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Expression mechanisms underlying long-term potentiation: a postsynaptic view

Roger A. Nicoll

Departments of Cellular and Molecular Pharmacology and Physiology, University of California San Francisco, San Francisco, CA 94143, USA (nicoll@cmp.ucsf.edu)

This review summarizes the various experiments that have been carried out to determine if the expression of long-term potentiation (LTP), in particular *N*-methyl-D-aspartate (NMDA) receptor-dependent LTP, is presynaptic or postsynaptic. Evidence for a presynaptic expression mechanism comes primarily from experiments reporting that glutamate overflow is increased during LTP and from experiments showing that the failure rate decreases during LTP. However, other experimental approaches, such as monitoring synaptic glutamate release by recording astrocytic glutamate transporter currents, have failed to detect any change in glutamate release during LTP. In addition, the discovery of silent synapses, in which LTP rapidly switches on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor function at NMDA-receptor-only synapses, provides a postsynaptic mechanism for the decrease in failures during LTP. It is argued that the preponderance of evidence favours a postsynaptic expression mechanism, whereby NMDA receptor activation results in the rapid recruitment of AMPA receptors as well as a covalent modification of synaptic AMPA receptors.

Keywords: long-term potentiation; plasticity; hippocampus; postsynaptic density; AMPA receptor; NMDA receptor

1. INTRODUCTION

It is remarkable that the debate over whether LTP is expressed presynaptically or postsynaptically persists after *ca*. 20 years. Such a protracted battle has indicated to some that a solution may never be found (Sanes & Lichtman 1999). While it is difficult not to get frustrated over the seemingly intractable nature of the problem, I believe that progress has been made and, perhaps more importantly, that the debate has stimulated a great deal of research into the fundamental mechanisms controlling transmitter release and postsynaptic responsiveness. This paper covers:

(i) the origins of the controversy;
(ii) the various types of experiments that have been designed to address the issue of whether the expression of LTP is pre- or postsynaptic;
(iii) the evidence for silent synapses; and
(iv) the mechanisms involved in the control of synaptic AMPA receptor trafficking.

2. SOME CAVEATS

It has been proposed that some of the confusion exists because LTP at different types of synapses might differ mechanistically. This is certainly the case for LTP at hippocampal mossy fibre synapses, which, unlike LTP at most other synapses, is independent of NMDA receptor activation (Nicoll & Malenka 1995). It also remains possible that differences exist for NMDA receptor-dependent LTP at different classes of synapse. In addition, there is considerable evidence that LTP at the same set of synapses may have different properties at different timepoints following induction. While both of these points are valid, even if observations are limited to LTP at CA1 hippocampal excitatory synapses, where much of the research has been carried out, and limited to the first hour following induction, much of the controversy remains. Thus, this review will primarily focus on studies performed in the CA1 region of the hippocampal slice and on the first hour following induction. Anyone who has studied LTP knows that the magnitude varies considerably from slice to slice, despite apparent control of all established variables. Thus, it is clear that we still have limited knowledge of this phenomenon, which opens the possibility that the precise conditions influence not only the magnitude of LTP but also perhaps some of the underlying mechanisms.

3. ORIGINS OF THE DEBATE

The mid-1980s witnessed a great outpouring of papers addressing the induction of LTP. These include the demonstration that:

(i) NMDA-receptor activation is required (Collingridge *et al.* 1983);
(ii) a rise in postsynaptic calcium is required (Lynch *et al.* 1983); and
(iii) depolarization of the postsynaptic cell is required (Malinow & Miller 1986; Wigstrom *et al.* 1986).

One contribution of 30 to a Theme Issue 'Long-term potentiation: enhancing neuroscience for 30 years'.

In addition, it was found that Mg^{2+} causes a voltage-dependent block of the NMDA receptor (Nowak *et al.* 1984), and the NMDA receptor is highly permeable to Ca^{2+} (MacDermott *et al.* 1986; Jahr & Stevens 1987; Ascher & Nowak 1988). Based on these observations a simple postsynaptic model for the induction of LTP emerged, in which activation of NMDA receptors, coupled with strong postsynaptic depolarization, causes a large rise in Ca^{2+} in the postsynaptic spine. The rise in Ca^{2+} initiates a series of steps that ultimately results in a persistent enhancement in synaptic transmission. This model is universally agreed upon. It is the site at which the persistent change resides that has been so difficult to resolve.

