Long-term potentiation: outstanding questions and attempted synthesis

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This article attempts an overview of the mechanism of NMDAR-dependent long-term potentiation (LTP) and its role in hippocampal networks. Efforts are made to integrate information, often in speculative ways, and to identify unresolved issues about the induction, expression and molecular storage processes. The pre/post debate about LTP expression has been particularly difficult to resolve. The following hypothesis attempts to reconcile the available physiological evidence as well as anatomical evidence that LTP increases synapse size. It is proposed that synapses are composed of a variable number of trans-synaptic modules, each having presynaptic release sites and a postsynaptic structure that can be AMPAfied by the addition of a hyperslot assembly that anchors 10–20 AMPA channels. According to a newly developed view of transmission, the quantal response is generated by AMPA channels near the site of vesicle release and so will depend on whether the module where release occurs has been AMPAfied. LTP expression may involve two structurally mediated processes: (i) the AMPAfication of existing modules by addition of hyperslot assemblies: this is a purely postsynaptic process and produces an increase in the probability of an AMPA response, with no change in the NMDA component; and (ii) the addition of new modules: this is a structurally coordinated pre/post process that leads to LTP-induced synapse enlargement and potentiation of the NMDA component owing to an increase in the number of release sites (the number of NMDA channels is assumed to be fixed). The protocol used for LTP induction appears to affect the proportion of these two processes; pairing protocols that involve low-frequency presynaptic stimulation induce only AMPAfication, making LTP purely postsynaptic, whereas high-frequency stimulation evokes both processes, giving rise to a presynaptic component. This model is capable of reconciling much of the seemingly contradictory evidence in the pre/post debate. The structural nature of the postulated changes is relevant to a second debate: whether a CaMKII switch or protein-dependent structural change is the molecular memory mechanism. A possible reconciliation is that a reversible CaMKII switch controls the construction of modules and hyperslot assemblies from newly synthesized proteins.

Keywords: long-term potentiation; long-term depression; protein synthesis; CaMKII; quantal analysis; memory

1. INTRODUCTION

In this article I will discuss some of the outstanding issues in the LTP field as we celebrate its 30th birthday. I will try to integrate the available information, often in speculative ways, and indicate the kind of experiments that may help to resolve important issues. For the sake of brevity, my discussion will focus on the best studied form of plasticity—the NMDAR-dependent form of LTP in the CA1 hippocampal region.

2. LTP/LTD/SILENT SYNAPSES: WHAT IS THE STATE DIAGRAM OF THE SYNAPSE?

As a field focused on the changes in the state of the synapse, it is crucial to know the number of states a synapse can have. In figure 1 I have summarized results from different publications that are relevant to this still unsolved issue. In the simplest case, the strength of synapses would be controlled by changes in a single process. It now appears, however, that LTP and LTD involve fundamentally different processes. The clearest evidence comes from the study of the GluR1 phosphorylation (Lee et al. 2000). Synapses start out in the ‘naive’ state (this is an operational definition meaning that the experimenter has not yet performed a manipulation). The induction of LTP then brings about an increase in the phosphorylation of the CaMKII site on GluR1, a process that can be reversed by ‘depotentiation’. By contrast, if LTD is induced from the ‘naive’ state, there is dephosphorylation of the PKA site on GluR1, a process that is reversed by ‘de-depression’. These findings suggest the state diagram shown in figure 1a. An important conclusion is that there must be two separate molecular memories at the synapse, one for LTD and one for LTP.

Note, however, that figure 1a contains no mention of ‘silent’ synapses (lacking AMPA, but not NMDA channels). Is this yet another state? Recent work (Montgomery & Madison 2002) studied the ‘unitary responses’ made by a single axon on a target cell and thus
gives a clearer view than previous work utilizing large populations of synapses. This study showed that LTD induction could often drive the synapse to a silent state. Perhaps then, there are two fundamental reversible processes, as illustrated in figure 1b:

(i) The unsilencing of synapses produces an 'active state'; when LTD is then induced, synapses can be silenced.

(ii) Activated synapses can be further potentiated, a process that can be reversed by depotentiation (interestingly, this occurs by a process that differs pharmacologically from 'silencing').

According to this view, the reason that experimentalists working on large populations of synapses cannot drive transmission to zero with LTD induction protocols is that some of the synapses are already in the potentiated state and that these undergo depotentiation, not LTD. The further study of unitary connections is a promising way to resolve this issue.

When a synapse undergoes LTP, does it undergo a gradual strengthening or is there a large discrete change? Petersen et al. (1998) used a repetitive induction protocol and found that the unitary response increased suddenly in an all-or-none manner (figure 1c). Importantly, the increase in synaptic current (as large as 15 pA) was much larger than could be carried by a single AMPA channel (ca. 1 pA), suggesting that a group of about 10–20 channels is involved. The term 'slot' has been used to denote the mechanism by which a channel is held in the synapse (Shi et al. 2001). I will use the term 'hyperslot' to describe an anchoring system capable of holding 10–20 AMPA channels. The average mushroom spine has 80 AMPA channels.

channels (Matsuzaki et al. 2001) and an area of 0.2 μm² (Harris et al. 1992). Based on these values, it can be estimated that a hyperslot diameter is ca. 0.2 μm, on the same order as the smallest CA1 synapses (Lisman & Harris 1993).

