THE ROYAL SOCIETY

Published online 6 March 2003

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 1*a*. 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 1*a* 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

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

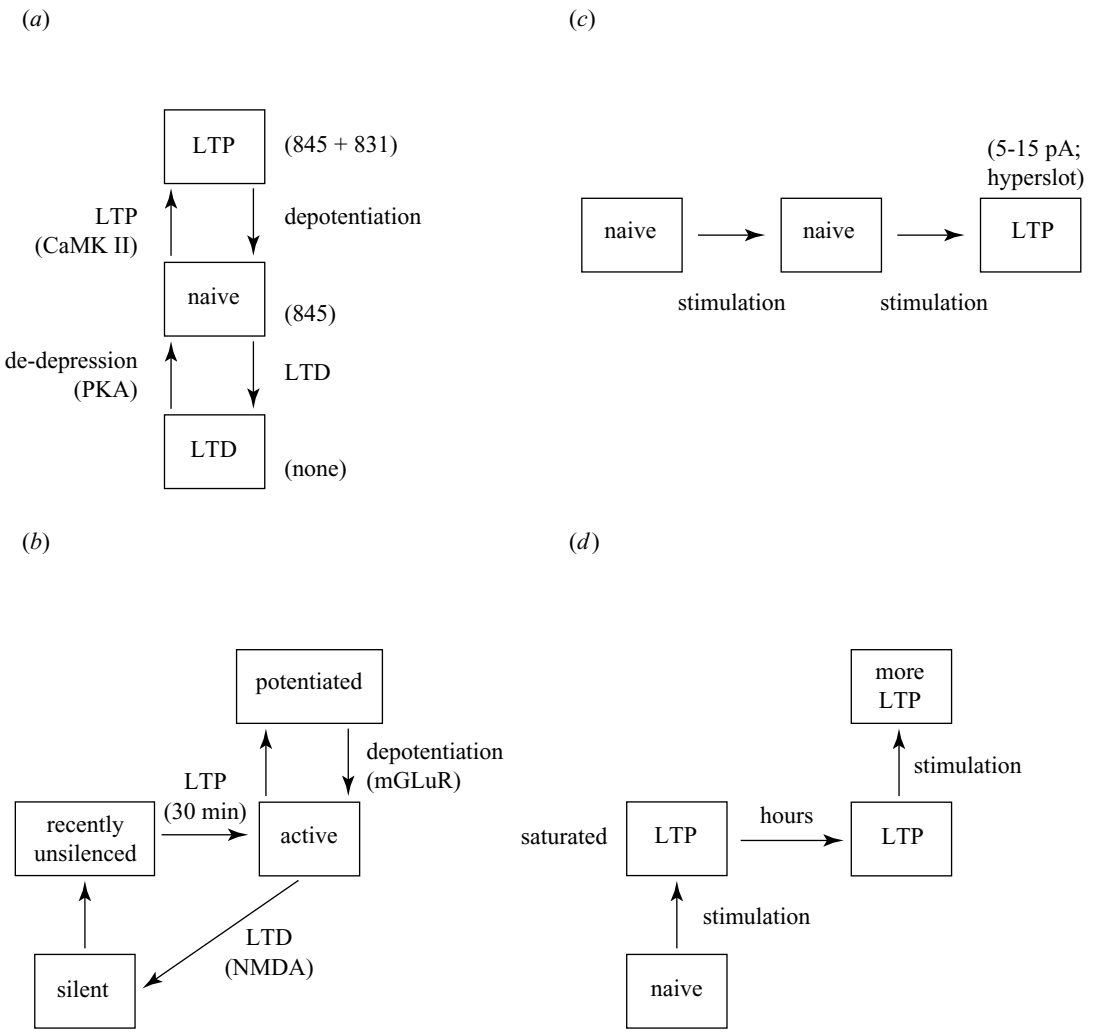

Figure 1. State diagrams of the synapse. (*a*) Three states having different GluR1 phosphorylation (845/831): states based on the phosphorylation changes of GluR1 produced by various stimulation protocols (Lee *et al.* 2000). (*b*) States of unsilenced synapse: states based on the study of unitary silent and active connections in the CA3 region (Montgomery & Madison 2002). After unsilencing, there is a 30 minute period before the synapse can be weakened. Note that depotentiation is mGluR dependent whereas LTD is NMDA dependent. (*c*) Potentiation occurs by discrete addition of a multi-channel unit: a unitary connection is given repeated moderate stimulation. At some point an all-or-none increase in the synaptic response occurs. The increase in current is much larger than can be carried by a single channel as it is envisioned to involve the incorporation of a 'hyperslot' into the synapse (Petersen *et al.* 1998). (*d*) Further LTP can be induced after delay: after LTP saturation, the synapse can again undergo LTP, but only after several hours (Frey *et al.* 1995). An additional state-change not illustrated here is that mGluR receptors are initially required for LTP induction, but are then not further required for subsequent LTP induction (Bortolotto *et al.* 1994).

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 1*b*:

(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 1*c*). 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 (Matsuzaki *et al.* 2001) and an area of 0.2 μm^2 (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.* 1995) it was found that it was possible to induce further LTP if several hours progressed after LTP saturation (figure 1*d*; 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: Ca^{2+} 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 Ca^{2+} entry through NMDA channels triggers LTP, but it remains unclear whether induction is caused by Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} sensor for LTP is CaMKII. Direct measurements show that CaMKII is activated and autophosphorylated 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 Ca^{2+} 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 Ca^{2+}-independent activity. Such autophosphorylation is much more likely to occur once the kinase is bound to the NMDA channel, both because the Ca^{2+} 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 Ca^{2+} 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; Bozdagi *et al.* 2000). Elevation of cAMP can work synergistically with CaMKII-dependent process. In particular, elevation of cAMP leads to inhibition of PP1, 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 Ca^{2+} sensors include PKC, calpain and NOS.