Shortly before the elucidation of the induction mechanism, Dolphin *et al.* (1982) measured extracellular glutamate *in vivo* from the dentate gyrus and reported that LTP is associated with an increase in glutamate overflow. Given that the induction is clearly postsynaptic this finding required the involvement of a retrograde messenger, which remains to be identified. A provocative and, in retrospect, prescient proposal advanced by Lynch & Baudry (1984) was that the expression of LTP is due to the 'insertion of glutamate receptors in the postsynaptic membranes'.

One of the first physiological studies (McNaughton 1982) to address the issue of whether the expression of LTP is pre- or postsynaptic examined PPF, in which the response to the second of two closely spaced stimuli is enhanced. This is a presynaptic phenomenon and manipulations that alter presynaptic release probability invariably alter PPF. As LTP of perforant path synapses was not associated with a change in PPF, a postsynaptic expression mechanism was proposed (McNaughton 1982). This finding has subsequently been confirmed in numerous studies (e.g. Manabe *et al.* 1993; Asztely *et al.* 1996), but also challenged (e.g. Schulz *et al.* 1994). The inability to reach a strong consensus on whether LTP is associated with a change in PPF is emblematic of the problem associated with virtually all observations made concerning the expression of LTP.

Another early physiological experiment examined the relative effects of LTP on the AMPA and NMDA components of the EPSC. The logic was quite simple: the NMDA receptors would serve as a separate bioassay for measuring the amount of glutamate release. If only the AMPA component changed this would be strong evidence for a selective postsynaptic modification, whereas if both changed to the same degree, this would be more consistent with a presynaptic modification. LTP was associated primarily with an enhancement in the AMPA component (Kauer *et al.* 1988; Muller *et al.* 1988; Perkel & Nicoll 1993). This finding was quickly challenged (e.g. Bashir *et al.* 1991) and a great deal has been published subsequently about this. However, recent studies have emphasized the selectivity in the enhancement of the AMPA component (e.g. Liao *et al.* 1995; Durand *et al.* 1996; Choi *et al.* 2000; Montgomery *et al.* 2001).

Within 2 years of the initial experiments that examined the effect of LTP on the NMDA component, two papers, one by Malinow & Tsien (1990) and the other by Bekkers & Stevens (1990), appeared concluding that LTP is expressed presynaptically. These authors found that LTP was associated with a decrease in the coefficient of variation and in the failure rate. Based on classical work at the neuromuscular junction, such changes were very powerful evidence for a presynaptic expression mechanism. Both the change in the coefficient of variation (Manabe *et al.* 1993) and the change in failure rate (Kullmann & Nicoll 1992) associated with LTP were readily confirmed. Indeed, there is little debate concerning these two observations and it is fair to say that these two papers swayed public opinion overwhelmingly to accepting a presynaptic expression mechanism.

4. EXPERIMENTS DESIGNED TO TEST WHETHER LTP IS ASSOCIATED WITH AN INCREASE IN TRANSMITTER RELEASE

However, a number of observations did not fit comfortably with a presynaptic expression mechanism. These experiments fell into two broad categories: those designed to test whether release increases during LTP and those designed to test whether the sensitivity and/or number of AMPA receptors increases during LTP. I begin by discussing the first category.

(i) The PPF data discussed in § 3 are not consistent with an increase in the probability of transmitter release, although it should be noted that interactions between release probability and LTP have been reported (Schulz 1997).

(ii) If LTP were due to an increase in the probability of transmitter release, then it should be possible to occlude LTP by maximally increasing the probability of release. This has been accomplished by applying the K^+ channel blocker 4-AP, which markedly enhances transmitter release by broadening the presynaptic action potential (Muller & Lynch 1989; Hjelmstad *et al.* 1997). In the presence of 4-AP other manipulations that enhance the probability of release, such as elevating Ca^{2+}, fail to further increase transmission, confirming that the probability of release is close to saturation. However, the magnitude of LTP evoked under these conditions is no different from that evoked under normal conditions (Hjelmstad *et al.* 1997). A tenfold change in transmitter release induced by blocking or activating presynaptic adenosine receptors also failed to alter LTP (Asztely *et al.* 1994).