Having undergone LTP, is this the end of the road for a synapse or is further potentiation possible? Petersen et al. (1998) did not observe further potentiation, but they did not wait very long. In a different study (Frey et al. 1996) it was found that it was possible to induce further LTP if several hours progressed after LTP saturation (figure 1d; see also Dixon et al. (2002)). Taken together, these results suggest that with repeated LTP episodes, multiple hyperslots can be added to the synapse. This interpretation meshes well with the finding that at the largest CA1 synapses there are over 100 AMPA channels (Matsuzaki et al. 2001). Other ideas about states of the synapse are not dealt with in figure 1 (see figure 1 caption and other ideas about synaptic growth introduced later in this paper). It is not yet possible to integrate all of these ideas in a simple way. Thus, the specification of a state diagram of the synapse must be considered to be a future goal of the field.

3. LTP INDUCTION: Ca²⁺ SENSORS

One of the major accomplishments in the LTP field has been to elucidate the role of the NMDA channel in LTP. There is general agreement that Ca²⁺ entry through NMDA channels triggers LTP, but it remains unclear whether induction is caused by Ca²⁺ elevation in the spine cytoplasm or in a local domain close to the mouth of the NMDA channel. A recent study analysed the effect of buffer concentration on LTP induction and concluded that during some induction protocols, a local domain is involved (Hoffman et al. 2002). Interestingly, different induction protocols were not only differentially sensitive to Ca²⁺ buffers, but also differentially sensitive to the knockout of GluR1. The kinetics of onset and decay were also different. These results suggest that there are multiple Ca²⁺ sensors, each of which is coupled to a different potentiation mechanism. More generally, it seems difficult to escape the conclusion that LTP is complex, with even the early phase of LTP involving multiple mechanisms.

The primary Ca²⁺ sensor for LTP is CaMKII. Direct measurements show that CaMKII is activated and auto-phosphorylated by LTP induction (Fukunaga et al. 1993). This activation is required for LTP induction (Otmakhov et al. 1997; Giese et al. 1998), as indicated by genetic and pharmacological experiments. Importantly, the block of LTP in mature animals is nearly complete, suggesting that all components and phases of LTP require CaMKII activation. Other experiments show that the introduction of active CaMKII induces potentiation that closely mimics LTP and occludes with it (Lledo et al. 1995). A broad range of results support the idea that CaMKII activation is necessary and sufficient for LTP induction and is integral to the synaptic plasticity processes that occur during development and learning (Lisman et al. 2002). The activation of CaMKII occurs in multiple steps and these may initiate different LTP expression mechanisms (figure 2). During synaptic activity, the kinase translocates from the cytoplasm to the PSD (Shen et al. 2000; Dosemeci et al. 2001). There, it binds to the NMDA channel where it becomes ideally positioned to sense the very high Ca²⁺ levels in the microdomain of the NMDA channel (Gardoni et al. 1998; Strack et al. 2000). The translocation requires the activation of CaMKII activity, but does not require autophosphorylation of the enzyme—the change that leads to persistent Ca²⁺-independent activity. Such autophosphorylation is much more likely to occur once the kinase is bound to the NMDA channel, both because the Ca²⁺ levels are higher there and because the binding to the NMDA channel itself promotes autophosphorylation (Bayer et al. 2001). Theoretical work suggests that there may be another step in CaMKII activation that requires the crossing of an additional threshold: the nearly full autophosphorylation of multiple (n) nearby CaMKII holoenzymes in the PSD (Lisman & Zhabotinsky 2001). Only in this condition, can a CaMKII switch remain ‘on’ despite phosphatase activity (see § 8), a necessary condition for a stable molecular memory. As suggested in figure 2, this form of CaMKII is likely to control the addition of hyperslots to the synapse.

A second Ca²⁺ sensor involved in LTP is the Ca/calmodulin-activated adenylate cyclase (Chetkovich & Sweatt 1993; Wong et al. 1999). The cAMP pathway apparently does not directly trigger potentiation, but stimulates neural activity that triggers LTP by the normal NMDA-dependent process (Ma et al. 1999; Makhinson et al. 1999; Bozdogi et al. 2000). Elevation of cAMP can work synergistically with CaMKII-dependent process. In particular, elevation of cAMP leads to inhibition of PIP₁, thereby enhancing CaMKII autophosphorylation and CREB activation (Brown et al. 2000; Genoux et al. 2002). The CREB pathways then trigger the synthesis of proteins that may be important for the structural component of CaMKII-dependent potentiation (see § 4). Other potential Ca²⁺ sensors include PKC, calpain and NOS.