Biophysical work is badly needed to measure the Ca^{2+} dynamics during LTP induction and to determine the properties of Ca^{2+} sensors. Although great progress has been made in Ca^{2+} imaging, there has not yet been a study of the Ca^{2+} 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 Ca^{2+} 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.* 1997*a*; 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

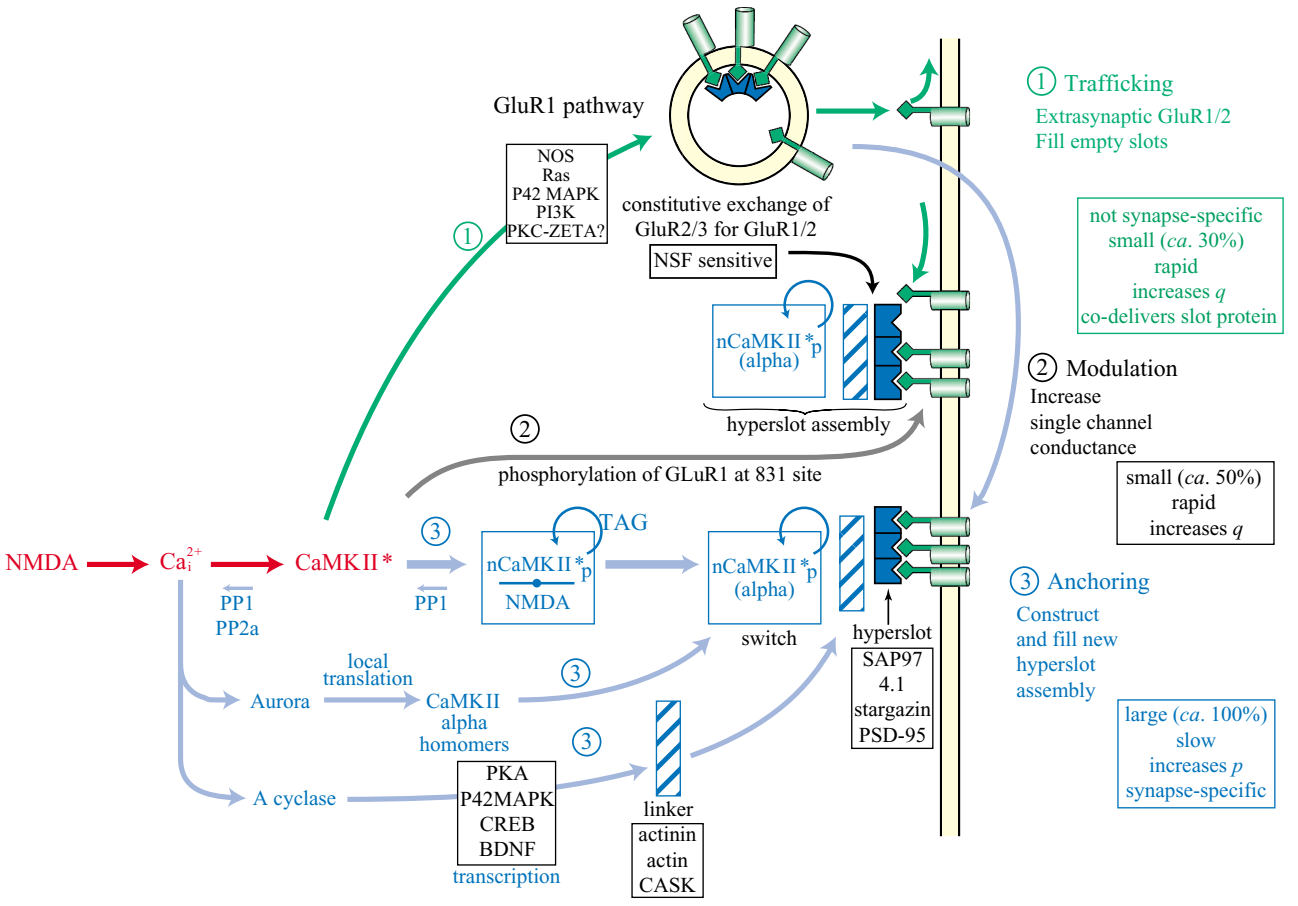

Figure 2. Three postsynaptic pathways by which CaMKII produces LTP expression. Two Ca^{2+} sensors are shown, CaMKII and adenylate cyclase (how Aurora is activated remains unclear). CaMKII has sequential activation states, which may be differentially coupled to three different expression mechanisms. (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 'on' 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). AMPAfication 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 depalmitolaytion 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 actinin 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 AMPAfication; 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.* 1996*a*; 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.* 1996*b*).

(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 3*a*,*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 NMDAR-mediated Ca^{2+} 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.* 1997*b*; 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 modulatory 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