(iii) The rate at which use-dependent antagonists block glutamate receptors can be used to measure the probability of glutamate release. The rate of receptor blockade during synaptic stimulation is directly related to the probability of transmitter release. Two types of experiments have been carried out. First, the irreversible NMDA receptor antagonist MK-801 has been used (Manabe & Nicoll 1994). The rate of block of NMDA EPSCs, upon repeated stimulation, was simultaneously compared in the same cell for control synapses and synapses expressing LTP. No difference was observed. Mimicking the change in synaptic strength seen with LTP by increasing the probability of transmitter release (e.g. PPF), demonstrated that this MK-801 assay had the necessary sensitivity to detect a change in release, if it had occurred. However, these results were later challenged and a presyn-

aptic mechanism proposed (Kullmann *et al.* 1996). Second, a similar set of experiments was carried out using polyamine compounds in the GluR2-lacking mouse (Mainen *et al.* 1998). GluR2-lacking AMPA receptors are reversibly blocked in a use-dependent manner by polyamine compounds. LTP had no effect on the rate of block, implying that no change in glutamate release occurred during LTP.

(iv) Astrocytic glutamate transporter currents have been used to monitor synaptically released glutamate during LTP (Diamond *et al.* 1998; Luscher *et al.* 1998). Astrocytes surround excitatory synapses, and Bergles & Jahr (1997) convincingly showed that astrocytic glutamate transporter currents are remarkably sensitive to synaptically released glutamate. Changing the probability of release or the number of activated synapses demonstrated that glial transporter currents have the necessary sensitivity to detect a change in release if occurring during LTP. However, LTP produced no change in the currents (Diamond *et al.* 1998; Luscher *et al.* 1998).

(v) FM1-43 has been used to examine directly the effects of LTP on presynaptic vesicle recycling (Zakharenko *et al.* 2001). The fluorescent marker FM1-43 reversibly binds to membranes and is taken up into endocytosed vesicles. After loading the vesicle pool and washing away the extracellular FM1-43, the probability of vesicle release can be assayed by measuring the rate of destaining of synaptic boutons during presynaptic stimulation. Standard NMDA receptor-dependent LTP (50 or 100 Hz tetanus) was not associated with any change in the rate of destaining and yet control experiments demonstrated that this technique had the necessary sensitivity to detect a change if it had occurred during LTP. This finding argues strongly that there is no presynaptic increase in release probability during NMDA receptor-dependent LTP. In this same paper the authors did find a change in the destaining curve for another form of LTP that is induced by 200 Hz stimulation and depends on both NMDA receptors and L-type Ca^{2+} channels.

Taken as a whole, the body of evidence reviewed here is very difficult to reconcile with a presynaptic expression mechanism. However, it must be acknowledged that there is not unanimous agreement among the experimental findings or in their interpretation. In addition, it can be argued that this conclusion is based on negative results, which typically are not as convincing as positive results. Nevertheless, in all cases the necessary controls were conducted to calibrate the sensitivity of the particular assay and thus ensure that the required sensitivity was present.

5. EXPERIMENTS DESIGNED TO TEST WHETHER LTP IS ASSOCIATED WITH AN INCREASE IN THE SENSITIVITY AND/OR NUMBER OF AMPA RECEPTORS

I now turn to experiments designed to test whether the sensitivity and/or number of AMPA receptors increase during LTP.

(i) An early experiment reported a delayed increase in the response to iontophoretically applied AMPA following the induction of LTP (Davies *et al.* 1989). This is a rather surprising result, given the large area of tissue that would be exposed to the 20 s application of AMPA used in most of this study compared with the very small number of synapses that would undergo LTP. Nevertheless, if we accept this finding, it not only establishes a delayed postsynaptic contribution to LTP, but also rules out a postsynaptic contribution early in LTP, because the late change establishes that the necessary sensitivity is present in this experiment to detect an early postsynaptic change. More recently, a rapid increase in the size of responses to exogenous AMPA has been reported (Montgomery *et al.* 2001) during LTP. Another approach to overcome the small fraction of synapses expressing LTP is to load pyramidal cells with a constitutively active CaMKII. A great deal of evidence exists to indicate that activation of CaMKII is sufficient for LTP (Soderling & Derkach 2000). By loading pyramidal cells with a constitutively active form of CaMKII (Lledo *et al.* 1995) all synapses would be exposed to the loaded CaMKII and potentiated. CaMKII not only enhanced EPSCs, as expected, but also enhanced the responses to iontophoretically applied AMPA. Furthermore, the enhancement of AMPA EPSCs occluded with LTP and CaMKII no longer enhanced synaptic transmission at synapses already expressing LTP.