Biophysical work is badly needed to measure the Ca²⁺ dynamics during LTP induction and to determine the properties of Ca²⁺ sensors. Although great progress has been made in Ca²⁺ imaging, there has not yet been a study of the Ca²⁺ dynamics during LTP or LTD induction at synapses that are demonstrably active during the process. It will also be important to understand more about calmodulin, the protein that couples Ca²⁺ to many enzymes. This adaptor molecule is not completely free in the cytoplasm, but can itself be ‘buffered’. A recent study shows that synaptic plasticity undergoes major changes after knockout of the postsynaptic calmodulin buffer, RC3 (Krucker et al. 2002).

4. CaMKII POTENTIATES BY THREE DIFFERENT POSTSYNAPTIC MECHANISMS: NEUROMODULATION, TRAFFICKING AND ANCHORING

It initially appeared as though LTP expression could be accounted for by a simple neuromodulatory process, the CaMKII-dependent phosphorylation of existing AMPA channels and the consequent increase in their single channel conductance (Barria et al. 1999a; Benke et al. 1998) (pathway 2 in figure 2). More recent work shows, however, that this component is relatively small (Poncer et al. 2002) and that LTP can still occur in its absence (Benke et al. 2002).
Figure 2. Three postsynaptic pathways by which CaMKII produces LTP expression. Two Ca2+ sensors are shown, CaMKII and adenylate cyclase (how Aurora is activated remains unclear). CaMKII has sequential activation states, which may be differentially coupled to three different expression mechanisms. (1) Trafficking: GluR1 vesicular delivery pathway produces quasi-local delivery of microclusters of GluR1 and associated slot proteins to the plasma membrane. An existing synapse is strengthened by filling an unfilled slot with an AMPAR. (2) Neuromodulation: strengthening may also occur by CaMKII-dependent phosphorylation of GluR1, leading to enhanced single channel conductance. (3) Anchoring: a hyperslot assembly may be added to a silent synapse or, as shown, to a synapse that already has one assembly. The building blocks for the hyperslot assembly may come from several sources, including the GluR1 containing vesicle. In addition, transcription and translation may provide building blocks, one of which is CaMKII alpha homomers. A hyperslot assembly includes the CaMKII switch in its ‘off’ phosphorylated state, AMPA channels, the hyperslot that anchors the channels at the synapse, and linker proteins that bind the switch to the hyperslot. A major goal is to determine the time course and magnitude of different expression mechanisms, as well as their effect on quantal analysis parameters. Tentative answers are given on the right-hand side.

et al. 1998; Hayashi et al. 2000; Lee et al. 2003), indicating that other mechanisms are also involved.

There is now good evidence for a second mechanism (pathway 1 in figure 2): CaMKII drives a ‘trafficking’ process that involves vesicular delivery of GluR1 into the extrasynaptic membrane. This is followed by the diffusion of channels to synapses and their anchoring there (Chen et al. 2000; Hayashi et al. 2000; Passafaro et al. 2001). The extrasynaptic delivery of GluR1 is likely to occur in a large dendritic region near the active synapse. This would explain the finding that LTP induction enhances the response to the quasi-local application of glutamate (Montgomery et al. 2001), an increase that is hard to understand if the only change in AMPA channels occurs at the small fraction of synapses that undergo LTP. It would seem likely that some of the slots that anchor AMPA channels at synapses (see below) are empty; increasing the extrasynaptic concentration of channels could fill these slots. As this could happen at any synapse near the site of GluR1 delivery, one can understand why there should be a component of LTP that is not specific to stimulated synapses (Engert & Bonhoeffer 1999). Consistent with the somewhat diffuse character of the GluR1 delivery mechanism is its mediation by a cascade involving Ras and the soluble MAPK amplification system (Zhu et al. 2002). One of the most remarkable manipulations of GluR1 delivery involves the effect of PI3K inhibitors. These block the delivery of GluR1 to the extrasynaptic membrane (Passafaro et al. 2001) and selectively interfere with transmission at synapses that have undergone LTP (Sanna et al. 2002). The effect of PI3K inhibitors is reversible, indicating that the process is driven by some upstream synaptic memory. PKC also appears to be involved in this process (Daw et al. 2002; Ling et al. 2002). According to the ideas developed by Malinow and his associates (Shi et al. 2001) this persistent ‘GluR1 pathway’ will eventually subside, to be replaced by a constitutive ‘GluR2 pathway’ for AMPA channel delivery. It will

be important to determine the timing of this transition. It will also be important to obtain direct evidence for the concept of an unfilled slot. So far there is only suggestive evidence: the gradual slide in the separation of quantal peaks observed during low-frequency depression (Larkman et al. 1997). A direct demonstration would be evidence for a change in the ratio of slot proteins to AMPA channels at the synapse.

