Neuron
Review

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A Brief History of Long-Term Potentiation

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http://dx.doi.org/10.1016/j.neuron.2016.12.015

Since the discovery of long-term potentiation (LTP) in 1973, thousands of papers have been published on this intriguing phenomenon, which provides a compelling cellular model for learning and memory. Although LTP has suffered considerable growing pains over the years, LTP has finally come of age. Here the rich history of LTP is reviewed. These are exciting times and the pace of discovery is remarkable.

Introduction

As the 45 year anniversary for long-term potentiation (LTP) is just around the corner, I thought it would be interesting to review the rich history of this field and where we are. Some of the most fascinating questions of our time involve how we learn and how our brain stores information. It is now accepted that memory is not a unitary process and can be broadly divided into declarative and nondeclarative forms (Milner et al., 1998). Declarative memory is what we ordinarily mean by the term memory and involves the conscious recollection of facts and events. Nondeclarative memory underlies the changes in skilled behavior and its improvement with practice. The cellular changes that underline these two forms of memory differ considerably, but both are thought to involve changes in the strength of neuronal connections as proposed by Cajal (Cajal, 1911) more than a century ago. This Review will focus on declarative memories. Based to a considerable degree on the profound memory loss observed in the patient H.M. (Scoville and Milner, 1957), who had a bilateral resection of the medial structures of the temporal lobe, attention was focused on the temporal lobes and particularly the hippocampus in its role in declarative memory. The notion that synaptic strength changes during learning and memory was refined into an elegant concrete model by Hebb in 1949 (Hebb, 1949), in which he postulated a synaptic modification for learning and memory that occurs as a consequence of coincidence between pre- and postsynaptic activity. However, as discussed below, experimental evidence that synapses are plastic in the mammalian brain had to wait almost 20 years, until the discovery of LTP (Bliss and Lomo, 1973; Lomo, 1966), in which brief, high-frequency stimulation, typically referred to as a tetanus, of hippocampal excitatory synapses produced a rapid and long-lasting increase in the strength of these synapses that could persist for many days (Bliss and Gardner-Medwin, 1973). LTP, which has been described at synapses throughout the brain, remains to this day one of the most attractive cellular models for learning and memory. Some of the confusion that has plagued the field of LTP may be due, in part, to the existence of multiple forms of LTP. The variables include the type of synapse, the stimulation parameters, the time analyzed after LTP induction, and the developmental age. Perhaps the most dramatic example of different forms is a comparison of LTP at CA1 hippocampal synapses and that at mossy fiber synapses onto CA3 pyramidal cells. LTP at CA1 synapses, which is broadly representative of LTP at excitatory synapses, is dependent on NMDA receptor activation and primarily involves a postsynaptic modification (see below). Mossy fiber LTP is independent of NMDA receptors and is entirely expressed presynaptically (Nicoll and Schmitz, 2005). There is even evidence that under certain conditions CA1 synapses can express an NMDA receptor-independent component to LTP (Grover and Teyler, 1990; Zakharenko et al., 2001). Finally, there are reports that there are mechanistic differences between neonatal animals (<P10) and more mature animals (Bolshakov and Siegelbaum, 1995; Palmer et al., 2004; Yasuda et al., 2003). However, as will be discussed below, it is specifically the LTP that requires NMDA receptor activation that holds the fascination of those working in this field because it provides a simple explanation for associative memory. Thus, this Review focuses solely on NMDA receptor-dependent LTP, and primarily on the first hour, since this has received the most attention.

The Discovery of LTP

The field began with Terje Lomo's publication of a single author abstract (Lomo, 1966), when he was a student in Per Andersen's lab. In this abstract Lomo concluded, "This represents an example of a plastic change in a neuronal chain, expressing itself as a long-lasting increase of synaptic efficacy. The effect, which may last for hours, is dependent on repeated use of the system." Lomo was occupied with finishing his thesis and did not pursue his finding. It is my understanding that Tim Bliss, who majored in psychology at McGill, talked to Andersen about his interest in learning and memory and Andersen said he should talk to his student Lomo "who has something that will interest you" (Lømo, 2016). Thus began the collaboration that resulted in the landmark paper by Bliss and Lomo (Bliss and Lomo, 1973), entitled *Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path*. This paper launched the field of LTP. It is impossible to overstate the importance of this paper; it is truly a landmark in the field of neuroscience and should be required reading for any student in the neurosciences. It elegantly outlines the logic for carrying out the experiments, which are remarkably well controlled and impeccably address virtually all of the possible artifacts that could confound their interpretation. They conclude that the long-lasting change they recorded is due to an increase in the strength of synaptic transmission. Furthermore, they make two additional fundamental discoveries, showing that LTP is saturable and that there is also an increase

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Photo: John Lisman

Figure 1. The Key Individuals in the Discovery of LTP
From left to right: Timothy Bliss, Per Andersen, and Terje Lomo. This picture was taken by John Lisman in 2003 at a Royal Society meeting celebrating the 30 year anniversary of the discovery of LTP.

in the coupling between the synaptic response and the firing of postsynaptic neurons. There is not a single controversial finding in this paper, which is a very remarkable thing in this field. They end their paper with a most prescient summary of where the field will go. First, they outline that the increase in strength could be either due to an increase in transmitter release (importantly, the transmitter was not known and would have to await the pharmacological tools that identified glutamate as the transmitter, see below) or to an increase in the sensitivity of the postsynaptic cell to the transmitter. Second, they raise the question of whether this cellular phenomenon has anything to do with learning and memory. Figure 1 shows a photograph of Tim Bliss, Per Andersen, and Terje Lomo taken in 2003 at the Royal Society in London for the 30th anniversary of the discovery of LTP.