(ii) Experiments have used quantitative autoradiography to measure the binding properties of AMPA and NMDA receptors in the dentate gyrus after inducing LTP in the perforant path *in vivo* (Maren *et al.* 1993). A selective increase in AMPA binding was observed and this was due to an increase in the number of AMPA binding sites. It is unclear if this increase in binding reflects AMPA receptor trafficking, assembly or synthesis.

(iii) Experiments have been designed to examine the effect of LTP on mEPSCs (Manabe *et al.* 1992; Oliet *et al.* 1996). Studies at the neuromuscular junction have shown that a change in size of miniature synaptic responses is due to a change in postsynaptic responsiveness. Analysis of quantal events in central nervous system neurons is complicated by the fact that an individual neuron receives thousands of synapses, whereas LTP is induced in only a tiny fraction of synapses. To circumvent this problem, extracellular Ca^{2+} was replaced with Sr^{2+}, which causes the asynchronous release of quanta only from activated synapses. Under these conditions a clear increase in size of mEPSCs was recorded (Oliet *et al.* 1996). An increase in apparent frequency was also observed which could be due to:

(1) an increase in the detection of events that were previously below threshold;
(2) the turning on of silent synapses; or
(3) an increase in the probability of transmitter release.

(iv) Another method for estimating quantal size relies on recording synaptic responses to minimal stimulation,

in which a single or a few presynaptic fibres are activated. Responses to such stimulation are composed of a mixture of failures and responses. If one removes the failures and averages together the trials in which a response is evoked, the average quantal size can be estimated, assuming that only a single synapse is being recorded. The average size of the successes is also referred to as potency (Stevens & Wang 1994). Isaac *et al.* (1996) and Stricker *et al.* (1996) have found that LTP is invariably associated with an increase in potency and this can occur in the complete absence of a change in failure rate. However, this finding contrasts with two other studies in which no change in potency was associated with LTP (Stevens & Wang 1994; Bolshakov & Siegelbaum 1995). The basis for this difference is unclear, but resolution of this disagreement is of critical importance, because a lack of change in potency is incompatible with a postsynaptic contribution to LTP.

(v) Nonstationary noise analysis has been used to estimate the single-channel conductance of AMPA receptors before and after LTP (Benke *et al.* 1998). LTP was often associated with an increase in single-channel conductance, indicating and postsynaptic change in AMPA receptor function during LTP.

(vi) Mice in which the GluRA/GluR1 AMPA receptor subunit has been deleted exhibit a striking deficit in LTP in the CA1 region (Zamanillo *et al.* 1999). This finding strongly implicates the AMPA receptor in the expression of LTP.

6. EVIDENCE FOR SILENT SYNAPSES

The results summarized in § 5 turned the spotlight directly on the change in synaptic failures; a unanimously agreed upon result and the single most compelling finding for a presynaptic expression mechanism. Might the change in synaptic failures have a postsynaptic explanation? A comparison of the coefficient of variation of the AMPA and NMDA component found that the variation was substantially higher for the AMPA component and that this difference decreased during LTP (Kullmann 1994). To account for these results Kullmann suggested that there might be a population of synapses that lacked functional AMPA receptors, while having the normal complement of NMDA receptors, and that during LTP these synapses would acquire AMPA receptor function. (Kullmann later emphasized the role of glutamate spillover, rather than differences in AMPA receptor composition, to account for these results (Kullmann *et al.* 1996).) A series of experiments were designed to test more directly for the existence of silent synapses (Isaac *et al.* 1995). Indeed, using minimal stimulation techniques a population of synapses was found that contained no detectable AMPA component, but had a normal NMDA component. Furthermore, an LTP-inducing protocol rapidly switched on these silent synapses. Liao *et al.* (1995) obtained virtually identical results. Numerous investigators have confirmed these findings. However, a recent study (Choi *et al.* 2000) indicated that silent synapses are due to the incomplete emptying of synaptic vesicles, so that the concentration of glutamate in the synaptic cleft is insufficient to activate AMPA receptors, but does activate NMDA receptors. LTP would result in more complete vesicle fusion. However, some of the results upon which this interpretation is based have been challenged (Montgomery *et al.* 2001) and such an increase in glutamate release should be detected by astrocytic glutamate transporter currents (see § 4).