The third CaMKII-dependent expression mechanism (pathway 3 in figure 2) involves the generation of new AMPA anchoring sites (‘hyperslots’) at synapses (Shi et al. 2001). AMPAification of silent synapses has been widely discussed, but as argued previously, it is also likely that hyperslots can be added to synapses that already have AMPA-mediated transmission. There is still no consensus on the identity of slot proteins. The candidates are proteins known to interact with AMPA channels including SAP97, protein 4.1, GRIP and APB. Recent work suggests that PSD95 may be a critical slot protein (Schnell et al. 2002). AMPA channels are linked to PSD95 by the protein, stargazin. Overexpression of PSD-95 can enhance synaptic transmission (El-Husseini et al. 2000) whereas delocalizing the protein from the synapse by depalmitoylation decreases transmission (El-Husseini et al. 2002). It remains unclear how the phosphorylated state of CaMKII can control the addition of hyperslots. It is known that CaMKII bound in the PSD is less than 30 nm from the channels (Peterson et al. 2003) so the distances involved are not large. One possible linkage is CaMKII to actin to actin to the protein 4.1/SAP97 complex (Lisman & Zhabotinsky 2001) because each of the individual binding interactions has been demonstrated. Whatever the exact structural linkage, it seems likely that there is an entire assembly of proteins that produce AMPAification; this includes the reversible CaMKII switch, the hyperslots that directly anchor AMPA channels and linker proteins that couple CaMKII to the hyperslots. I term this entire structure the hyperslot assembly (figure 2). In addition, there must be a trans-synaptic structure that ensures that the hyperslot assembly is added to the postsynaptic membrane in proper alignment with the presynaptic structures. Changes in the number of such structures could give rise to a presynaptic component of LTP—a possibility that will be discussed in § 6.

5. A STRUCTURALLY EXPLICIT THEORY OF QUANTAL ANALYSIS

The discovery that synapses can be ‘silent’ revolutionized the interpretation of quantal analysis by demonstrating the existence of a postsynaptic mechanism that could change the probability of transmission, a change that was previously interpreted to imply a presynaptic mechanism. Here, I will briefly describe further revisions of quantal analysis that we have developed in my laboratory. In essence what we are proposing is that AMPA-mediated transmission at large synapses is modular; multiple vesicles can be released, each acting preferentially on the local pool of AMPA receptors near the site of release. Some local regions of the synapse may lack AMPA receptors. Such synapses can be considered ‘partly silent’ since vesicles released at non-AMPAfied subregions will not generate a substantial AMPA response.

Central to this new view are five concepts.

(i) Quantal size can be estimated from the separation of quantal peaks in amplitude histograms (Foster & McNaughton 1991; Larkman et al. 1991; Malinow 1991; Kuhnt et al. 1992; Kullmann & Nicoll 1992; Liao et al. 1992; Stricker et al. 1996a; Magee & Cook 2000). The quantal size of 5–10 pA can be accounted for by the opening of about 10–20 AMPA channels (Magee & Cook 2000). Importantly, the narrowness of quantal peaks implies that the CV of the quantal response is small (<0.2) (Stricker et al. 1996b).

(ii) Average mEPSCs (<10 pA) activate only a small fraction of the total channels at a synapse (Liu et al. 1999; McAllister & Stevens 2000). Large mEPSCs are probably multiquantal (Bolshakov et al. 1997).

(iii) EPSCs at large CA1 synapses are multiquantal, with the number of vesicles released being potentially greater than 10 (Oertner et al. 2002; Conti & Lisman 2003). Quanta summate nearly linearly; the variability of the number of effective release events accounts for the very high trial-to-trial variation of the AMPAR-mediated EPSC (4–40 pA) and NMDAR-mediated Ca$^{2+}$ entry (Conti & Lisman 2003).

(iv) The rise time of small mEPSCs recorded with improved methodology is very fast (<50 μs) (Magee & Cook 2000). In this brief period, glutamate remains highly concentrated near the site of vesicle release (100 nm). This ‘spike’ of glutamate is efficient at activating the AMPA channels in this region (Raghavachari & Lisman 2003). As glutamate spreads, its concentration falls and AMPA channels are no longer efficiently activated, particularly because low concentrations drive desensitization better than activation. The net result is that each vesicle release causes a hotspot of channel activation ca. 200 nm in diameter. This hotspot is of the same order as our estimate of the dimension of a hyperslot, which contains a sufficient number of AMPA channels to generate a quantal event. Thus, synapses may be modular, with AMPA transmission occurring independently in each module.