The Mechanistic Dissection of LTP

With the discovery of LTP, three key questions immediately came to the forefront. (1) What occurs during the brief tetanus ($\sim$1 s) that initiates LTP? This process is referred to as "induction." (2) In what way are the synapses altered following LTP induction? This process is referred to as "expression." (3) Is LTP involved in learning and memory? I have provided a timeline for what I consider to be some of the key discoveries that have propelled the field forward (Figure 2). I have purposely stopped the timeline at 2005, because I believe that considerable time is required to accurately weigh the importance of new discoveries.

What Occurs during the Tetanus: Induction?

It is interesting to note that very little attention ($\sim$50 citations) was given to LTP for a decade after its discovery. The reason for this was primarily 2-fold. First, and most importantly, neither the neurotransmitter nor the receptors were known for these excitatory synapses that expressed this remarkable plasticity. Second, the development of the in vitro hippocampal slice preparation was essential for rigorous pharmacological and biophysical studies. Nevertheless, some fundamental discoveries were made during this time, which set the foundation for this field. McNaughton et al. (McNaughton et al., 1978) and shortly thereafter, Levy and Stewart (Levy and Steward, 1979), reported that LTP had the property of "cooperativity" and "associativity." A weak input, in which only a few excitatory synapses were tetanized, failed to induce LTP, whereas a strong input reliably induced LTP (cooperativity), although only in the tetanized pathway ("input specificity"). In addition, the simultaneous activation of two separate inputs, one of which is weak and fails to undergo LTP on its own, exhibits robust LTP when tetanized together with a strong input (associativity). These findings established LTP as a Hebbian process and raised a fundamental question central to LTP: how does the strong input communicate to the weak input? Clues to the answer came with two further observations. First, injecting depolarizing current into the postsynaptic cell could substitute for a strong tetanus (Gustafsson et al., 1987; Kelso et al., 1986; Wigstrom et al., 1986) and, second, preventing depolarization during a strong tetanus by hyperpolarizing (Malinow and Miller, 1986) or voltage clamping (Kelso et al., 1986) the cell prevented LTP. These findings indicate that there are only two requirements for LTP: postsynaptic depolarization coupled with synaptic stimulation. There is no need to stimulate the synapses at high frequency. This is shown experimentally in Figure 3.

Making sense of these early observations had to await a deeper understanding of excitatory synaptic transmission. Due in large part to the lifetime work of Jeff Watkins, the pharmacology of these synapses was revealed (Watkins and Jane, 2006). This involved the design of a number of highly selective glutamate receptor agonists and antagonists. With these tools in hand, the transmitter was established to be glutamate, which acts primarily on NMDA receptors and non-NMDA receptors (AMPA receptors). AMPA receptors are responsible for the reliable moment-to-moment transmission. The NMDA receptors were an enigma. They were clearly expressed on neurons because the application of NMDA evoked strong responses. However, when the selective NMDA receptor antagonist APV was applied it had no effect on excitatory postsynaptic potentials

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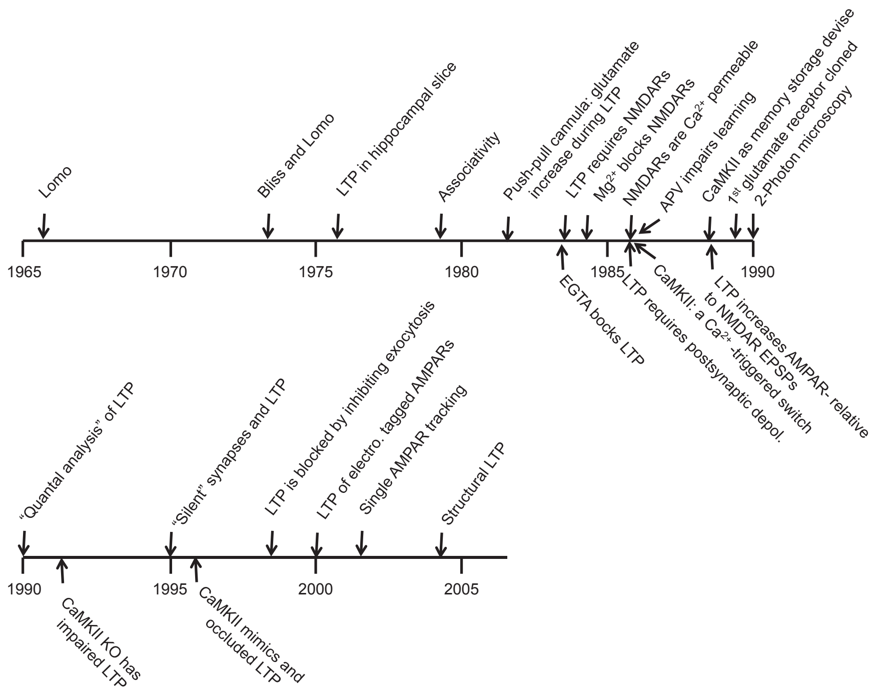