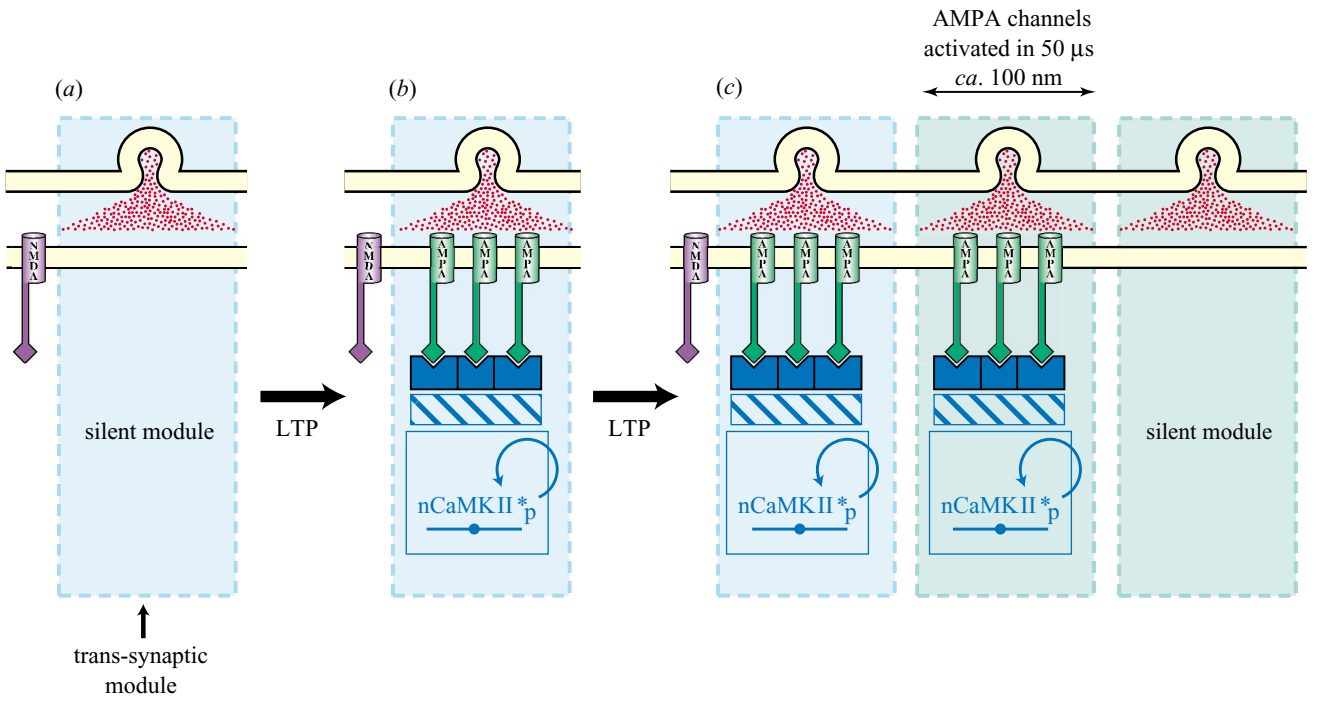

Figure 3. Both presynaptic and postsynaptic processes can contribute to LTP at synapses composed of trans-synaptic modular elements. (*a*) A silent synapse containing a single 'silent' module. LTP induction leads to its AMPAfication (*b*). This is a structural process that occurs through the addition 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 AMPAfied 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 homomers 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 A′) 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 Ca^{2+} 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 Ca^{2+} permeability of the

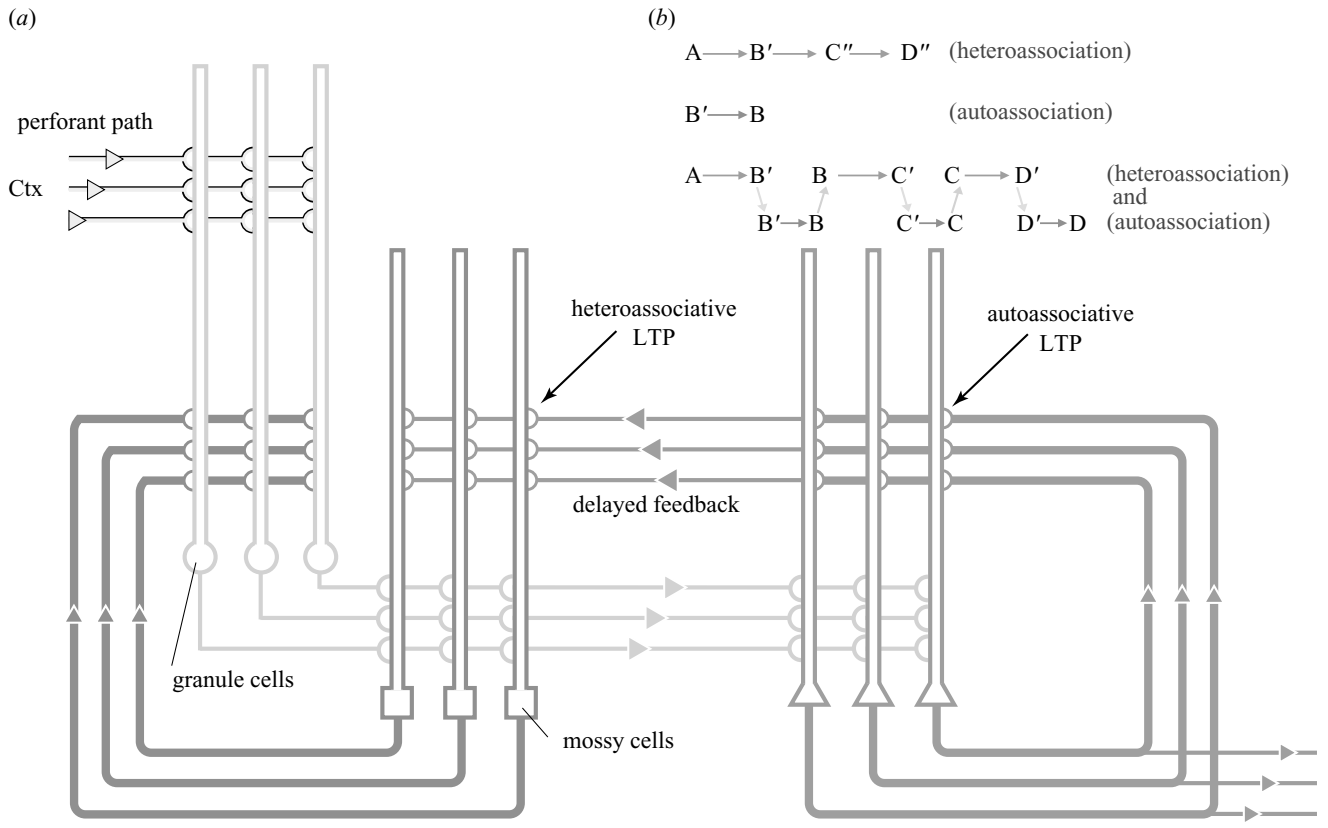

Figure 4. The (*a*) dentate and (*b*) CA3 hippocampal networks responsible for the storage of heteroassociative and autoassociative memory. The known bidirectional interconnections between these networks is appropriate for sequence recall (see § 11). Of particular note is that the transmission of information 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).