(v) Some modules may be ‘silent’, whereas others are not; thus synapses can be ‘partly silent’. Vesicles released in ‘silent’ modules generate an NMDA component (which does not depend on the location of vesicle release), but not a significant AMPA component. Other vesicles released over AMPAfied modules would generate both components. This could explain two remarkable recent findings. The first is that the AMPA/NMDA ratio of evoked responses at single synapses can vary dramatically from trial to trial (Renger et al. 2001). Lack of an AMPA response could be explained if release occurred at a silent module. The second result is that block of desensitization can dramatically increase the probability of response at low p synapses (Diamond & Jahr 1995; Choi et al. 2000; Gasparini et al. 2000). Our simulations indicate that that this can be explained because the glutamate released at silent modules can affect distant AMPA channels.

when desensitization is blocked (an alternative explanation (Choi et al. 2000) is that some vesicles release their content too slowly to activate AMPA channels).

In summary, many vesicles may be released at large synapses, with each activating an independent group of channels near the site of vesicle release (i.e. within a module).

This view of transmission has substantial impact on the way quantal analysis is interpreted. Importantly, the progressive addition of hyperslots and AMPA channels will not substantially affect quantal size, which is determined by other factors including the single channel conductance, the fraction of unfilled slots and the transmitter content of the vesicles. The probability of the response can be affected either by AMPAfication of modules or by changes in the probability of release. Thus, neither quantal size nor the probability of response can be used to unambiguously determine the locus of LTP expression. Several further implications should be noted. First, the shortcut method for monitoring synaptic strength by measuring mEPSP size is now dubious; there seems no alternative but to directly measure the average evoked responses, a technically demanding task at single synapses. Second, the concept of partly silent synapses makes it possible to understand how the pairing protocol for LTP induction can routinely produce massive potentiation (up to 400%) through a purely postsynaptic process (see §6) and do so under conditions (age 2–3 weeks) where there are relatively few (ca. 25%) silent synapses (Nusser et al. 1998; Petralia et al. 1999; Takumi et al. 1999).

6. LTP EXPRESSION: A UNIFYING PRE/POST HYPOTHESIS

Figure 3a,b shows a silent synapse and its AMPAfication by the addition of a hyperslot assembly. I have drawn a box through the pre- and postsynaptic region to emphasize that the entire structure must be a trans–synaptic module with a given number of release sites on the presynaptic side and the ability to bind a hyperslot assembly into the postsynaptic side. In addition, the synapse contains several NMDA channels, which are placed outside the modules.

Consider now how to draw this picture if LTP causes additional hyperslot assemblies to be added to the same synapse. One view would be that the number of modules (not shown) is fixed; all that need occur is that more and more of the existing modules become AMPAfied. But according to this view, LTP will produce no change in synapse size, contrary to the available evidence. Recent work (Ostroff et al. 2002) provides the strongest indication so far that LTP induction produces an increase in the size of synapses measured 2 h after induction, consistent with previous work (Geinisman et al. 1995; Buchs & Muller 1996; Bozdagi et al. 2000; Weeks et al. 2003). If this is true, there must be a change in the size of the presynaptic active zone, an increase in the number of release sites and thus a presynaptic component of LTP.

A simple model that can account for both the structural changes and the evidence for silent synapses is as follows. The synapse consists of a variable number of trans–synaptic modules. The presynaptic side of the module contains a certain number of release sites; the postsynaptic side

may or may not contain a hyperslot assembly and thus may be AMPAfied or silent. LTP expression involves two structural processes.

(i) Addition of ‘silent’ trans–synaptic modules: this produces a growth in the size of the synapse and an increase in the number of presynaptic release sites.

(ii) Postsynaptic AMPAfication of ‘silent’ modules.

It is envisioned that these processes occur together or independently, depending on the induction conditions, and that the number of NMDA channels is not strongly affected by either process (Racca et al. 2000). If only AMPAfication occurs, LTP will appear postsynaptic; if module addition occurs, there will be enhanced presynaptic release owing to the increase in number of release sites. In essence what is being proposed is a hybrid of the silent synapse model and the model of Bolshakov et al. (1997). The proposal that synapses grow by the addition of trans–synaptic modules provides a simple explanation for one of the most striking features of synapse architecture: the exact registration of the presynaptic active zone and the PSD despite wide variation in synapse size (Lisman & Harris 1993).

Of particular importance to resolving the pre/post debate is that different induction protocols may evoke process 1 and 2 in different proportions. When LTP is induced by standard pairing protocols (low-frequency presynaptic stimulation and strong postsynaptic depolarization), there is little or no change in the NMDA component (Kullmann 1994; Selig et al. 1995; Montgomery et al. 2001). This is what would be expected by process 2 alone and provides one of the strongest lines of support for the purely postsynaptic model of LTP. However, LTP of the NMDA component is large when LTP is induced by high-frequency stimulation (Bashir et al. 1991; Asztely et al. 1992; Xie et al. 1992). This would be consistent with the idea that high-frequency presynaptic activity is required for the addition of trans–synaptic modules and the consequent increase in presynaptic release. Thus, a potential resolution of the pre/post debate would be that under some induction conditions only a postsynaptic component of expression is involved, whereas under others condition both pre- and postsynaptic components are involved.

