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Authors
Granger, Adam J
Nicoll, Roger A

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Author for correspondence:
Roger A. Nicoll
e-mail: roger.nicoll@ucsf.edu

Expression mechanisms underlying long-term potentiation: a postsynaptic view, 10 years on

Adam J. Granger[1] and Roger A. Nicoll[2]

[1]Neuroscience Graduate Program, and [2]Department of Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, CA 94158, USA

This review focuses on the research that has occurred over the past decade which has solidified a postsynaptic expression mechanism for long-term potentiation (LTP). However, experiments that have suggested a presynaptic component are also summarized. It is argued that the pairing of glutamate uncaging onto single spines with postsynaptic depolarization provides the final and most elegant demonstration of a postsynaptic expression mechanism for NMDA receptor-dependent LTP. The fact that the magnitude of this LTP is similar to that evoked by pairing synaptic stimulation and depolarization leaves little room for a substantial presynaptic component. Finally, recent data also require a revision in our thinking about the way AMPA receptors (AMPARs) are recruited to the postsynaptic density during LTP. This recruitment is independent of subunit type, but does require an adequate reserve pool of extrasynaptic receptors.

1. Introduction

The review for this meeting 10 years ago marvelled at the fact that the debate over whether LTP was expressed presynaptically or postsynaptically had gone on for 20 years [1]. The fact that we are still having this debate is unbelievably frustrating. However, as we discuss, the past 10 years have been very good for those supporting a postsynaptic expression mechanism. In this review, we begin by discussing issues that have contributed to the confusion in the LTP field. This is followed by a discussion addressing evidence for a presynaptic expression mechanism. We then summarize the relevant data that have appeared during the past 10 years supporting postsynaptic expression of LTP. Finally, recent experiments on the trafficking of AMPA receptors (AMPARs) conclude the review.

2. Multiple forms of long-term potentiation

Part of the confusion with LTP is that the field has never settled on a precise definition. The broadest definition is a long-lasting enhancement in synaptic strength following a brief high-frequency stimulation. With such a broad definition, it is clear that mechanistically distinct forms of LTP exist, at least at different types of synapses. The most dramatic example of this is mossy fibre LTP in the CA3 region of the hippocampus compared with LTP at Schaffer collateral synapses in the CA1 region. There is general agreement that mossy fibre LTP is independent of NMDA receptors (NMDARs) and is induced and expressed presynaptically [2,3], whereas LTP in the CA1 region is induced postsynaptically and has a strong component that is expressed postsynaptically [1,4–7]. However, this heterogeneity across different types of synapses does not account for most of the disagreements. Even when we limit the discussion to the same population of synapses, i.e. the Schaffer collateral/CA1 pyramidal cell synapse, it has been argued that multiple mechanistic forms of LTP can exist at the same synapse. Although the criterion for distinguishing among these forms has not been rigorously defined, the variables that have been proposed include the frequency of stimulation, the pattern of stimulation and the

strength of the stimulus. It has further been proposed that the expression mechanism changes during development and during different times after the tetanus. For instance, protein synthesis is thought to be required for the maintenance of LTP at some point (approx. 1 h) following induction [8–10]. Although rarely cited, there are also a number of very well-controlled studies that have found no effect of protein synthesis inhibition for up to 8 h following the tetanus [11,12]. Finally, the fact that well over a 100 proteins have been claimed to be involved in LTP has led some investigators to lament that understanding this phenomenon will remain forever elusive [13].

How does one grapple with such confusion? In a recent review [14] an attempt was made to remove as many layers of complexity as possible while maintaining the core aspects of LTP. First, the unique property of coincidence detection provided by the NMDAR is what makes NMDAR-dependent LTP so appealing as a cellular model for learning and memory. Thus, it makes sense to focus our attention on NMDAR-dependent form(s) of LTP. Second, as synapses made by the Schaffer collateral/CA1 pyramidal cell synapse express the most robust NMDAR-dependent LTP and have been the focus of most of the work on LTP, it makes sense to focus on these synapses specifically.

Another source of confusion is the often overlooked fact that the induction of LTP involves two distinct questions. The first question is what controls the activation of the NMDAR, the second is what happens after the receptor is activated? It is generally accepted that there are only two requirements for the induction of LTP: binding of glutamate to the NMDAR and depolarization of the postsynaptic membrane. The degree of postsynaptic depolarization will depend on a wide variety of variables, including the frequency of stimulation, the pattern of stimulation, the strength of the stimulus, the amount of inhibition, the excitability of the postsynaptic membrane, the probability of transmitter release and many more variables. Any manipulation that alters any of these variables will influence the induction of LTP. While understanding how neuronal circuits control the activation of the NMDAR is of critical importance in understanding the physiological context in which plasticity is engaged, it tells us nothing about the mechanism of LTP expression. To bypass all of these variables, the experimenter needs to take control of the postsynaptic membrane with intracellular Cs^+ to block K^+ channels. This allows the experimenter to hold the cell at a constant membrane potential and induce a minimal 'pairing' protocol to induce LTP: depolarizing the cell to 0 mV while stimulating synapses. With this protocol, there are only two ways that a manipulation can alter LTP. First, it can directly interfere with the NMDAR, in which case we learn nothing about LTP expression mechanisms. Second, the manipulation alters LTP without any effect on the NMDAR. Such a result is of considerable value in terms of probing the downstream mechanisms underlying LTP expression.