Figure 2. Timeline for the Major LTP Discoveries

Lomo (Lomo, 1966). Bliss and Lomo (Bliss and Lomo, 1973). LTP in slice (Schwartzkroin and Wester, 1975). Associativity (Levy and Steward, 1979; McNaughton et al., 1978). LTP requires NMDARs (Collingridge et al., 1983). EGTA blocks LTP (Lynch et al., 1983). Push-pull cannula: glutamate increase during LTP (Dolphin et al., 1982). Mg^{2+} blocks NMDARs (Mayer et al., 1984; Nowak et al., 1984). LTP requires postsynaptic depolarization (Gustafsson et al., 1987; Kelso et al., 1986; Malinow and Miller, 1986). MNDARs are Ca^{2+} permeable (Ascher and Nowak, 1988; MacDermott et al., 1986). APV impairs learning (Morris et al., 1986). CaMKII: a Ca^{2+} trigger switch (Miller and Kennedy, 1986). CaMKII as a memory storage devise (Lisman and Goldring, 1988). LTP increases AMPAR-relative to NMDAR-EPSPs (Kauer et al., 1988; Muller et al., 1988). First glutamate receptor cloned (Hollmann et al., 1989). Two-photon microscopy (Denk et al., 1990). "Quantal analysis" of LTP (Bekkers and Stevens, 1990; Malinow and Tsien, 1990). CaMKII KO has impaired LTP (Silva et al., 1992). "Silent" synapses and LTP (Isaac et al., 1995; Liao et al., 1995). CaMKII mimics and occludes LTP (Lledo et al., 1995; Pettit et al., 1994). LTP is blocked by inhibiting exocytosis (Lledo et al., 1998). LTP of electrophysiologically tagged AMPARs (Hayashi et al., 2000). Single AMPAR tracking (Borgdorff and Choquet, 2002). Structural LTP (Matsuzaki et al., 2004).

(EPSPs), leading Collingridge et al. (Collingridge et al., 1983) to conclude that, "The firmest conclusion that can be drawn from the antagonist studies is that the NMA (now referred to as NMDA) receptor is not involved in mediating synaptic excitation in the Schaffer collateral-commissural pathway." In the same series of experiments, and adding to the confusion, Collingridge et al. found that application of APV blocked LTP. They conclude the paper as follows: "the present study has shown that the NMA receptor plays no role in the mediation of synaptic transmission but may be involved in the generation of l.t.p." What could be the explanation for the seeming incompatibility of these two observations?

Within a year the answer to this conundrum jumped out with a most amazing and simple discovery by Ascher (Nowak et al., 1984) and independently by Mayer and Westbrook (Mayer et al., 1984). The NMDA receptor is profoundly voltage dependent and thus conducts little current at resting membrane potentials. Ascher and Mayer/Westbrook showed that this was due to a voltage-dependent block of extracellular Mg^{2+} and that as the cell was depolarized this block was relieved. These studies involved the application of glutamate agonists onto dissociated neurons. The findings were quickly linked to excitatory synapses by Dale and Roberts (Dale and Roberts, 1985), who showed that unitary EPSPs in *Xenopus* embryos were composed of a fast nonNMDA receptor component and a slow NMDA receptor component. Combining the voltage sensitivity of the NMDA receptor with the requirement of postsynaptic depolarization, which occurs during a tetanus, not only explained the previously published baffling pharmacological results (Collingridge et al., 1983), but also suggested that the Hebbian mechanism underlying LTP resides in the NMDA receptor itself, laying a mechanistic foundation for LTP as a compelling logical link to associative learning. A seemingly complex phenomenon turns out to be extraordinarily simple. So the NMDA receptor is critical for LTP, but in what way? The answer was not long in coming and it came, again, from the same players: Ascher (Ascher and Nowak, 1988) and Mayer/Westbrook (MacDermott et al., 1986). Again, the answer was disarmingly simple. Unlike the AMPA receptor, the NMDA receptor is highly permeable to Ca^{2+}. This finding immediately explained a previous finding by Lynch (Lynch et al., 1983) reporting that chelating postsynaptic Ca^{2+} prevents LTP. So within a matter of a few years, the answer to what occurs during the 1 s tetanus, i.e., induction, was solved (Figure 4). These were heady times for the LTP field. We were well on our way to providing the biophysical basis for learning and memory.

In What Way Are the Synapses Altered following LTP Induction: Expression?