Before this resolution can be accepted, it will be necessary to strengthen the case that the LTP of the NMDA component is indeed caused by enhanced release rather than by a postsynaptic change in the number or conductance of NMDA channels. There has been surprisingly little work on this issue, but the available evidence points to a presynaptic mechanism. Kullmann et al. (1996) reported that LTP of the NMDA component is associated with a decrease in the CV, consistent with a presynaptic change. More directly, a preliminary report (Dixon et al. 2002) indicates that the probability of the NMDA–mediated Ca2+ signals at single spines increases after LTP induction. If confirmed, these results would provide strong support for an increase in release.

7. LTP MAINTENANCE: SYNAPTIC MEMORY VERSUS ATTRACTORS

One of the major goals of the study of LTP is to understand the molecular basis of synaptic memory. A key ques-
tion is whether our ability to remember something for a lifetime requires that synaptic memory be stable for that duration. This may not be the case. One interesting alternative (Wittenberg & Tsien 2002) is that the molecular memory of a synapse is relatively short (perhaps only months) and that longer-term storage relies on the ability of autoassociative attractor networks to refresh this memory. What stores autoassociative information in an attractor network is the mutual enhancement of excitatory transmission between the subgroup of cells that represent a memory. It is exactly this kind of mutual enhancement that can be produced by the Hebbian form of LTP. Once synapses are strengthened in this way, the subgroup of cells that represent a memory form an ensemble that can be easily reactivated and fire persistently. Owing to the redundancy of the connections, such reactivation can occur even after partial decay of LTP. The reactivation could strengthen weakened synapses through additional LTP, restoring the strength of the original memory. There is increasing evidence that memories are in fact reactivated periodically, probably during sleep. Thus, the requirement at the synaptic level is that the average stability of LTP be long compared with the interval at which memories are reactivated. Unfortunately, no estimate is yet available for this interval.

8. MOLECULAR MEMORY MECHANISMS: CaMKII AND PROTEIN-SYNTHESIS-DEPENDENT STRUCTURAL CHANGE

There have been two main proposals regarding the molecular basis of synaptic memory, the CaMKII hypothesis (Lisman & Zhabotinsky 2001) and protein synthesis hypothesis (Kandel 2001). I will first discuss these separately and then suggest ways in which they can be reconciled.

CaMKII is a potential memory molecule because it undergoes persistent activation after LTP induction (Fukunaga et al. 1993). Importantly, a single amino-acid substitution at Thr286 that blocks the generation of persistent activation, blocks LTP and interferes strongly with memory in behavioural tests (Giese et al. 1998). It has been argued that the kinase acts as a reversible memory switch localized at each synapse. Detailed models now exist showing how the biochemically established properties of the kinase could give rise to a stable memory switch (Lisman & Zhabotinsky 2001). The key properties are as follows.

(i) The ability of phosphorylation of Thr286 to make the subunit display Ca\(^{2+}\)-independent activity (Miller & Kennedy 1986).

(ii) The ability of an active subunit to phosphorylate a neighbouring subunit, thereby providing a positive feedback autocatalytic reaction (Hanson et al. 1994).

(iii) The demonstration that the PP1 held in the PSD is the only phosphatase allowed access to PSD-associated CaMKII (Strack et al. 1997). This implies that the relevant chemistry is local and has the important consequence that PP1 will be saturated when CaMKII becomes hyperphosphorylated.

Simulations of these reactions show that a group of CaMKII holoenzymes in the PSD can have the stable ‘on’ and ‘off’ states required of a memory switch. Furthermore, the PP1 saturation provides a novel mechanism for interactions among holoenzymes that is important for long-term stability; during protein turnover a newly inserted holoenzyme will become phosphorylated if PP1 is saturated by neighbouring phosphorylated holoenzymes. Because of this process, a switch of this kind can be stable despite the protein turnover of its constituents. As noted earlier, there is growing evidence that CaMKII acts as a reversible memory switch; its phosphorylation is increased during LTP and decreased by depotentiation (Barria et al. 1997b; Huang et al. 2001). An important outstanding question is whether inhibition of PSD CaMKII can reverse LTP or memory maintenance, as would be expected if CaMKII is a memory molecule.

According to the protein synthesis model, LTP induction activates transcription and translation. The newly synthesized proteins somehow produce stable synapses, perhaps by promoting their growth. The key experimental support is that transcription and translation inhibitors applied just before LTP induction interfere with the late phase of LTP (Frey et al. 1988). Conversely, the late phase can be enhanced by constitutive activation of CREB and the resulting enhanced synthesis of proteins (Barco et al. 2002). Newly synthesized molecules are not targeted exclusively to the synapses whose activity induced their synthesis, but rather can be captured by any synapse that has undergone LTP. This was nicely demonstrated in experiments where LTP was induced in the presence of protein synthesis inhibitors (Frey & Morris 1997, 1998). These synapses nevertheless expressed late LTP if other synapses were allowed to stimulate protein synthesis either immediately before or just after the period of protein synthesis inhibition. It thus appears that synapses that have undergone LTP contain a ‘tag’ that allows them to capture newly synthesized proteins. Once captured, the potentiation of these synapses becomes stable. The identity of the captured proteins and the mechanism by which they make potentiation stable is not specified in this model.