3. Evidence from the past 10 years for a presynaptic long-term potentiation expression mechanism

The most significant line of evidence of the past 10 years supporting a presynaptic locus of LTP expression comes from a technique dubbed optical quantal analysis [15–18]. In this technique, neurons are loaded with a Ca^{2+}-sensitive dye and intracellular voltage is monitored by sharp electrodes. This method is designed to overcome the difficulty of monitoring unitary synaptic transmission, when stimulation of even a single afferent fibre may cause neurotransmitter release at multiple unique active zones. In these studies, synapses activated by electrical stimulation of Schaffer collateral axons were identified by the presence of excitatory postsynaptic Ca^{2+} transients (EPSCaTs). Surprisingly, these EPSCaTs require AMPAR-mediated spine depolarization and Ca^{2+} release from intracellular calcium stores [15]. Though they were unable to detect Ca^{2+} entry through NMDARs directly, they do report that some NMDAR-mediated Ca^{2+} is required to induce release from intracellular stores. Nevertheless, they provide evidence that this indirect assay is capable of approximating the probability of presynaptic neurotransmitter release (P_r) by measuring the probability of eliciting an EPSCaT (P_{Ca}) from electrical stimulation. They find that LTP induction caused P_{Ca} to increase, leading to the conclusion that P_r has also increased [16,18], except in silent synapses, where LTP expression is mediated entirely by AMPAR insertion [17], i.e. a purely postsynaptic modification. While the presence of an EPSCaT at a single spine and a global excitatory postsynaptic potential (EPSP) are normally decoupled in these studies owing to limitations in imaging every spine in a neuron's dendritic arbour, Enoki *et al*. [18] do report three cells where the presence of an EPSCaT correlates perfectly with an evoked EPSP. Based upon these three spines, and other experiments where they subtract the EPSP amplitude during EPSCaT failures from the EPSP recorded during EPSCaT successes, they conclude that the unitary EPSP amplitude does not increase during LTP, and therefore the increased global EPSP is owing entirely to changes in P_r.

These conclusions rely on the assumption that EPSCaTs reliably indicate presynaptic release of neurotransmitter, instead of an increased coupling between neurotransmitter release and postsynaptic depolarization by AMPARs, resulting in Ca^{2+} influx through NMDARs. Indeed, this possibility is suggested by the fact that the EPSCaT can be eliminated by blocking AMPARs or NMDARs [15]. These papers provide several controls to explore this possibility, including using low concentrations of the AMPAR-antagonist CNQX to show that significant block of the EPSP does not decrease the frequency or amplitude of EPSCaTs [16], and by using CPA to deplete intracellular Ca^{2+} stores, demonstrating a reduction in EPSCaTs even in Mg^{2+}-free solution, ruling out NMDARs as the sole source of Ca^{2+} [17]. However, CNQX acts as a partial agonist and depolarizes neurons [19], which may actually result in increased Ca^{2+} through NMDARs. Also, numerous other studies failed to find a major role for intracellular stores in synaptically evoked calcium transients [20–22], instead finding that the main source of Ca^{2+} is through NMDARs [23,24]. Because of the well-accepted block of NMDARs by Mg^{2+} at resting membrane potentials, other studies examining synaptically evoked Ca^{2+} transients therefore record either in Mg^{2+}-free solution [23] or at depolarized potentials to ensure reliable and direct coupling of glutamate release and Ca^{2+} transients [20]. This discrepancy may be partially attributed to the use of whole-cell patch clamp [20,21], which washes out signalling molecules that induce Ca^{2+} release from intracellular stores [25]. The use of sharp intracellular electrodes prevents this wash-out, but introduces its own significant caveats, as it adds a large leak conductance and has decreased

signal-to-noise ratio compared with whole-cell patch recordings [26,27], making it unlikely that small synaptic events can be recorded. Thus, it is unfortunate that the authors did not design their experiments so that they could directly monitor calcium influx through the NMDAR or maximize their ability to record small-amplitude synaptic events.