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

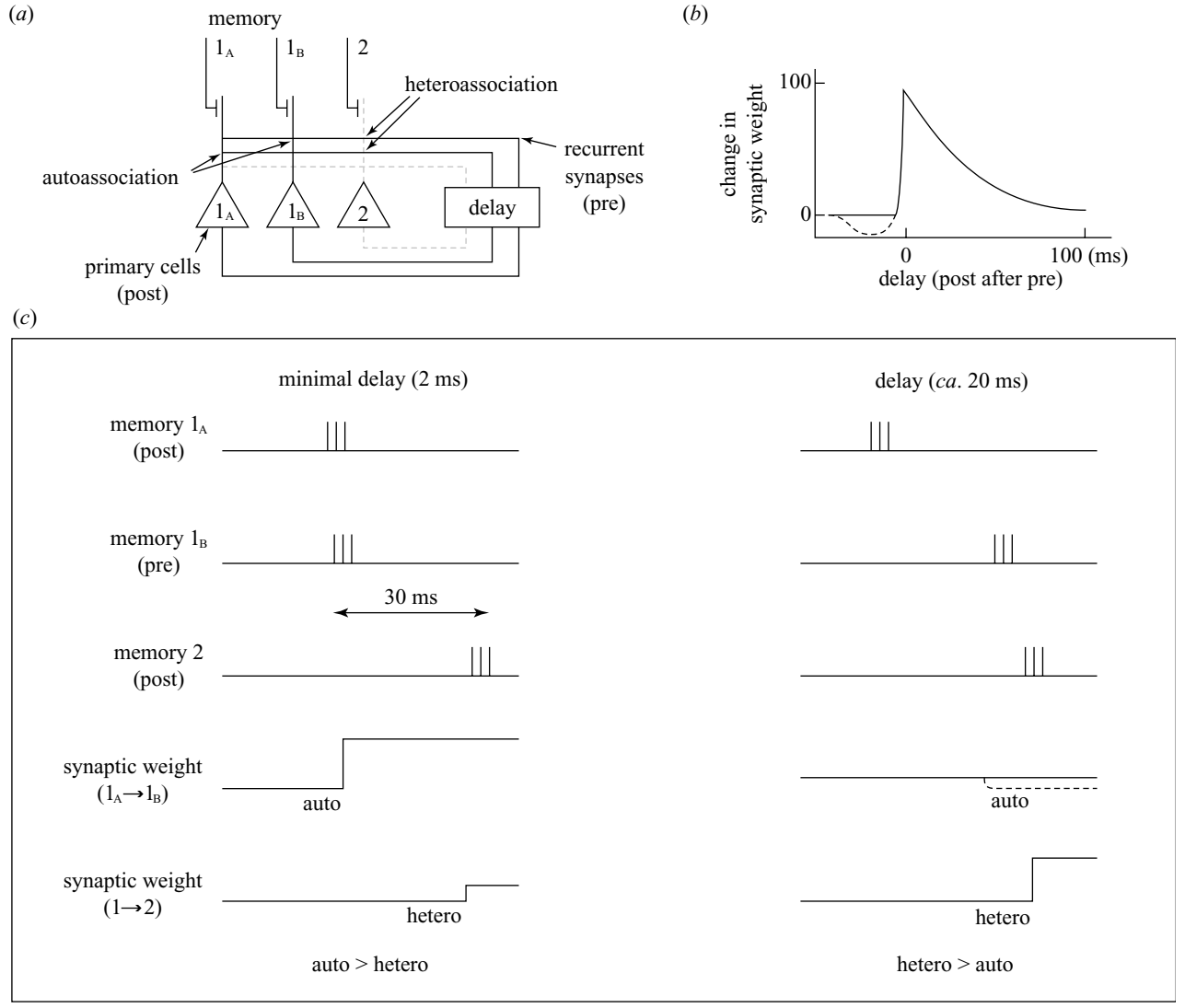

Figure 5. An argument that a single LTP learning rule (*b*) can perform autoassociation or heteroassociation depending on the delay of the feedback in recurrent networks (*a*). The autoassociation produces connections between cells 1_A and 1_B that encode two aspects of the same memory. The heteroassociation process provides connections between cells that encode sequential memories, 1 and 2. According to the learning rule, if postsynaptic firing occurs within a time window after presynaptic input, the NMDA channel will be activated and LTP will occur. If the order is opposite, either no LTP will occur or there may be weakening. Sequential memories are applied to the network at 30 ms intervals. (*c*) If the delay in the recurrent pathway is small, autoassociation will occur whereas if the delay is larger, heteroassociation will occur. The delay required for heteroassociation may be provided by sending information from the dentate to CA3 and back again to the dentate (see figure 4).

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.

The author thanks N. Otmakhov and A. Zhabotinsky for comments on the paper and J. Cook of the Woods Hole Oceanographic Institute Graphics Department for the figures. This work was supported by the National Institute of Neurological Disorders and Stroke grant nos. RO1 NS-27337 and RO1 NS-35083, and a grant from the David and Lucille Packard Foundation's Interdisciplinary Science Program. The author gratefully acknowledges the support of the W. M. Keck Foundation.

REFERENCES

Asztely, F., Wigstrom, H. & Gustafsson, B. 1992 The relative contribution of NMDA receptor channels in the expression of long-term potentiation in the hippocampal CA1 region. *Eur. J. Neurosci.* **4**, 681–690.

Barco, A., Alarcon, J. M. & Kandel, E. R. 2002 Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. *Cell* **108**, 689–703.

Barria, A., Derkach, V. & Soderling, T. 1997*a* Identification of the Ca^{2+}/calmodulin-dependent protein kinase II regulatory phosphorylation site in the alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate-type glutamate receptor. *J. Biol. Chem.* **272**, 32 727–32 730.

