

A synaptic model of memory: long-term potentiation in the hippocampus

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Long-term potentiation of synaptic transmission in the hippocampus is the primary experimental model for investigating the synaptic basis of learning and memory in vertebrates. The best understood form of long-term potentiation is induced by the activation of the *N*-methyl-D-aspartate receptor complex. This subtype of glutamate receptor endows long-term potentiation with Hebbian characteristics, and allows electrical events at the postsynaptic membrane to be transduced into chemical signals which, in turn, are thought to activate both pre- and postsynaptic mechanisms to generate a persistent increase in synaptic strength.

THE assumption that information is stored in the brain as changes in synaptic efficiency emerged about a century ago following the demonstration by Cajal that networks of neurons are not in cytoplasmic continuity but communicate with each other at the specialized junctions which Sherrington called synapses. External events are represented in the brain as spatio-temporal patterns of neural activity, and it is these patterns of activity which must themselves be the agents of synaptic change. The location of storage, the engram of learning and memory, must therefore be found among those synapses which support activity-dependent changes in synaptic efficiency. These ideas were refined in the late 1940s by Hebb¹ and Konorski², who proposed a coincidence-detection rule in which the synapse linking two cells is strengthened if the cells are active at the same time. The first such synapses to be identified in the mammalian brain were the excitatory connections made by perforant path fibres onto granule cells of the hippocampus, a cortical structure required for the formation of conscious memories in man. Brief trains of high-frequency stimulation to monosynaptic excitatory pathways in the hippocampus cause an abrupt and sustained increase in the efficiency of synaptic transmission. This effect, first described in detail in 1973^{3,4}, is called long-term potentiation (LTP). LTP has since been found in all excitatory pathways in the hippocampus, as well as in several other regions in the brain, and there is growing evidence that it underlies at least certain forms of memory^{5,6}. In the past 10 years, LTP in the hippocampus has become the dominant model of activity-dependent synaptic plasticity in the mammalian brain, and much progress has been made in elucidating the mechanisms underlying its induction and expression.

Properties of hippocampal LTP

Activity-dependent synaptic potentiation occurs within milliseconds and can persist for many hours in the anaesthetized animal or in the *in vitro* hippocampal slice preparation, and for days when induced in the freely moving animal. This time span incorporates a number of mechanistically distinct temporal components, which include post-tetanic potentiation (PTP), short-term potentiation (STP) and LTP. Activity-dependent potentiation can also be classified on the basis of whether or not its induction is blocked by antagonists of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor (Box 1). In this article, by LTP we mean synaptic potentiation, which is both NMDA receptor-dependent and lasts for more than an hour.

LTP is expressed as a persistent increase in the size of the synaptic component of the evoked response, recorded from individual cells or from populations of neurons. It can be induced in a number of ways, most conveniently by delivering a tetanus (typically a train of 50–100 stimuli at 100 Hz or more) to the pathway of interest (Fig. 1). LTP can also be induced by more modest stimulus parameters, providing the patterns of

stimulation fall within certain critical ranges. (Two particularly efficient recipes are termed 'theta-burst stimulation'⁷ (for example, several bursts of 4 shocks at 100 Hz delivered at an interburst interval of 200 ms) and 'primed-burst stimulation'⁸ (for example, a single priming stimulus followed at 200 ms by a single burst of 4 shocks at 100 Hz). The significance of these protocols is that synchronized firing patterns at similar frequencies occur in the hippocampus during learning⁹.)