These experiments also stand in stark contrast to a number of studies in the past addressing the possibility of an increase in transmitter release during LTP. There are a variety of approaches that have been used to monitor transmitter release and these were discussed at the previous meeting [1]. These include a lack of change in paired pulse facilitation, a sensitive assay for changes in the probability of release [28], the finding that LTP is normal when evoked at synapses in which the probability of transmitter release is saturated [29,30], the lack of effect of LTP on the rate of block of the use-dependent and irreversible NMDAR antagonist MK-801 [31], the lack of effect of LTP on the rate of block of use-dependent polyamine antagonists on GluA2-lacking AMPARs [32], the lack of change in the glutamate transporter currents recorded from astrocytes which ensheath excitatory synapses, another sensitive assay for synaptic glutamate release [33,34], and finally the lack of change in the presynaptic FM1-43 destaining [35] or the rate of exocytosis of a pH-sensitive fluorophore attached to the synaptic vesicle protein VAMP2 during normal NMDAR-dependent LTP [36], although LTP elicited with a stronger induction protocol, which recruits an NMDAR-independent, L-type Ca^{2+}-channel-dependent component, did alter the rate of destaining and exocytosis.

Taken together, the results summarized above are seemingly incompatible with a change in transmitter release during NMDAR-dependent LTP. The strength of these 'negative' results relies entirely on the sensitivities of each of these assays. In each case, the assays were calibrated and quantified with various manipulations known to increase transmitter release. In general, these assays provided a sensitivity that would have detected a roughly 10% change had it occurred, while LTP roughly increases synaptic transmission twofold.

4. Evidence from the past 10 years for a postsynaptic long-term potentiation expression mechanism

Most LTP research of the past decade has not explicitly focused on identifying a presynaptic or postsynaptic locus of expression. Since the discovery of silent synapses [37], attention has largely shifted to studies on CaMKII signalling [38], or AMPAR trafficking [39], with an implicit assumption of a postsynaptic expression mechanism. However, we feel that there are several key studies of the past decade that unequivocally demonstrate a primarily postsynaptic locus of LTP expression through increased insertion of AMPARs.

The simplest way to probe a postsynaptic mechanism of LTP expression is to remove the presynaptic terminal entirely. Technological advances in the past 10 years have allowed precisely that, such that experimenters can now apply glutamate with high enough spatial and temporal resolution to mimic release from single synaptic vesicles. This is done with an inert, caged derivative of glutamate, MNI-glutamate, that can undergo photolysis following two-photon excitation, resulting in a release of active glutamate with 1 µm-spatial resolution at a submillisecond timescale. By adjusting the size and intensity of light excitation, uncaging glutamate onto a dendritic spine can produce uncaging excitatory postsynaptic currents (uEPSCs) with the same amplitude and kinetics as miniature EPSCs (mEPSCs) produced by neurotransmitter release [40]. Additionally, 2-photon uncaging can specifically activate synaptic receptors with minimal contamination from glutamate spillover onto extrasynaptic AMPARs, as demonstrated by studies that specifically activated NMDARs with no AMPAR-mediated uEPSC in silent synapses [41,42]. Several studies have since demonstrated that pairing postsynaptic depolarization with repetitive glutamate uncaging causes an enhancement in AMPAR-mediated currents, with an associated increase in the volume of the spine [43–46]. This plasticity is blocked completely by NMDAR antagonists and partially by CaMKII antagonists [43,46] and is synapse specific [45], mechanistically the same as LTP. The magnitude of AMPAR potentiation is also similar to that seen in LTP, which combined with the complete removal of any contribution from the presynaptic terminal, leaves little doubt as to a major contribution to LTP expression from increased current through postsynaptic AMPARs.

5. AMPA receptor trafficking during long-term potentiation

Observations and manipulations of the AMPAR complex themselves also show profound effects on LTP, which are difficult to explain in models that rely on primarily presynaptic LTP expression. Andrasfalvy & Magee [47] were able to study outside-out patches pulled from the dendrites of CA1 neurons that had undergone LTP, and through non-stationary fluctuation analysis found an increase in the AMPAR number, but not single-channel conductance. The first AMPAR-specific manipulation to have an effect on LTP was constitutive deletion of the AMPAR subunit GluA1 [48], which prevented LTP expression in the hippocampus, reminiscent of the lack of LTP seen with pharmacological block of NMDARs. However, it should be noted that subsequent studies have found that at juvenile ages [49] or with alternative LTP induction protocol [50], some potentiation could still be observed in these mice. In a similar fashion to GluA1 deletion, knockout of the transmembrane AMPAR regulatory protein (TARP) γ-8 also blocks LTP expression [51], as did deletion of cornichon-2 and cornichon-3 (CNIH-2/3), a recently discovered addition to the AMPAR complex [52].