Barria, A., Muller, D., Derkach, V., Griffith, L. C. & Soderling, T. R. 1997*b* Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. *Science* **276**, 2042–2045.

Bashir, Z. I., Alford, S., Davies, S. N., Randall, A. D. & Collingridge, G. L. 1991 Long-term potentiation of NMDA receptor-mediated synaptic transmission in the hippocampus. *Nature* **349**, 156–158.

Bayer, K. U., De Koninck, P., Leonard, A. S., Hell, J. W. & Schulman, H. 2001 Interaction with the NMDA receptor locks CaMKII in an active conformation. *Nature* **411**, 801–805.

Benke, T. A., Luthi, A., Isaac, J. T. & Collingridge, G. L. 1998 Modulation of AMPA receptor unitary conductance by synaptic activity. *Nature* **393**, 793–797.

Bolshakov, V. Y., Golan, H., Kandel, E. R. & Siegelbaum, S. A. 1997 Recruitment of new sites of synaptic transmission during the cAMP-dependent late phase of LTP at CA3–CA1 synapses in the hippocampus. *Neuron* **19**, 635–651.

Bortolotto, Z. A., Bashir, Z. I., Davies, C. H. & Collingridge, G. L. 1994 A molecular switch activated by metabotropic glutamate receptors regulates induction of long-term potentiation. *Nature* **368**, 740–743.

Bozdagi, O., Shan, W., Tanaka, H., Benson, D. L. & Huntley, G. W. 2000 Increasing numbers of synaptic puncta during late-phase LTP: N-cadherin is synthesized, recruited to synaptic sites, and required for potentiation. *Neuron* **28**, 245–259.

Brown, G. P., Blitzer, R. D., Connor, J. H., Wong, T., Shenolikar, S., Iyengar, R. & Landau, E. M. 2000 Long-term potentiation induced by theta frequency stimulation is regulated by a protein phosphatase-1-operated gate. *J. Neurosci.* **20**, 7880–7887.

Buchs, P. A. & Muller, D. 1996 Induction of long-term potentiation is associated with major ultrastructural changes of activated synapses. *Proc. Natl Acad. Sci. USA* **93**, 8040–8045.

Burgin, K. E., Waxham, M. N., Rickling, S., Westgate, S. A., Mobley, W. C. & Kelly, P. T. 1990 *In situ* hybridization histochemistry of Ca^{2+}/calmodulin-dependent protein kinase in developing rat brain. *J. Neurosci.* **10**, 1788–1798.

Chen, L., Chetkovich, D. M., Petralia, R. S., Sweeney, N. T., Kawasaki, Y., Wenthold, R. J., Bredt, D. S. & Nicoll, R. A. 2000 Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. *Nature* **408**, 936–943.

Chetkovich, D. M. & Sweatt, J. D. 1993 NMDA receptor activation increases cyclic AMP in area CA1 of the hippocampus via calcium/calmodulin stimulation of adenylyl cyclase. *J. Neurochem.* **61**, 1933–1942.

Choi, S., Klingauf, J. & Tsien, R. W. 2000 Postfusional regulation of cleft glutamate concentration during LTP at 'silent synapses'. *Nature Neurosci.* **3**, 330–336.

Conti, R. & Lisman, J. 2003 The high variance of AMPA and NMDA responses at single hippocampal synapses: evidence for multiquantal release. *PNAS* (In the press.)

Daw, M. I., Bortolotto, Z. A., Saulle, E., Zaman, S., Collingridge, G. L. & Isaac, J. T. 2002 Phosphatidylinositol 3 kinase regulates synapse specificity of hippocampal long-term depression. *Nature Neurosci.* **5**, 835–836.

Diamond, J. S. & Jahr, C. E. 1995 Asynchronous release of synaptic vesicles determines the time course of the AMPA receptor-mediated EPSC. *Neuron* **15**, 1097–1107.

Dixon, D. B., Bliss, T. V. P. & Fine, A. 2002 Individual hippocampal synapses express incremental (analog) and bi-directional long-term plasticity. *Soc. Neurosci. Abstr.* Program No. 150.3.

Dosemeci, A., Tao-Cheng, J. H., Vinade, L., Winters, C. A., Pozzo-Miller, L. & Reese, T. S. 2001 Glutamate-induced transient modification of the postsynaptic density. *Proc. Natl Acad. Sci. USA* **98**, 10 428–10 432.

El-Husseini, A. E., Schnell, E., Chetkovich, D. M., Nicoll, R. A. & Bredt, D. S. 2000 PSD-95 involvement in maturation of excitatory synapses. *Science* **290**, 1364–1368.

El-Husseini, A. E., Schnell, E., Dakoji, S., Sweeney, N., Zhou, Q., Prange, O., Gauthier-Campbell, C., Aguilera-Moreno, A., Nicoll, R. A. & Bredt, D. S. 2002 Synaptic strength regulated by palmitate cycling on PSD-95. *Cell* **108**, 849–863.

Engert, F. & Bonhoeffer, T. 1999 Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature* **399**, 66–70.

Fortin, N. J., Agster, K. L. & Eichenbaum, H. B. 2002 Critical role of the hippocampus in memory for sequences of events. *Nature Neurosci.* **5**, 458–462.

Foster, T. C. & McNaughton, B. L. 1991 Long-term enhancement of CA1 synaptic transmission is due to increased quantal size, not quantal content. *Hippocampus* **1**, 79–91.

Frey, U. & Morris, R. G. 1997 Synaptic tagging and long-term potentiation. *Nature* **385**, 533–536.

Frey, U. & Morris, R. G. 1998 Weak before strong: dissociating synaptic tagging and plasticity-factor accounts of late-LTP. *Neuropharmacology* **37**, 545–552.

Frey, U., Krug, M., Reymann, K. G. & Matthies, H. 1988 Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region *in vitro*. *Brain Res.* **452**, 57–65.

Frey, U., Schollmeier, K., Reymann, K. G. & Seidenbecher, T. 1995 Asymptotic hippocampal long-term potentiation in rats does not preclude additional potentiation at later phases. *Neuroscience* **67**, 799–807.