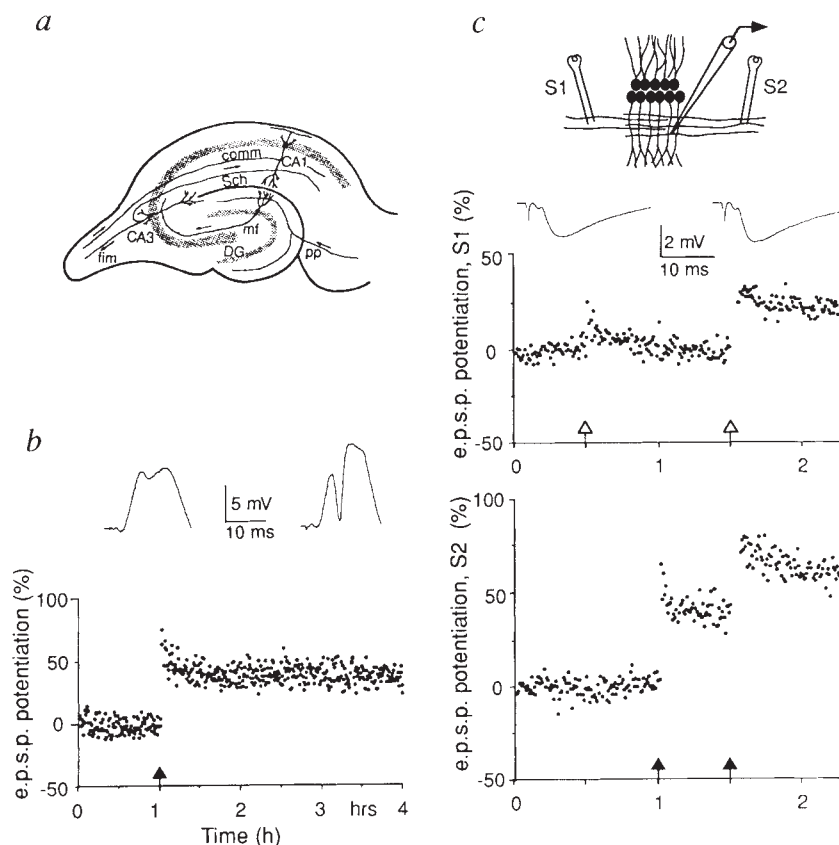
LTP is characterized by three basic properties: cooperativity, associativity and input-specificity. Cooperativity describes the existence of an intensity threshold for induction; 'weak' tetani, activating relatively few afferent fibres, do not trigger LTP¹⁰. The threshold for inducing LTP is a complex function of the intensity and pattern of tetanic stimulation; between 'weak' trains which produce only PTP and 'strong' trains which induce LTP, lies an intermediate range of activation which engages STP^{11,12}. LTP is associative in the sense that a 'weak' input can be potentiated if it is active at the same time as a strong tetanus to a separate but convergent input^{10,13}. Finally, LTP is input-specific, because other inputs that are not active at the time of the tetanus do not share in the potentiation induced in the tetanized pathway^{14,15}. Associativity provides a cellular analogue of classical conditioning, and is an implicit property of the Hebb synapse, the computing element that lies at the heart of the current interest in neural computation. The three properties can be explained on the assumption that a synapse will be potentiated if, and only if, it is active at a time when the region of dendrite on which it terminates is sufficiently depolarized. Validation of this induction rule was provided in 1986 by experiments showing that low-frequency (1 Hz), low-intensity stimuli could produce robust LTP if repeatedly paired with depolarizing pulses delivered through an intracellular recording electrode^{16–18}. In the limit, LTP can be produced in this way between pairs of synaptically coupled neurons¹⁹. Conversely, the induction of LTP can be blocked by limiting the depolarization of the cell during a tetanus^{16,20}.

What is now needed to complete a mechanistic description of the induction requirements for associative LTP is a molecular coincidence detector, able to respond to the conjunction of activity in afferent fibres and adequate depolarization in target dendrites. Compelling evidence that the NMDA receptor performs this function is reviewed in the next section.

The induction of LTP

The role of amino-acid receptors in the induction of LTP. The involvement of several amino-acid receptor subtypes in the induction of LTP has been determined largely by the use of antagonists and is described in Box 2. The key role of the NMDA receptor channel complex relies on several of its special properties, in particular the voltage-dependent block of its channel by Mg²⁺ (ref. 21). It is this that allows the NMDA receptor to