9. RECONCILING THE CaMKII AND PROTEIN SYNTHESIS MODELS

As mentioned earlier, it was initially thought that LTP expression mechanisms might be modular in nature, but it now appears clear that structural processes are also involved. If hyperslot assemblies and trans-synaptic modules are added to synapses, new building blocks will be required and these may have to be newly synthesized. Recent work provides evidence that the synthesis of one component of the hyperslot assembly, CaMKII (Miller et al. 2003) is in fact required for late LTP. CaMKII is one of the small group of proteins that is synthesized in the dendrites and whose synthesis is rapidly and locally induced by LTP induction (Burgin et al. 1990; Ouyang et al. 1999; Huang et al. 2002). This occurs as a result of the rapid translation of pre-existing mRNA (Huang et al. 2002). Miller et al. (2003) modified the targeting signals on CaMKII mRNA, abolishing its entry into the dendrites. This produced no change in early LTP, but a marked reduction in the stability of late LTP. Furthermore, PSDs isolated from these animals had enormously reduced CaMKII content (17% of normal; total CaMKII
Figure 3. Both presynaptic and postsynaptic processes can contribute to LTP at synapses composed of trans-synaptic modular elements. (a) A silent synapse containing a single ‘silent’ module. LTP induction leads to its AMPAification (b). This is a structural process that occurs through the addition of a hyperslot assembly under the control of an ‘on’ CaMKII switch. The number of NMDA channels and the number of release sites is not changed; thus only AMPA-mediated transmission is enhanced. A second LTP induction (c) leads to the addition of two new modules, enlarging the synapse pre- and postsynaptically. Furthermore, one of these modules becomes AMPAified whereas the other does not (it is unclear what determines the relative proportion of these two processes). Because vesicles primarily activate the AMPA channels in the module where they are released, the rightmost module, which lacks AMPA channels will be ‘silent’. The synapse as a whole is therefore ‘partly silent’. The enhancement of the number of release sites will produce LTP of the NMDA component of transmission. At large synapses, release is multiquantal. The details of the LTP induction procedure may determine the relative proportion of the two LTP processes (see §6).

is only reduced 50%). It is important to realize that dendritically synthesized CaMKII is a novel form: the holoenzymes are homomeric α whereas the somatically synthesized form contains both α and β subunits. These observations suggest the following scenario: during LTP induction pre-existing CaMKII α/β heteromers are translocated to the PSD where they become autophosphorylated and serve as the tag. Induction also activates the local synthesis of alpha homomers. These interact with the tag to somehow stabilize the late phase of LTP. Why homo- 

meres would have this special ability is unclear, but the fact that they, unlike heteromers, can interact with the densin/actinin complex in the PSD is intriguing (Walikonis et al. 2001). In any case, the fact that synaptic strengthening involves structural modification, both for adding hyperslot assemblies to existing modules and for adding new modules, clearly must require new proteins. Thus, a simple unifying model would be that the CaMKII switch is the molecular storage device, but that the structural process of building a more powerful synapse cannot be completed without protein synthesis.

10. LTP IN AUTO-ASSOCIATIVE NETWORKS

I now turn to a discussion of the role of LTP in actual learning processes. The recent paper from Tonegawa’s laboratory is a milestone in the study of LTP because it studies the effect of modifying LTP in a specific network (CA3), the behavioural function of which can be understood in terms of established neural network concepts (Nakazawa et al. 2002). It has long been assumed that memories are stored in the distributed synapses of recurrent networks. A critical requirement for such autoassociative memory networks is that the synapses must display a Hebbian form of LTP. It has thus been very satisfying that the CA3 network is a recurrent one (figure 4) and that the LTP found at these synapses is the NMDA-dependent Hebbian form. It is this broad hypothesis that has now been tested by genetically disabling NMDA-dependent LTP in the CA3 region. According to the theory of autoassociative networks, when a partial or degraded form of the memory A (designated A₁) is provided as input to the network, the output will be the correct memory, A. Consistent with this, normal animals can recognize a partial stimulus whereas mutant animals lacking NMDA channels in CA3 cannot.

As technically impressive as this experiment is, the bar can always be raised. CA3 cells have two types of synapse with NMDA-dependent LTP: the recurrent synapses and the perforant path synapses. In the ‘ultimate’ experiment, LTP would be disabled only at the recurrent synapses. A further complicating factor is that NMDA channels not only produce the Ca²⁺ entry that triggers LTP, but also produce slow EPSPs that can be important for information transmission. Thus, an ‘ultimate, ultimate’ experiment might specifically delete the Ca²⁺ permeability of the
channel required for LTP induction while leaving intact the Na⁺ permeability required for EPSP generation.