Fukunaga, K., Stoppini, L., Miyamoto, E. & Muller, D. 1993 Long-term potentiation is associated with an increased activity of Ca^{2+}/calmodulin-dependent protein kinase II. *J. Biol. Chem.* **268**, 7863–7867.

Gardoni, F., Caputi, A., Cimino, M., Pastorino, L., Cattabeni, F. & Di Luca, M. 1998 Calcium/calmodulin-dependent pro-

tein kinase II is associated with NR2A/B subunits of NMDA receptor in postsynaptic densities. *J. Neurochem.* **71**, 1733–1741.

Gasparini, S., Saviane, C., Voronin, L. L. & Cherubini, E. 2000 Silent synapses in the developing hippocampus: lack of functional AMPA receptors or low probability of glutamate release? *Proc. Natl Acad. Sci. USA* **97**, 9741–9746.

Geinisman, Y., Detoledo-Morrell, L., Morrell, F. & Heller, R. E. 1995 Hippocampal markers of age-related memory dysfunction: behavioral, electrophysiological and morphological perspectives. *Prog. Neurobiol.* **45**, 223–252.

Genoux, D., Haditsch, U., Knobloch, M., Michalon, A., Storm, D. & Mansuy, I. M. 2002 Protein phosphatase 1 is a molecular constraint on learning and memory. *Nature* **418**, 970–975.

Giese, K. P., Fedorov, N. B., Filipkowski, R. K. & Silva, A. J. 1998 Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. *Science* **279**, 870–873.

Hanson, P. I., Meyer, T., Stryer, L. & Schulman, H. 1994 Dual role of calmodulin in autophosphorylation of multifunctional CaM kinase may underlie decoding of calcium signals. *Neuron* **12**, 943–956.

Harris, K. M., Jensen, F. E. & Tsao, B. 1992 Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. [Published erratum appears in *J. Neurosci.* **12**(8): following table of contents.] *J. Neurosci.* **12**, 2685–2705.

Hayashi, Y., Shi, S. H., Esteban, J. A., Piccini, A., Poncer, J. C. & Malinow, R. 2000 Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science* **287**, 2262–2267.

Hoffman, D. A., Sprengel, R. & Sakmann, B. 2002 Molecular dissection of hippocampal theta-burst pairing potentiation. *Proc. Natl Acad. Sci. USA* **99**, 7740–7745.

Huang, C. C., Liang, Y. C. & Hsu, K. S. 2001 Characterization of the mechanism underlying the reversal of long-term potentiation by low-frequency stimulation at hippocampal CA1 synapses. *J. Biol. Chem.* **276**, 48 108–48 117.

Huang, Y. S., Jung, M. Y., Sarkissian, M. & Richter, J. D. 2002 *N*-methyl-d-aspartate receptor signaling results in Aurora kinase-catalyzed CPEB phosphorylation and alpha CaMKII mRNA polyadenylation at synapses. *EMBO J.* **21**, 2139–2148.

Jensen, O. & Lisman, J. E. 1996 Hippocampal CA3 region predicts memory sequences: accounting for the phase precession of place cells. *Learning Memory* **3**, 279–287.

Kandel, E. R. 2001 The molecular biology of memory storage: a dialogue between genes and synapses. *Science* **294**, 1030–1038.

Krucker, T., Siggins, G. R., McNamara, R. K., Lindsley, K. A., Dao, A., Allison, D. W., De Lecea, L., Lovenberg, T. W., Sutcliffe, J. G. & Gerendasy, D. D. 2002 Targeted disruption of RC3 reveals a calmodulin-based mechanism for regulating metaplasticity in the hippocampus. *J. Neurosci.* **22**, 5525–5535.

Kuhnt, U., Hess, G. & Voronin, L. L. 1992 Statistical analysis of long-term potentiation of large excitatory postsynaptic potentials recorded in guinea pig hippocampal slices: binomial model. *Exp. Brain Res.* **89**, 265–274.

Kullmann, D. M. 1994 Amplitude fluctuations of dual-component EPSCs in hippocampal pyramidal cells: implications for long-term potentiation. *Neuron* **12**, 1111–1120.

Kullmann, D. M. & Nicoll, R. A. 1992 Long-term potentiation is associated with increases in quantal content and quantal amplitude. *Nature* **357**, 240–244.

Kullmann, D. M., Erdemli, G. & Asztely, F. 1996 LTP of AMPA and NMDA receptor-mediated signals: evidence for presynaptic expression and extrasynaptic glutamate spillover. *Neuron* **17**, 461–474.

Larkman, A., Stratford, K. & Jack, J. 1991 Quantal analysis of excitatory synaptic action and depression in hippocampal slices. *Nature* **350**, 344–347.

Larkman, A. U., Jack, J. J. & Stratford, K. J. 1997 Quantal analysis of excitatory synapses in rat hippocampal CA1 *in vitro* during low-frequency depression. *J. Physiol.* 505(Part 2), 457–471.

Lee, H. K., Barbarosie, M., Kameyama, K., Bear, M. F. & Huganir, R. L. 2000 Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* **405**, 955–959.

Lee, H. K. (and 11 others) 2003 Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. *Cell* (Submitted.)

Liao, D., Jones, A. & Malinow, R. 1992 Direct measurement of quantal changes underlying long-term potentiation in CA1 hippocampus. *Neuron* **9**, 1089–1097.

Ling, D. S., Benardo, L. S., Serrano, P. A., Blace, N., Kelly, M. T., Crary, J. F. & Sacktor, T. C. 2002 Protein kinase Mzeta is necessary and sufficient for LTP maintenance. *Nature Neurosci.* **5**, 295–296.

Lisman, J. E. 1999 Relating hippocampal circuitry to function: recall of memory sequences by reciprocal dentate-CA3 interactions. *Neuron* **22**, 233–242.