With the problem of induction solved, the field, fortified by its rapid successes, quickly moved on to tackling the underlying changes that occur at the synapse, once LTP is induced, i.e., "expression." More specifically the issue is whether LTP is expressed as a postsynaptic change in the sensitivity to glutamate or as an increase in the release of glutamate. The closest analogy that I can come up with is Napoleon's very long freezing winter of 1812 in the middle of Russia. However, unlike Napoleon, this winter lasted for over a decade, although no lives were lost. What went wrong? In many respects the problem was similar to the prolonged winter that occurred after the discovery of LTP—ignorance. Our understanding of synaptic transmission in the brain was just too rudimentary. We were not as smart as

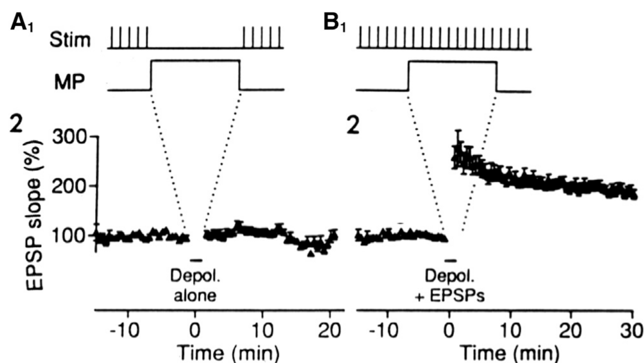

Figure 3. Minimum Requirements for Induction of LTP
The top series of diagrams (A1 and B1) illustrates schematically, at an expanded timescale, the stimulation of the excitatory synapses (Stim) and the control of the membrane potential (MP). The graphs below (A2 and B2) plot the maximal initial slope of the EPSP. In (A), synaptic stimulation was stopped and the cells were depolarized to 0 mV for 2 min. In (B), synaptic stimulation continued throughout the experiment and the cells were depolarized to 0 mV for 2 min. The recording electrode contained cesium to allow depolarization of the membrane. Each graph averages the results from 8–12 cells. Each slope measurement in an individual experiment was normalized to the average value of all points on the baseline (at least 10 min prior to each manipulation) for that experiment. Experiments were then divided into 20 s bins, each of which was averaged. Data are shown as mean ± SE (modified from Malenka et al., 1989).

we thought we were. We had a lot to learn and this took time. The question underlying expression is very simple: is the change in synaptic strength due to an increase in the release of glutamate or is it due to an increase in the postsynaptic response to glutamate? As you will see, arriving at the answer to this question was anything but simple. One might argue that this debate was much ado about nothing. However, this could not be further from the truth. To understand the molecular underpinning of LTP, it was absolutely essential to know which side of the synapse undergoes the change. No progress could be made without resolving this issue. The long debate that ensued left many people, both inside and outside the field disillusioned, wondering whether there would ever be a resolution to this mess. For instance, Sanes and Lichtman (Lisman et al., 2003; Sanes and Lichtman, 1999) felt compelled to vent their frustration about the endless claims and counterclaims in the field. More specifically they asked "this alarming question… Does LTP exist?" and posited, mockingly, that "when humans go looking for something they often find it—even when it is not there…. seeing a….Mickey Mouse in a peculiar arrangements of clouds." However, in hindsight, while there were, admittedly, many layers of conflicting claims made, it is most refreshing to realize that for the most part there was actually little disagreement on the truly key experimental results marshaled by both sides—the experiments were easily reproducible. It was the interpretation of the results, which were based on our understanding of the neuromuscular junction (NMJ). It turned out that the hippocampal synapse threw us a curve ball—it was more sophisticated than the NMJ, as well as the investigators studying it. So LTP has been humbling and has taught us a great deal about the subtleties and beauty of the central nervous system synapse. Please keep this in mind as I wade through some of the nitty-gritty. Trust me, it is simple and it is beautiful!

With the discovery that activation of postsynaptic NMDA receptors is essential for the induction of LTP, it was clear from the outset that LTP is *induced* postsynaptically. Thus, either the postsynaptic signaling cascade initiated by NMDA receptor activation remained in the postsynaptic cell and resulted in a postsynaptic modification, or alternatively, a retrograde signal was generated that acted back on the presynaptic terminal to increase the amount of glutamate released. Based on push-pull cannula experiments (Dolphin et al., 1982), actually carried out just prior to the evidence showing the postsynaptic role of NMDA receptors, it was proposed that LTP is associated with an increase in glutamate release, necessitating a retrograde messenger. This presynaptic expression mechanism dominated the field at the time. Although, it is now widely accepted that the expression of LTP is largely postsynaptic (see below), a presynaptic component may well contribute under some conditions (see Bliss and Collingridge, 2013; Emptage et al., 2003; Ward et al., 2006). This is particularly the case for NMDA receptor-independent forms of LTP (Grover and Teyler, 1990; Nicoll and Schmitz, 2005; Zakharenko et al., 2001).