FIG. 1 Basic properties of LTP: cooperativity, input-specificity and associativity. *a*, Simplified diagram of a transverse section through the hippocampus of the rat, showing the principal neuronal fields (granule cells of the dentate gyrus (DG) and the pyramidal cells of areas CA3 and CA1), and the main excitatory afferent projections (the perforant path (pp) from entorhinal cortex to granule cells, the mossy fibre projection (mf) from granule cells to CA3 cells, and the Schaffer collateral (Sch)-commissural (comm) system which connects ipsilateral and contralateral CA3 cells to CA1 cells). Interneurons, which are found in all hippocampal subfields and which form powerful inhibitory connections with principal cells through feed-forward and feedback loops, have been omitted. *b*, An example of LTP in the perforant pathway recorded *in vivo*. The graph plots the slope of the rising phase of the evoked response (population e.p.s.p.), recorded from the cell body region in response to constant test stimuli, for 1 h before and 3 h following a tetanus (250 Hz, 200 ms), delivered at the time indicated by the arrow. Representative traces before and after the induction of LTP are illustrated above the graph. Note the increase in slope of the population e.p.s.p. and the increase in size of the superimposed population spike (downward deflection). *c*, Demonstration of the properties of cooperativity, input specificity and associativity. The diagram at the top shows the experimental arrangement in area CA1 of the hippocampal slice preparation. Two independent sets of afferent fibres converging on a common population of cells are activated by stimulating electrodes (S1 and S2) placed either side of the extracellular recording electrode. The stimulus intensities are adjusted so that S1 activates fewer fibres than S2. The slope of the population e.p.s.p.s, in response to stimuli delivered alternately to S1 and S2 at 15-s intervals, are plotted as a function of time. Arrows denote episodes of tetanic stimulation to S1 (the 'weak' pathway, open arrows) or S2 (the 'strong' pathway, solid arrows). The tetanus to S1 produced a rapidly decaying phase of PTP, lasting 2–3 min, with a small tail of STP, but no stable increase in synaptic transmission; the intensity of the tetanus was below the cooperativity threshold for LTP. The stronger tetanus to S2 (first



filled arrow) produced PTP and robust LTP, but there was no transfer of the effect to the first input (test shocks to S1 were out of phase with the high-frequency bursts to S2), demonstrating the input-specificity of LTP. Finally, tetani to S1 and S2 were delivered together. The coincident activation of a weak, subthreshold input with a strong input induced associative LTP in the weak input. The traces above the graph illustrate field e.p.s.ps, evoked by test shocks in S1 and recorded in the synaptic layer, before and after the induction of associative LTP.

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behave as a molecular coincidence detector. For the NMDA channel to open, and thus to trigger the induction of LTP, it is necessary for two events to occur simultaneously: the membrane must be sufficiently depolarized to expel Mg^{2+} from NMDA channels at the same time that L-glutamate has, by binding to NMDA receptors, promoted their opening. The slow time course and voltage-dependence of the NMDA receptor-mediated conductance makes it particularly susceptible to the hyperpolarizing influence of synaptic inhibition²²; this susceptibility, together with the frequency-dependent depression of inhibition itself, largely accounts for the frequency-dependence of the induction of LTP²³.

The properties of cooperativity, associativity and input-specificity can now easily be explained. The cooperativity threshold follows from the need for depolarization to reduce the level of the Mg^{2+} block of the NMDA channel. 'Weak' stimuli, activating only a few fibres, fail to induce LTP not because insufficient L-glutamate is released to activate NMDA receptors, but because the level of depolarization provided by the weak input does not produce an adequate reduction of the Mg^{2+} block. When many fibres are activated in synchrony by a 'strong' stimulus, depolarization spreads between neighbouring synapses to enhance the unblocking of NMDA channels. Associativity has a similar explanation except that the required depolarization is provided by a different set of afferent fibres; in theory, these

'helper' inputs could use any neurotransmitter that promotes depolarization, and, experimentally, depolarization is often provided by injecting current into the cell. Input-specificity is explained by the need for the presynaptic terminal to provide a sufficient concentration of L-glutamate to activate adequate numbers of NMDA receptors. (It follows that there can be little activation of NMDA receptors by ambient or spontaneously released L-glutamate, otherwise LTP would be induced by depolarization alone.)