Lisman, J., Schulman, H. & Cline, H. 2002 The molecular basis of CaMKII function in synaptic and behavioural memory. *Nature Rev. Neurosci.* **3**, 175–190.

Lisman, J. E. & Harris, K. M. 1993 Quantal analysis and synaptic anatomy—integrating two views of hippocampal plasticity. *Trends Neurosci.* **16**, 141–147.

Lisman, J. E. & Zhabotinsky, A. M. 2001 A model of synaptic memory: a CaMKII/PP1 switch that potentiates transmission by organizing an AMPA receptor anchoring assembly. *Neuron* **31**, 191–201.

Liu, G., Choi, S. & Tsien, R. W. 1999 Variability of neurotransmitter concentration and nonsaturation of postsynaptic AMPA receptors at synapses in hippocampal cultures and slices. *Neuron* **22**, 395–409.

Lledo, P. M., Hjelmstad, G. O., Mukherji, S., Soderling, T. R., Malenka, R. C. & Nicoll, R. A. 1995 Calcium/calmodulin-dependent kinase II and long-term potentiation enhance synaptic transmission by the same mechanism. *Proc. Natl Acad. Sci. USA* **92**, 11 175–11 179.

Ma, L., Zablow, L., Kandel, E. R. & Siegelbaum, S. A. 1999 Cyclic AMP induces functional presynaptic boutons in hippocampal CA3–CA1 neuronal cultures. *Nature Neurosci.* **2**, 24–30.

McAllister, A. K. & Stevens, C. F. 2000 Nonsaturation of AMPA and NMDA receptors at hippocampal synapses. *Proc. Natl Acad. Sci. USA* **97**, 6173–6178.

Magee, J. C. & Cook, E. P. 2000 Somatic EPSP amplitude is independent of synapse location in hippocampal pyramidal neurons. *Nature Neurosci.* **3**, 895–903.

Makhinson, M., Chotiner, J. K., Watson, J. B. & O'Dell, T. J. 1999 Adenylyl cyclase activation modulates activity-dependent changes in synaptic strength and Ca^{2+}/calmodulin-dependent kinase II autophosphorylation. *J. Neurosci.* **19**, 2500–2510.

Malinow, R. 1991 Transmission between pairs of hippocampal slice neurons: quantal levels, oscillations and LTP. *Science* **252**, 722–724.

Matsuzaki, M., Ellis-Davies, G. C., Nemoto, T., Miyashita, Y., Iino, M. & Kasai, H. 2001 Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nature Neurosci.* **4**, 1086–1092.

Miller, S., Yasuda, M., Coats, J. K., Jones, Y., Martone, M. E. & Mayford, M. 2003 Disruption of dendritic translation of CaMKII-alpha impairs stabilization of synaptic plasticity and memory consolidation. *Neuron* (In the press.)

Miller, S. G. & Kennedy, M. B. 1986 Regulation of brain type II Ca^{2+}/calmodulin-dependent protein kinase by autophosphorylation: a Ca^{2+}-triggered molecular switch. *Cell* **44**, 861–870.

Montgomery, J. M. & Madison, D. V. 2002 State-dependent heterogeneity in synaptic depression between pyramidal cell pairs. *Neuron* **33**, 765–777.

Montgomery, J. M., Pavlidis, P. & Madison, D. V. 2001 Pair recordings reveal all-silent synaptic connections and the postsynaptic expression of long-term potentiation. *Neuron* **29**, 691–701.

Nadasdy, Z., Hirase, H., Czurko, A., Csicsvari, J. & Buzsaki, G. 1999 Replay and time compression of recurring spike sequences in the hippocampus. *J. Neurosci.* **19**, 9497–9507.

Nakazawa, K. (and 10 others) 2002 Requirement for hippocampal CA3 NMDA receptors in associative memory recall. *Science* **297**, 211–218.

Nusser, Z., Lujan, R., Laube, G., Roberts, J. D., Molnar, E. & Somogyi, P. 1998 Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. *Neuron* **21**, 545–559.

Oertner, T. G., Sabatini, B. L., Nimchinsky, E. A. & Svoboda, K. 2002 Facilitation at single synapses probed with optical quantal analysis. *Nature Neurosci.* **10**, 10.

O'Keefe, J. & Recce, M. L. 1993 Phase relationship between hippocampal place units and the EEG theta rhythm. *Hippocampus* **3**, 317–330.

Ostroff, L. E., Fiala, J. C., Allwardt, B. & Harris, K. M. 2002 Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron* **35**, 535–545.

Otmakhov, N., Griffith, L. C. & Lisman, J. E. 1997 Postsynaptic inhibitors of calcium/calmodulin-dependent protein kinase type II block induction but not maintenance of pairing-induced long-term potentiation. *J. Neurosci.* **17**, 5357–5365.

Ouyang, Y., Rosenstein, A., Kreiman, G., Schuman, E. M. & Kennedy, M. B. 1999 Tetanic stimulation leads to increased accumulation of Ca(2+)/calmodulin-dependent protein kinase II via dendritic protein synthesis in hippocampal neurons. *J. Neurosci.* **19**, 7823–7833.

Passafaro, M., Piech, V. & Sheng, M. 2001 Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. *Nature Neurosci.* **4**, 917–926.

Petersen, C. C., Malenka, R. C., Nicoll, R. A. & Hopfield, J. J. 1998 All-or-none potentiation at CA3-CA1 synapses. *Proc. Natl Acad. Sci. USA* **95**, 4732–4737.

Peterson, J., Chen, X., Dosemici, A., Lisman, J. & Reese, T. 2003 Localization of PSD95 and CaMKII in the PSD. (In preparation.)

Petralia, R. S., Esteban, J. A., Wang, Y. X., Partridge, J. G., Zhao, H. M., Wenthold, R. J. & Malinow, R. 1999 Selective acquisition of AMPA receptors over postnatal development suggests a molecular basis for silent synapses. *Nature Neurosci.* **2**, 31–36.