Focus on the site of expression of NMDA receptor-dependent LTP was heightened by two papers published in 1988 (Kauer et al., 1988; Muller et al., 1988), one with the title *A persistent postsynaptic modification mediates long-term potentiation in the hippocampus* (Kauer et al., 1988). These papers reported that LTP is expressed primarily on the AMPA receptor compared to the NMDA receptor. It was argued, and shown experimentally, that increasing synaptic glutamate release by post-tetanic potentiation caused an identical increase in both components, indicating that the NMDA receptors can detect an increase in glutamate release and are not saturated. These findings pointed to a *selective* postsynaptic modification of AMPA receptor transmission during LTP. While there was some debate as to whether there was any change in the NMDA component, virtually all subsequent studies have confirmed that LTP is primarily expressed on the AMPA receptor component (e.g., Asztely et al., 1992; Choi et al., 2000; Durand et al., 1996; Kullmann, 1994; Liao et al., 1995; Mainen et al., 1998; Montgomery et al., 2001; Muller et al., 1992; Perkel and Nicoll, 1993; Selig et al., 1995; Watt et al., 2004). This finding remains extremely difficult to explain by a presynaptic mechanism. Nevertheless, within little over a year two papers appeared, one entitled *Presynaptic mechanism for long-term potentiation in the hippocampus* (Bekkers and Stevens, 1990) and the other *Presynaptic enhancement shown by whole-cell recordings of long-term potentiation in hippocampal slices* (Malinow and Tsien, 1990). Both papers used "quantal analysis" to examine the statistical properties of synaptic transmission before and after LTP. Based on the classical studies of Del Castillo and Katz (Del Castillo and Katz, 1954) at the neuromuscular junction (NMJ) neurotransmitter is released as packets from vesicles and the number of vesicles released per stimulus varies probabilistically from trial-to-trial, and, on occasions, no vesicles are released, which is referred to as "failures." One can calculate the coefficient of variation (CV) before and after alterations in synaptic strength to determine whether the change is mediated by a presynaptic phenomenon, reflected in a change in CV. Alternatively, postsynaptic changes would not be accompanied by changes in CV. A more direct way to monitor changes in

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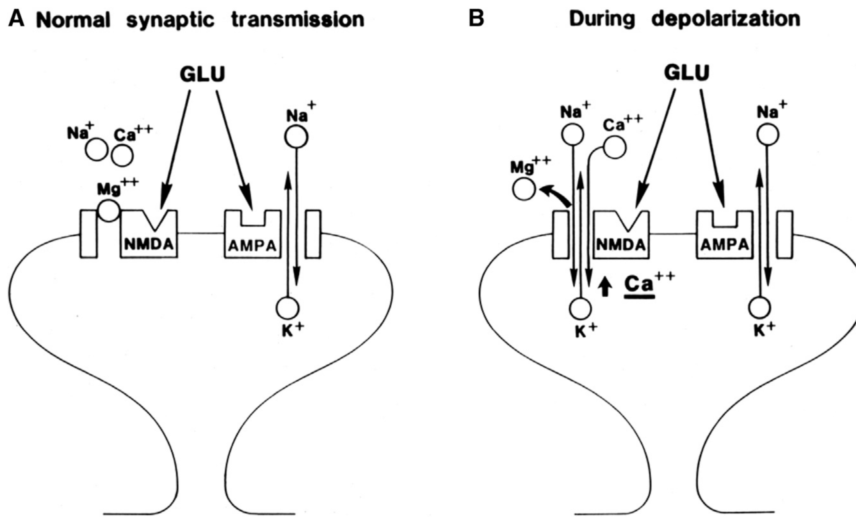

Figure 4. Model for the Induction of LTP in the CA1 Region of the Hippocampus
(A) The events occurring during low-frequency synaptic transmission. Glutamate is released from the presynaptic terminal and binds to both NMDA and AMPA receptors. Na^+ and K^+ flow through the AMPA receptor channel but not through the NMDA receptor channel, due to Mg^{2+} block of this channel.
(B) The events occurring when the postsynaptic membrane is depolarized, as would occur during a high-frequency tetanus. The depolarization relieves the Mg^{2+} block of the NMDA receptor channel, allowing Na^+, K^+, and most importantly Ca^{2+} to flow through the channel. The primary target of the rise in Ca^{2+} in dendritic spines is the calcium-calmodulin-dependent kinase II (CaMKII) (modified from Nicoll et al., 1988).

release is to measure the rate of synaptic failures, in which no response is evoked. Both of these studies clearly demonstrated that procedures known to result in modifications of either presynaptic or postsynaptic function behaved as predicted. When they applied their analysis to LTP, both the changes in the CV and failures indicated a presynaptic increase in transmitter release during LTP. These two papers played a pivotal role in driving research on LTP. Based on these papers, a presynaptic expression mechanism was immediately embraced by the neuroscience community at large. These experiments were quickly repeated by numerous labs (e.g., Kullmann and Nicoll, 1992; Liao et al., 1992; Manabe et al., 1993), and thus the findings were unimpeachable. So what could be the basis for the seeming contradiction?

