide that interferes with the interaction of NSF and GluR2 causes a decrease in surface AMPA receptor density [62•]. Injection of the same peptide in neurons causes a rapid 30–50% depression of transmission [54•,55•,61•–63•], suggesting the possibility of a remarkably rapid turnover of the pool of receptors. This action of the peptides is consistent with the depressed transmission seen in mice lacking GluR2 (Z Mainen, R Malinow, unpublished data). In addition to depressing transmission, these peptides also prevent the induction of some forms of long-term depression (LTD) [61•,63•]. However, the effect on LTD may be through actions other than the prevention of NSF–GluR2 interactions, as mice lacking GluR2 show LTD (as well as LTP) [64]. In contrast to GluR2 knockout animals, mice lacking GluR1 have relatively normal transmission but lack LTP ([65•]; LTD has not been examined).

Trafficking model for plasticity

The dichotomy between the properties of GluR1/4 and GluR2/3 subunits, with respect to carboxy-tails, interaction proteins, and apparent roles in basal transmission and plasticity, leads us to suggest a model for subunit-specific trafficking of AMPA receptors that can account for a wide range of experimental observations regarding LTP. The basic principle of our model is that two distinct regulatory mechanisms govern the local insertion and removal of AMPA receptors from the synapse: a constructive pathway and a maintenance pathway (Figure 2). The constructive pathway is inactive during periods in which no plasticity is occuring and is rapidly and transiently turned on by activity. The maintenance pathway is always on and is responsible for the constant turnover of receptors. The constructive pathway is thus responsible for the formation of memories, whereas the maintenance pathway is responsible for their persistence.

We posit that the activity-dependent constructive pathway sets new levels of AMPA receptor numbers at synapses through receptor insertion (LTP). This process is driven by transient events localized to one or a few synapses (such as a rise in intracellular Ca^{2+} concentration in spines) that change the number of receptors at those synapses, thereby storing new information. A destructive mechanism for receptor removal (LTD) operates to reverse the insertion process, also modifying information. The maintenance pathway, in contrast, simply replaces existing postsynaptic receptors in a one-for-one manner with receptors from a reserve pool (either newly synthesized or recycled). This process does not increase or decrease the number of synaptic receptors, but rather maintains the existing structure (and information). Thus, synapses lacking AMPA receptors (silent synapses) stay that way in the absence of activity. The exchange of a new receptor for an old might be accomplished in a single biochemical reaction. Alternatively, there may be receptor 'slots', also delivered

Figure 2



A model showing two pathways for AMPA receptor synaptic delivery. Receptors containing a GluR1 or a GluR4 subunit plus any other subunit (GluR1/4,GluRX) are retained in the dendrite and require a calcium stimulus for delivery to or removal from the postsynaptic membrane. Receptors with GluR2 and 3 (GluR2,GluR3) are constitutively exchanged for synaptic receptors containing any subunit composition (GluRX,GluRX) that is made available to the maintenance pathway. While GluR2,GluR3 receptors are always in the maintenance pathway, GluRX synaptic GluR1/4,GluRX receptors enter the maintenance pathway through special signaling (**?**). Replacement of GluR1/4,GluRX with a permanently recycling GluR2,GluR3 allows for persistence of increased numbers of synaptic AMPA receptors. This model can preserve transient (regulated) changes in synaptic receptor number despite protein turnover.

by the constructive pathway, that are temporarily opened when an old receptor is removed and filled when the new receptor is added by the maintenance process.

We postulate that the AMPA-receptor subunit composition dictates the availability of receptors for delivery to or removal from synapses through the constructive or the maintenance pathways (Figure 2). Receptors containing a GluR1 subunit depend on the constructive pathway for delivery. (Once inserted, they can also be removed by activity that triggers LTD.) A number of results are consistent with a role for GluR1 in activity-dependent plasticity. GluR1 knockout mice lack LTP [65•]. Synaptic delivery of recombinant GluR1 subunits can be detected optically [38•] or electrophysiologically (Hayashi *et al*., unpublished data) after LTP, and mutations in their carboxy-tails that block interactions with PDZ-domain proteins prevent this delivery (Hayashi *et al*., unpublished data). LTD removes GluR1-containing receptors from synapses [33•]. We predict that mice lacking GluR1 will not demonstrate LTD. Meanwhile, in this model, receptors lacking GluR1 (containing GluR2 and GluR3) are not eligible for insertion and removal through LTP and LTD. Instead, they are trafficked through the maintenance pathway. A potential mechanism for the differential handling of receptors is that GluR1 subunits have a retention signal (preventing insertion into the synapse) that is relieved by activity, while GluR2 subunits have a permissive delivery signal allowing them to be delivered into the postsynaptic membrane. Indeed, GluR1–GFP is restricted from entering spines until LTP-inducing stimuli are delivered [38•] (Hayashi *et al*., unpublished data). We would predict that GluR2–GFP would enter spines in the absence of activity (via the maintenance pathway). Peptides blocking the necessary GluR2–NSF interactions would block delivery in this pathway; removal of synaptic GluR2/GluR3 receptors could proceed and depress transmission [54•,55•,61•].

Because GluR4 contains a carboxy-tail similar to that of the GluR1 subunit, we expect that receptors containing GluR4 act like GluR1-containing receptors with respect to trafficking. Differences between GluR1 and GluR4 may include the pattern and time course of expression throughout development, and perhaps the specific activity requirements for mobilization. Under some conditions, GluR4-mediated plasticity may substitute for that mediated by GluR1, potentially explaining why mice lacking GluR1 show no deficit in learning [65•]. We would likewise expect GluR3 to be handled similarly to GluR2, given the similarity of the intracellular domains of these receptors.

In the proposed model, the interaction of constructive and maintenance receptor trafficking pathways forms the key foundation for a persistent and self-maintaining mechanism for synaptic modifications. Once synaptic GluR1-containing receptors are replaced with GluR1-lacking receptors, they are continually renewed by the maintenance pathway and are not subject to decay or removal (LTD). In other words, the memory sticks: an increase in synaptic receptor number produced by a transient event can outlast protein turnover. This view can help explain the observation that LTP is less sensitive to depression by LTD-inducing stimuli with time [66]. This model predicts that the GluR2 knockout mouse, lacking constitutive replacement, would lack long-lasting LTP despite robust initial potentiation. We expect that the availability of GluR1-containing synaptic receptors to the maintenance pathway is under strict control, potentially by kinase activity, transient synthesis of specific proteins, or other mechanisms. This step probably requires special input signals (e.g. spaced training protocols [67]).

Conclusion

In the future, we expect that there will be much work examining in detail how signal transduction mechanisms

interact with the machinery that regulates the trafficking of AMPA receptors and their associated proteins. This should lead to an elucidation of the roadways used to achieve 'AMPA-fication', and ultimately a satisfactory molecular understanding of LTP.

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The work referred to in the text as (Hayashi *et al.*, unpublished data) has now been published: Hayashi Y, Shi S-H, Esteban JA, Piccini A, Poncer J-C, Malinow R: **Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction.** *Science* 2000, **287**:2262-2267.