Poncer, J. C., Esteban, J. A. & Malinow, R. 2002 Multiple mechanisms for the potentiation of AMPA receptor-mediated transmission by alpha-Ca^{2+}/calmodulin-dependent protein kinase II. *J. Neurosci.* **22**, 4406–4411.

Racca, C., Stephenson, F. A., Streit, P., Roberts, J. D. & Somogyi, P. 2000 NMDA receptor content of synapses in stratum radiatum of the hippocampal CA1 area. *J. Neurosci.* **20**, 2512–2522.

Raffone, A., Talamini, L. & Lisman, J. 2003 Sequence learning and recall by hippocampal networks: NMDA-mediated LTP can produce both autoassociative and heteroassociative linkages. (In preparation.)

Raghavachari, S. & Lisman, J. 2003 Generation of fast-rising mEPSCs by activation of AMPA channels close to the site of transmitter release. (Submitted.)

Renger, J. J., Egles, C. & Liu, G. 2001 A developmental switch in neurotransmitter flux enhances synaptic efficacy by affecting AMPA receptor activation. *Neuron* **29**, 469–484.

Sanna, P. P., Cammalleri, M., Berton, F., Simpson, C., Lutjens, R., Bloom, F. E. & Francesconi, W. 2002 Phosphatidylinositol 3-kinase is required for the expression but not for the induction or the maintenance of long-term potentiation in the hippocampal CA1 region. *J. Neurosci.* **22**, 3359–3365.

Schnell, E., Sizemore, M., Karimzadegan, S., Chen, L., Bredt, D. S. & Nicoll, R. A. 2002 Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. *Proc. Natl Acad. Sci. USA* **99**, 13 902–13 907.

Selig, D. K., Hjelmstad, G. O., Herron, C., Nicoll, R. A. & Malenka, R. C. 1995 Independent mechanisms for long-term depression of AMPA and NMDA. *Neuron* **15**, 417–426.

Shen, K., Teruel, M. N., Connor, J. H., Shenolikar, S. & Meyer, T. 2000 Molecular memory by reversible translocation of calcium/calmodulin-dependent protein kinase II. *Nature Neurosci.* **3**, 881–886.

Shi, S., Hayashi, Y., Esteban, J. A. & Malinow, R. 2001 Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. *Cell* **103**, 331–343.

Skaggs, W. E. & McNaughton, B. L. 1996 Replay of neuronal firing sequences in rat hippocampus during sleep following spatial experience. *Science* **271**, 1870–1873.

Skaggs, W. E., McNaughton, B. L., Wilson, M. A. & Barnes, C. A. 1996 Theta phase precession in hippocampal neuronal populations and the compression of temporal sequences. *Hippocampus* **6**, 149–172.

Strack, S., Barban, M. A., Wadzinski, B. E. & Colbran, R. J. 1997 Differential inactivation of postsynaptic density-associated and soluble Ca^{2+}/calmodulin-dependent protein kinase II by protein phosphatases 1 and 2A. *J. Neurochem.* **68**, 2119–2128.

Strack, S., Robison, A. J., Bass, M. A. & Colbran, R. J. 2000 Association of calcium/calmodulin-dependent kinase II with developmentally regulated splice variants of the postsynaptic density protein densin-180. *J. Biol. Chem.* **275**, 25 061–25 064.

Stricker, C., Field, A. C. & Redman, S. J. 1996*a* Changes in quantal parameters of EPSCs in rat CA1 neurones *in vitro* after the induction of long-term potentiation. *J. Physiol.* (*Lond.*) **490**, 443–454.

Stricker, C., Field, A. C. & Redman, S. J. 1996*b* Statistical analysis of amplitude fluctuations in EPSCs evoked in rat CA1 pyramidal neurones in vitro. *J. Physiol.* **490**, 419–441.

Takahashi, T., Svoboda, K. & Malinow, R. 2002 Experience strengthens transmission in barrel cortex by driving Glur1-containing AMPA receptors into synapses. *Soc. Neurosci. Abstr.* Program No. 713.3.

Takumi, Y., Ramirez-Leon, V., Laake, P., Rinvik, E. & Ottersen, O. P. 1999 Different modes of expression of AMPA and NMDA receptors in hippocampal synapses. *Nature Neurosci.* **2**, 618–624.

Tsodyks, M. V., Skaggs, W. E., Sejnowski, T. J. & McNaughton, B. L. 1996 Population dynamics and theta rhythm phase precession of hippocampal place cell firing: a spiking neuron model. *Hippocampus* **6**, 271–280.

Walikonis, R. S., Oguni, A., Khorosheva, E. M., Jeng, C. J., Asuncion, F. J. & Kennedy, M. B. 2001 Densin-180 forms a ternary complex with the (alpha)-subunit of

Ca^{2+}/calmodulin-dependent protein kinase II and (alpha)-actinin. *J. Neurosci.* **21**, 423–433.
Weeks, A. C., Ivanco, T. L., Leboutillier, J. C., Marrone, D. F., Racine, R. J. & Petit, T. L. 2003 Unique changes in synaptic morphology following tetanization under pharmacological blockade. *Synapse* **47**, 77–86.
Wittenberg, G. & Tsien, J. 2002 An emerging molecular and cellular framework for memory processing by the hippocampus. *Trends Neurosci.* **25**, 501.
Wong, S. T., Athos, J., Figueroa, X. A., Pineda, V. V., Schaefer, M. L., Chavkin, C. C., Muglia, L. J. & Storm, D. R. 1999 Calcium-stimulated adenylyl cyclase activity is critical for hippocampus-dependent long-term memory and late phase LTP. *Neuron* **23**, 787–798.
Xie, X., Berger, T. W. & Barrionuevo, G. 1992 Isolated NMDA receptor-mediated synaptic responses express both LTP and LTD. *J. Neurophysiol.* **67**, 1009–1013.
Zhu, J. J., Qin, Y., Zhao, M., Van Aelst, L. & Malinow, R. 2002 Ras and Rap control AMPA receptor trafficking during synaptic plasticity. *Cell* **110**, 443–455.

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