At the time there were a substantial number of studies implicating a postsynaptic change during LTP (Nicoll, 2003) and the only widely agreed upon evidence for a presynaptic change involved the results of the quantal analysis. Thus, a number of studies appeared using alternative approaches to interrogate the release of glutamate during LTP (Diamond et al., 1998; Hjelmstad et al., 1997; Lüscher et al., 1998; Manabe and Nicoll, 1994; Manabe et al., 1993). All of these challenges came up empty handed. Despite the high sensitivity of these assays, no change in the amount of glutamate released during LTP could be detected. The findings in one study (Manabe and Nicoll, 1994) were summarized as follows, "How can the present results be reconciled with studies using quantal analysis? The decrease in failures is usually interpreted as an increase in P_r (probability of transmitter release). Alternatively, the decrease in failures could reflect the appearance of patches of functional AMPA receptors on the postsynaptic cell." Within a year two papers appeared (Isaac et al., 1995; Liao et al., 1995), providing experimental evidence for the presence of "silent synapses." Remarkably, a substantial number of hippocampal synapses are silent, in that there are no functional AMPA receptors, but they do contain a normal compliment of NMDA receptors. LTP rapidly unsilences these synapses with the all-or-none insertion of a population of AMPA receptors (Isaac et al., 1995; Liao et al., 1995). This finding not only provides a postsynaptic explanation for the change in CV and in failure rate following LTP, but also for the selective increase in the AMPA EPSC during LTP. These findings have been repeated in many subsequent studies and quickly swayed public opinion to a postsynaptic explanation for LTP. Since the publication of these findings, most work on LTP has focused on the nature of the postsynaptic modification (see below). It is important to note that, in addition to the unsilencing there is good evidence that synapses that already contain AMPA receptors can also undergo LTP. For instance, LTP is associated with an increase in the size of "quantal" miniature EPSCs, as well as frequency (Manabe et al., 1992; Oliet et al., 1996). In addition, two-photon microscopy experiments (see below) have shown that spines that generate AMPA responses to glutamate uncaging can undergo LTP (Harvey and Svoboda, 2007; Matsuzaki et al., 2004; Tønnesen et al., 2014). A caveat with the uncaging experiments is that uncaged glutamate is likely to activate perisynaptic AMPA receptors, in addition to those at the PSD, thus likely underestimating the prevalence of silent synapses. The relative importance of unsilencing and the addition of AMPA receptors to synapses that already contain AMPA receptors is not entirely clear. Although the existence of silent synapses has been clearly established, both with immunogold electron microscopy (Nusser et al., 1998; Petralia et al., 1999; Takumi et al., 1999) and with two-photon uncaging experiments (Béïque et al., 2006; Busetto et al., 2008), their presence rapidly declines with age, suggesting that enhancement of preexisting synaptic AMPA receptor responses assumes a more important role with age. In summary, although the pathway to a postsynaptic expression mechanism seemed torturous and bewildering, the answer on the site of LTP expression, at least for the first hour, turned out to be quite simple.

So, what have we learned about the changes in the postsynaptic side of the synapse? In brief, the decade-long logjam was finally broken and remarkable and exciting progress has been made. Most excitatory presynaptic terminals form synapses on small protuberances, referred to as spines, which stud the dendrites of cortical pyramidal cells. Spines provide both an anatomical and biochemical isolation of the synapse, presumably providing the basis for synapse specificity for LTP.

The postsynaptic side of the synapse has a prominent electron-dense thickening referred to as the postsynaptic density (PSD). This specialization contains the receptors, scaffolding proteins, adhesion proteins, cytoskeletal proteins, and numerous signaling proteins. A significant advance has come with the introduction of two-photon microscopy (Denk, 1994; Denk et al., 1990). By coupling fluorescent microscopy measurements (e.g., Ca^{+2} transients, EGFP, FRET, etc.) with the uncaging of glutamate onto single spines, direct interrogation of single spines is now possible. In particular, FRET-based assays permit biochemical studies in real time on an intact cubic micron of tissue. Finally, the uncaging of glutamate removes the presynaptic terminal from the equation, greatly simplifying the phenomenon. At most spines uncaging glutamate activates both AMPA and NMDA receptors. By combining glutamate uncaging with postsynaptic depolarization, LTP of the glutamate response occurs, whose properties are remarkably similar to LTP induced by presynaptic stimulation, both in terms of time course and magnitude (Harvey and Svoboda, 2007; Lee et al., 2009; Matsuzaki et al., 2004; Tønnesen et al., 2014). Particularly fascinating is the finding that accompanying electrophysiological LTP there is a dramatic enlargement of the spine (Matsuzaki et al., 2004), which is similar to both the onset and time course of electrophysiological LTP. Given the tight linkage between structural LTP (sLTP) and functional LTP, many of the recent advances have been made focusing on sLTP. The sequence of events is as follows: Ca^{+2} entry through the NMDA receptor activates calcium-calmodulin-dependent kinase II (CaMKII), which may be necessary and sufficient for LTP (Lisman et al., 2012). As demonstrated by a number of studies, CaMKII engages the actin cytoskeleton, resulting in the spine enlargement (Bosch et al., 2014; Herring and Nicoll, 2016b; Patterson and Yasuda, 2011). Many of the steps involved in this enlargement also appear to be involved in functional LTP.