Because the induction of LTP by tetanic stimulation is prevented by a variety of NMDA antagonists, including those which act at the receptor (such as 2-amino-5-phosphonopentanoate (AP5)²⁴), in the channel (for example, MK-801 (ref. 25)) and at the allosteric glycine site (for example, 7-chlorokynurenic acid²⁶), it is clear that activation of these receptors is an essential trigger for the process. But, the application of NMDA itself is not usually sufficient to induce LTP, though it readily induces STP^{24,27}. A possible reason for this relates to the paradoxical finding that a level of activation of the NMDA receptor system, which is itself inadequate for producing LTP, can result in a subsequent impairment in the ability to generate LTP^{28–30}. Thus, with the application of NMDA there may be two opposing processes at work, one promoting and the other suppressing the induction of LTP. Alternatively, factors in addition to NMDA receptor activation, which could be either pre- or post-synaptic,

may be required to facilitate or allow the induction of LTP. In this respect, there has been interest in the possible role of metabotropic glutamate receptors (mGluRs), prompted by the observation that the mGluR antagonists 2-amino-4-phosphonobutanoate (AP4) and 2-amino-3-phosphonopropionate (AP3) reduce the duration of LTP^{31,32}. These compounds are, however, very weak mGluR antagonists of poorly defined specificity, and the observation will need to be confirmed as soon as more potent and selective mGluR antagonists are developed. A second indication that these receptors might be involved in the induction of LTP has come from the finding that aminocyclopentane dicarboxylate (ACPD), the 1S,3R-enantiomer of which is a specific agonist for mGluRs, can augment tetanus-induced potentiation³³. In addition, ACPD enables sub-threshold³⁴, or low-frequency stimuli (in conjunction with the application of NMDA)³⁵, to induce LTP. It does this in at least two ways. First, ACPD augments responses of hippocampal neurons to NMDA³⁴. Second, it can elicit an NMDA receptor-independent potentiation of slow onset which adds to STP to produce a potentiation that closely resembles tetanus-induced LTP³⁶.

The role of Ca^{2+} in the induction of LTP. In an important early study, it was found that the induction of LTP could be blocked by the intracellular injection of the Ca^{2+} chelator EGTA³⁷. This result implicated the postsynaptic cell, and in particular Ca^{2+} signalling in the induction process. Because NMDA channels are permeable to Ca^{2+} (refs 21, 38, 39) it is widely assumed, but not proven, that permeation through these channels during tetanic stimulation provides the Ca^{2+} signal necessary for the induction of LTP. Because NMDA receptors are assumed to be located on dendritic spines, it is believed that spines may act to localize the Ca^{2+} signal. Spines can restrict the diffusion of Ca^{2+} (ref. 40); however, whether they do so in LTP is not known.

Using Ca^{2+} -imaging techniques it has been shown that tetanic stimulation elevates Ca^{2+} within dendrites and spines^{41,42}. Part

of this signal depends on the synaptic activation of NMDA receptors and reflects, at least in part, Ca^{2+} entry through NMDA channels and voltage-gated Ca^{2+} channels. In one study⁴² the tetanically induced rise in Ca^{2+} persisted for several minutes, and it was proposed that sustained Ca^{2+} gradients might be important for memory processing. But it is unlikely that rises in Ca^{2+} of this duration are necessary for the induction of LTP in view of the demonstration that LTP can still be induced even if the duration of the post-tetanic rise in Ca^{2+} is restricted to less than 3 s, using a photo-activatable caged Ca^{2+} chelator⁴³. Complementary data have come from combining Ca^{2+} imaging with whole-cell recording⁴⁴. Although, for technical reasons, LTP could not be induced, this preparation allowed Ca^{2+} signals to be correlated directly with the synaptic response. Strong tetanic stimulation, which evoked large NMDA receptor-mediated synaptic currents, produced Ca^{2+} transients lasting only a few seconds. This combination of techniques has also enabled the Ca^{2+} signal that permeates NMDA channels on dendritic spines to be detected (Fig. 2).