The results described above strongly suggest that NMDAR-dependent LTP is necessary for actual learning; a related important discovery is that a natural learning process strengthens synapses by the same molecular processes that mediate LTP in the slice preparation (Takahashi et al. 2002). Specifically, it was shown that when cells in barrel cortex are virally transfected with GluR1 receptors (recognizable by their altered rectification), these receptors are driven into the synapse when the animal uses its whiskers, but not if the whiskers are removed. This result further strengthens the idea that LTP is involved in behaviourally meaningful synaptic modification.

11. LTP IN HETEROASSOCIATIVE NETWORKS (STORAGE OF MEMORY SEQUENCES BY THE HIPPOCAMPUS)

Although it has long been clear that NMDAR-dependent LTP is ideally suited for the formation of autoassociative memories, this is not the only form of memory. A second type, heteroassociative memory, deals with the links between temporally separated items. Can NMDAR-dependent LTP also subserve this function or is some entirely different mechanism required? This is a topic that can be discussed in terms of very specific findings regarding the anatomy, physiology and function of the hippocampus. There is now strong evidence that the overall memory function of the hippocampus is more than just a simple autoassociative memory for isolated memory items. Rather, the hippocampus may be a specialized device for the storage and recall of memory sequences. Behavioural tests of odour sequences show that hippocampal animals fail to recognize odour sequences even though they can recognize individual odours (Fortin et al. 2002). Recordings from hippocampal place cells have revealed the phenomenon of ‘phase precession’ (O’Keefe & Recce 1993). This appears to be the hippocampus caught in the act of a high-speed recall of the sequence of places along a path (Jensen & Lisman 1996; Skaggs et al. 1996; Tsodyks et al. 1996). Finally, during sleep, sequences that occurred during wakefulness are replayed (Skaggs & McNaughton 1996; Nadasdy et al. 1999).

To store and recall sequences, there must be a synaptic modification process that forms the heteroassociative linkages between cells encoding sequential memory items (linking A to B). Such linkages should not store autoassociative linkages (linking elements of A; e.g. A1 and A2). As shown in figure 5, standard LTP can selectively form ‘pure’ heteroassociative links provided there is a delay in the recurrent feedback. In ongoing work, we (Raffone et al. 2003) are attempting to determine how the multiple fields of the hippocampus could work together to encode autoassociative and heteroassociative synaptic weights and thereby enable the system to perform sequence storage and recall. Figure 4 describes our current view. The general idea is that a heteroassociative network alone could not produce correct sequence recall

Figure 4. The (a) dentate and (b) CA3 hippocampal networks responsible for the storage of heteroassociative and autoassociative memory. The known bidirectional interconnections between these networks is appropriate for sequence recall (see § 11). Of particular note is that the transmission of information from the dentate to CA3 and back again provides the delay needed to produce the heteroassociative synaptic linkages required for sequence learning and recall (see figure 5).
because a step in recall produces slight corruption (B’ instead of B). If B’ is used as a basis for the next step in the sequence this leads to an even more corrupted item, C’. This concatenation of errors can, however, be avoided if there is an interplay of autoassociative and heteroassociative networks: B’ is sent to an autoassociative network where it is converted to B; B is then sent to the heteroassociative network where it produces C’, etc. (Lisman 1999). The dentate and CA3 are both recurrent networks that are bidirectionally connected (figure 4). We now envision that CA3 is doing the autoassociation whereas the heteroassociation occurs in the dentate. The back and forth flow of information between these networks could produce correct sequence recall. The fact that the phase precession occurs in both the dentate and CA3 is consistent with this model. Importantly, the time that it takes information to flow from the dentate to CA3 and back to dentate could provide exactly the kind of delay needed to promote pure heteroassociative links in the dentate feedback synapses (figure 5). Thus, under different conditions, the same NMDA-dependent form of LTP may be able to subserve both autoassociative and heteroassociative memory. A critical test of this hypothesis would be to disable NMDA function at feedback synapses in the dentate; this should lead to deficits in sequence recall without interfering with autoassociative memory.

12. CLOSING REMARKS

An unhealthy field becomes closed and progress ceases. Just the opposite has been true for LTP. A field that began with simple extracellular recordings has now made pro-
ductive interfaces with biophysics, pharmacology, biochemistry, molecular biology, neural network theory and behavioural psychology. Although the controversies still abound, unifying ideas are emerging. This should be a very happy birthday for LTP.

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**GLOSSARY**

AMPA: (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid

CV: coefficient of variation

LTD: long-term depression

LTP: long-term potentiation

NMDAR: N-methyl-D-aspartate receptor

NOS: nitric acid synthase

PI3K: phosphoinositol-3 kinase

PKA: cyclic AMP-dependent protein kinase

PP1: protein phosphatase-1

PSD: postsynaptic density

RC3: neurogranin