So finally we come to the last step in LTP—the rapid accumulation of AMPA receptors at the synapse. Perhaps the most direct early study involved the trafficking of overexpressed homomeric GluA1 receptors (Hayashi et al., 2000). Unlike endogenous receptors, these homomeric receptors are rectifying. Using rectification as an electrophysiological ‘‘tag,’’ Hayashi et al. showed that LTP drove these receptors into the synapse. The source of the AMPA receptors appears to be 2-fold. First, LTP triggers an activity-dependent exocytosis (Jurado et al., 2013; Kennedy and Ehlers, 2011; Lledo et al., 1998; Makino and Malinow, 2009; Patterson et al., 2010). Second, largely based on single receptor tracking studies (Choquet and Triller, 2003; Opazo et al., 2012), but also with pH-sensitive tagged glutamate receptors (Makino and Malinow, 2009; Patterson et al., 2010), there is a pool of freely diffusible extrasynaptic receptors that can be captured by the PSD. The relative importance of these two processes remains to be established. A great deal of work over the past 15 years has focused on the way in which CaMKII engages these two processes. Most of this work has examined phosphorylation sites and protein-protein interaction sites in the cytoplasmic C termini of AMPA receptor subunits as well as their associated auxiliary subunits that control the trafficking of AMPA receptors and the single channel conductance of the receptors (Barry and Ziff, 2002; Collingridge et al., 2004; Henley and Wilkinson, 2016; Huganir and Nicoll, 2013; Jackson and Nicoll, 2011; Malinow and Malenka, 2002; Sheng and Kim, 2002; Shepherd and Huganir, 2007). In addition, CaMKII can target proteins in the postsynaptic density, such as scaffolding proteins, creating slots to accommodate AMPA receptors.

Linking LTP to Learning and Memory

What makes LTP such an appealing phenomenon is that it has all of the hallmarks expected for the cellular processes underlying learning and memory. However, linking these two phenomena together has been difficult. The first effort to link the two phenomena showed that infusion of the NMDA receptor antagonist APV into the hippocampus impairs learning (Morris et al., 1986). One limitation of this experiment is the realization that NMDA receptors play two roles. The one most often linked to NMDA receptors is LTP. However, these receptors also play a crucial role in the control of moment-to-moment synaptic transmission, independent of LTP (the Mg^{2+} block is not 100% at resting potentials). So an alternative explanation is that APV disrupts hippocampal function. This caveat was circumvented by Giese et al. (Giese et al., 1998), who generated a knockin mouse containing the point mutant T286A of CaMKII. This prevents autophosphorylation and thereby blocks CaM-independent persistent activity of the enzyme. In this mouse, NMDA receptor function was normal, but LTP was absent and learning and memory were strongly impaired. As with any deletion experiment, it is difficult to entirely exclude other roles that these proteins might play. An alternative approach has been to look for LTP during learning. Such a correlation has been reported for fear conditioning (Rogan et al., 1997) and for one-trial inhibitory avoidance learning (Whitlock et al., 2006). Moreover, saturating LTP with repeated tetanic stimulation interferes with memory (Moser et al., 1998). An intriguing recent paper (Nabavi et al., 2014) reports that in the amygdala an associative memory can be inactivated and reactivated with LTD and LTP, respectively. Taken together, these studies provide compelling evidence for a role in learning and memory.

Future Directions

Much has been learned since the discovery of LTP, but many mysteries remain. First, numerous investigators have proposed the existence of ‘‘slots’’ in the PSD that capture AMPA receptors during LTP. This is primarily a concept with little direct experimental evidence for their existence. Given that AMPA receptors are largely excluded from the center of the PSD and form an annulus (Chen et al., 2015; Kharazia and Weinberg, 1997; Takumi et al., 1999), presumably the slots would populate the same territory. The trafficking of AMPA receptors to the synapse requires the interaction between a PDZ binding motif present on AMPA receptor auxiliary subunits to PDZ domain containing proteins (Chen et al., 2000; Jackson and Nicoll, 2011). The MAGUK family of scaffolding proteins are the most abundant PDZ domain-containing synaptic proteins and therefore have repeatedly been proposed as obvious candidates for slot proteins. However, MAGUK proteins play many roles and it has been difficult to establish them as the slot proteins. For instance, knocking down all MAGUK proteins results in the dissolution of the PSD with the loss of both AMPA and NMDA receptors (Chen et al.,

2015). In such a condition, effects on LTP would be hard to interpret. A further issue concerns the saturation of LTP as first reported by Bliss and Lomo (Bliss and Lomo, 1973). The MAGUKs are in large excess to AMPARs and thus are not in limited supply. One of the key downstream targets of CaMKII is the Rho GTPases and their engagement of the actin cytoskeleton (Bosch et al., 2014; Herring and Nicoll, 2016a; Murakoshi and Yasuda, 2012; Okamoto et al., 2009). Thus, it is possible that there is a physical rearrangement of the PSD, exposing buried slots. It is important to keep in mind that kainate receptors, which are not normally expressed at CA1 synapses and differ considerably from AMPA receptors, nonetheless express normal LTP when expressed at CA1 synapses (Granger et al., 2013). Thus, it would appear that the slots are rather promiscuous, although there must be some constraints. Defining these constraints might help in charactering the nature of the slots.