Since the discovery of GluA1's requirement for LTP, much research has focused on its cytoplasmic carboxy terminal (c-tail), driven by a convincing model of AMPAR trafficking developed by Malinow and co-workers [53,54]. In this model, GluA2 traffics to the synapse constitutively, whereas GluA1 is excluded from the synapse until induction of LTP. This difference depends on the c-tails of the individual subunits, as swapping the c-tails of GluA1 and GluA2 also swaps their trafficking behaviour. The requirement of the GluA1 c-tail for LTP was supported by the finding that wash-in or expression of a soluble GluA1 c-tail peptide showed impaired LTP expression 30 min following induction [54]. A similar result was seen in knockin mice with two targeted phosphonull mutations in the GluA1 c-tail [55], and multiple other c-tail phosphorylation sites have been implicated including CaMKII [56], PKC [57], PKA [58] and an interaction with the

protein 4.1N [59]. However, none of these manipulations on the GluA1 c-tail resulted in an immediate or total block of LTP. Also, further analysis on the mechanism by which these phosphorylation sites or protein interaction domains promote synaptic delivery has yet to be provided.

In a recently published paper, we attempted to define the precise requirement of the GluA1 c-tail for LTP using a single-cell molecular replacement technique where all endogenous AMPARs were replaced with recombinant GluA1 subunits [60,61]. In this technique, CA1 neurons with the genes for GluA1, GluA2 and GluA3 flanked by loxP sites are co-transfected with Cre recombinase, resulting in complete removal of the endogenous AMPAR subunits and a replacement subunit. After replacing with various GluA1 subunits with truncated or mutated c-tails, we failed to find any single region that was required for LTP, instead showing that replacement with GluA2 or the kainate-receptor subunit GluK1 were sufficient to support LTP. Only under conditions with limited AMPAR surface expression was LTP impaired [61]. One criticism of our study is that our manipulations may be too artificial, and that by completely deleting all endogenous receptors, we may be fundamentally altering the normal signalling mechanisms used by the cell to regulate synaptic strength [62,63]. We argue that our manipulations are no more artificial than overexpression on a wild-type background, and that at no time during our experiments are the synapses devoid of AMPARs. This is because the exogenous receptor is expressed many days before the loss of the endogenous receptors. In particular, we report one condition where we expressed GluA1/A2 heteromers in which the GluA1 subunit lacked its c-tail (GluA1ΔC), and saw normal expression of LTP. This condition most closely mimics the endogenous situation, where surface and synaptic transmission are dominated by GluA1/A2 heteromers [64], differing only in the absence of the GluA1 c-tail.

Another criticism is that our study demonstrated that the GluA1ΔC receptor is actually impaired at getting to the synapse when the endogenous receptors are present, meaning that the c-tail may impart some competitive advantage [63]. We interpret this result differently, noting that GluA1ΔC is also profoundly impaired at trafficking to the surface, which would supersede any specific synaptic-targeting defect. This is a minor point, however, and elides the main controversy of that particular set of experiments—specifically that overexpressed GluA1 readily traffics to the synapse under basal conditions. This is in stark contrast to the foundational observation regarding subunit–unit-specific trafficking of AMPARs, that GluA1 only enters the synapse following LTP [53,54]. Further studies are needed to resolve this fundamental difference. Given the effects on trafficking seen from several GluA1 c-tail knockin mice [55], and the effects that manipulating the c-tail can have on overexpressed receptors [57,58], it is clear that the GluA1 c-tail does have a modulatory role in synaptic transmission. However, we maintain that it plays a minimal, if any role, for expression of LTP.

Given our findings that multiple different glutamate receptor subtypes can support LTP expression and that impaired surface trafficking correlates with impaired LTP, we propose a model whereby LTP primarily requires a large pool of extrasynaptic surface receptors. This is supported by the finding that deleting GluA1 dramatically decreases surface expression [64,65], suggesting that the observed requirement for GluA1 is in fact a requirement for a large pool of extrasynaptic receptors. Additionally, the (TARP) γ-8 and CNIH-2/3 deletions both severely limit the total amount of AMPAR protein available to the cell [51,52], which may explain their requirement for LTP.

If the glutamate receptor subtype is not important, what is changing at the synapse to allow potentiation? We believe that the answer may lie in the discovery of structural plasticity that accompanies LTP expression [43]. This suggests that LTP involves a broader reorganization of the synapse, probably involving the entire postsynaptic density and spine, allowing it to recruit or capture more AMPARs to potentiate synaptic transmission. Future research should therefore focus on the proteins involved in anchoring glutamate receptors to the synapse and how that interaction is altered following LTP.

6. Conclusion

Despite the substantial advances over the past 10 years, we still actually know very little about the core mechanisms of LTP. There is now near universal acceptance of a postsynaptic expression mechanism, and recent evidence indicates that LTP involves a dramatic change in the size and structure of the synapse, allowing it to anchor a variety of glutamate receptor subtypes. With advancing technology allowing greater control over the complement of synaptic proteins and precise observation and manipulation of individual spines, one can only hope that the pace quickens during the next 10 years.

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