7. REGULATION OF SYNAPTIC AMPA RECEPTORS

The demonstration of silent synapses and their rapid upregulation by LTP raised a host of questions. For instance, what is a silent synapse? Are AMPA receptors present at the synapse but functionally inactive? Or are AMPA receptors actually missing from the synapse? To answer these and many related questions on receptor localization and trafficking, a more cellular and molecular biological approach was needed. A number of immunocytochemical studies in neuronal cultures have analysed the distribution of synaptic glutamate receptors. While virtually all excitatory synapses contained NMDA receptors, only a portion of synapses stained for AMPA receptors (Rao & Craig 1997; Gomperts *et al.* 1998; Liao *et al.* 1999). Furthermore, using single cell cultures these anatomical results could be correlated to the presence of NMDA receptor only miniature synaptic currents (Gomperts *et al.* 1998). Immunogold ultrastructural localization studies have found that, by contrast to the uniform synaptic distribution of NMDA receptors, the number of AMPA receptors varies widely and some excitatory synapses appear to lack AMPA receptors (Nusser *et al.* 1998; Petralia *et al.* 1999; Takumi *et al.* 1999).

Much of the recent work on excitatory synapses has focused on the mechanisms involved in the trafficking of synaptic AMPA receptors and the role of activity in this process (Malenka & Nicoll 1999; Braithwaite *et al.* 2000; Scannevin & Huganir 2000; Malinow *et al.* 2000; Sheng 2001; Malinow & Malenka 2002; Barry & Ziff 2002). Perhaps the most elegant experiments have used electrophysiologically tagged AMPA receptors to dissect out the mechanisms involved in the delivery of AMPA receptors to the synapse (Malinow *et al.* 2000; Shi *et al.* 2001). Our own recent studies have concentrated on two synaptic proteins, stargazin and PSD-95, which play important roles in controlling the number of synaptic AMPA receptors. Stargazin, the protein deleted in the mutant mouse stargazer, is essential, not only for the localization of AMPA receptors to the synapse, but also for the surface expression of these receptors (Chen *et al.* 2000). Overexpression of PSD-95 in dissociated neuronal cultures enhances synapse maturation, increasing synaptic size and enhancing AMPA receptor synaptic responses (El-Husseini *et al.* 2000). In slice cultures PSD-95 causes a rapid and selective increase in AMPA EPSCs (Schnell *et al.* 2002). This enhancement is mediated by the direct binding of stargazin to both AMPA receptors and PSD-95 which then rapidly delivers AMPA receptors to the synapse (Schnell *et al.* 2002).

While attention has focused primarily on the trafficking of AMPA receptors during LTP, there is also considerable evidence that covalent modification of AMPA receptors by CaMKII can increase the single channel conductance

of the receptor (Benke *et al.* 1998; Soderling & Derkach 2000).

8. CONCLUSIONS

In this brief review I have summarized the history of the controversy of whether the expression of LTP is pre- or postsynaptic. Although I have tried to be as objective as possible, there is, nevertheless, an unmistakable postsynaptic bias in terms of the data selected for inclusion and in the interpretation of these data. I believe that I speak for many researchers in the field when I say that the study of LTP has been a humbling experience. LTP is vastly more complex than anyone could have imagined. However, I do feel strongly that, fuelled by the LTP debate, our basic understanding of the properties of excitatory synapses has progressed enormously during the past decade and that with this knowledge the detailed molecular mechanisms underlying LTP will inevitably emerge.

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GLOSSARY

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
4-AP: 4-aminopyridine
CaMKII: calcium/calmodulin-dependent protein kinase II
EPSC: excitatory postsynaptic current
LTP: long-term potentiation
mEPSC: miniature excitatory postsynaptic current
NMDA: *N*-methyl-D-aspartate
PPF: paired pulse facilitation