Second, and perhaps the most enigmatic aspect of LTP is the molecular basis underlying its persistence, i.e., the memory. Based on the remarkable biochemical properties of CaMKII, it has long been hypothesized that CaMKII is the "memory molecule" (Lisman and Goldring, 1988). In brief, CaMKII is activated by Ca^{2+}/CM, resulting in its autophosphorylation, which makes the enzyme constitutively active and independent of the continued presence of Ca^{2+} (Kim et al., 2016; Lisman et al., 2012; Miller and Kennedy, 1986). Furthermore, new subunits can assemble with the activated holoenzyne and undergo intersubunit autophosphorylation (Lisman and Raghavachari, 2015; Stratton et al., 2013). Thus, the "memory" can be maintained by subunits that never experienced the initial Ca^{2+}/CM activation of the enzyme. As attractive as this model is, it has been difficult to test experimentally. For instance, one would expect that blocking CaMKII activity following LTP should reverse LTP. Evidence for this has not been entirely compelling, although a recent study using a membrane-permeable peptide inhibitor of CaMKII is consistent with the erasure of established LTP (Sanhueza et al., 2011). On the other hand, recent studies using a FRET-based CaMKII sensor have concluded that the bulk CaMKII remains active for no longer than a minute after LTP induction (Lee et al., 2009). A number of issues have been raised, e.g., the experiments image CaMKII in the entire spine and the persistently active pool may be small and confined to the PSD. Another issue to keep in mind is that the FRET assay does not directly measure kinase activity (Hell, 2014; Kim et al., 2016). Thus, there are still some open issues regarding whether persistent CaMKII activity is required for maintaining LTP. Recent studies have emphasized the fact that CaMKII serves a structural role at the PSD as well as its enzymatic role. The initial activation of CaMKII results in its translocation to the PSD and binding to the GluN2B C terminus (Leonard et al., 1999; Lisman and Raghavachari, 2015; Strack and Colbran, 1998). Preventing this binding impairs LTP (Barria and Malinow, 2005). Once present in the PSD, it could serve as a structural protein, perhaps even in the absence of continued enzymatic activity. This is not a new idea. With the discovery of CaMKII and the fact that it accounts for ~2% of brain protein (Lisman et al., 2012), it was a puzzle as to why an enzyme would be present at such high amounts, raising speculation that it might well have structural roles. Given the diverse interacting partners, CaMKII has been proposed to serve as a signaling hub at the PSD (Kim et al., 2016).

There have been other proposed mechanisms for maintaining LTP. A number of studies from the Sacktor lab have proposed that persistent PKMζ activity may underlie memories (Pastalkova et al., 2006). PKMζ is an atypical protein kinase C isoform, which is persistently active following LTP. However, recent reports have found that LTP and memory are normal in the PKMζ knockout mice, diminishing enthusiasm in this model (Lee et al., 2013; Volk et al., 2013). Finally, based largely on the studies of Kandel (Si and Kandel, 2016), it is proposed that prion-like proteins, and, in particular, cytoplasmic polyadenylation element-binding protein (CPEB), are involved in the stabilization of memory. Work in *Aplysia*, *Drosophila*, and mouse suggests that CPEB can acquire a prion-like state and control protein synthesis at the synapse and thereby stabilize long-lasting changes. This is an intriguing proposal with many interesting questions to pursue. In summary, the field is left with rather few models to explain long-term information storage, each of which have its strengths and weaknesses. On balance, based on the preponderance of evidence, CaMKII still remains the most attractive model.

Third, much of the focus in the LTP field has concerned the first hour and whether LTP is expressed pre- or postsynaptically. As discussed in this Review, it is now generally accepted that it is expressed postsynaptically during the first hour. However, there must be more to the story. Although EM studies show an enormous variety of excitatory synapses in terms of shapes and sizes, the one thing that is constant is the precise matching of the shape and size of the presynaptic active zone and the PSD (Lisman and Harris, 1993; Schikorski and Stevens, 1997). The increase in spine size accompanying LTP is associated with an increase in the size of the PSD, which occurs approximately an hour after induction (Bosch et al., 2014; Meyer et al., 2014). At this point one would expect a mismatch between the enlarged PSD and the presynaptic active zone. This would be expected to exert a strain, via adhesion proteins that bridge the synaptic cleft, such as neuroligins/neurexins, on the presynaptic active zone leading to its expansion and matching with the enlarged PSD. There are data indicating that there is, indeed, a slow increase in the size of the presynaptic bouton, although the size of the active zone has not been addressed (Meyer et al., 2014). Interestingly, there is a good correlation between the size of the active zone and the number of docked vesicles. Furthermore, there is a correlation between the number of docked vesicles and the release probability (Murthy et al., 1997; Schikorski and Stevens, 1997). Thus, one ends up with a larger synapse that has more AMPARs and a higher probability of transmitter release. Such a modified and stable synaptic structure provides an appealing model for information storage. As attractive as this scenario is, much more work is needed, especially with the final stages of the "matching" process.

Conclusion

We have come a long way since the discovery of LTP in 1973. It is safe say that, despite some turbulent periods, LTP has finally come of age. These are exciting times. The progress during the past 5 years has been remarkable and the pace quickens.

ACKNOWLEDGMENTS

R.A.N. thanks Drs. John Lisman, Terje Lomo, and Eric Kandel and for their valuable comments on the manuscript. Work in my lab has been funded by grants from the National Institutes of Mental Health MH070957, MH080379, and MH038256.

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