There are indications from Ca^{2+} imaging experiments that the Ca^{2+} which permeates NMDA channels is augmented by Ca^{2+} release from intracellular stores (see Box 3). The Ca^{2+} transient associated with the synaptic activation of NMDA receptors is substantially reduced in the presence of ryanodine or thapsigargin⁴⁴, drugs which inhibit Ca^{2+} -induced Ca^{2+} release and deplete intracellular Ca^{2+} stores, respectively. That this Ca^{2+} might be important for the induction of LTP is suggested by the observations that dantrolene, which acts at the ryanodine receptor, and thapsigargin can both inhibit the induction of LTP^{36,45,46}. It is likely that inositol 1,4,5-trisphosphate (InsP_3) generated as the result of the activation of mGluRs, as well as the Ca^{2+} which permeates through NMDA channels, is involved in releasing Ca^{2+} from intracellular stores. Moreover, activation of mGluRs can induce LTP by a thapsigargin-sensitive mechanism, even if NMDA receptors are blocked³⁶. This suggests that

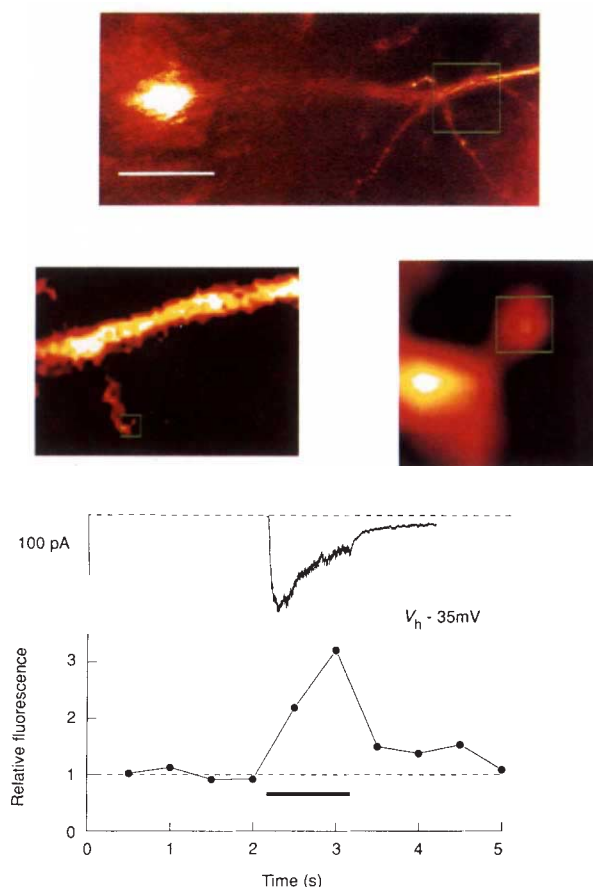


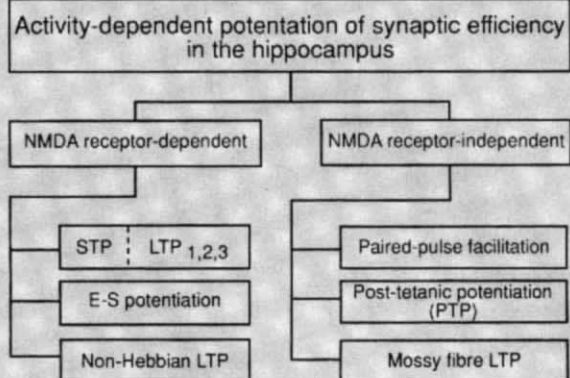
FIG. 2 Ca^{2+} permeates NMDA channels to produce a transient signal in spines in response to tetanic stimulation. Confocal images of a CA1 pyramidal neuron in a hippocampal slice. The upper image shows part of the soma and dendrites as they emerge into the plane of the optical section. The boxed region is enlarged to show a dendritic branch and spine-like structure. This is further enlarged to show the spine in more detail and the boxed region from which the fluorescence measurements were obtained. (The box is $\sim 1 \mu\text{m}^2$.) The graph plots the relative fluorescence, emitted by the indicator fluo-3, as a function of time. The tetanus (100 Hz, 1s), delivered for the duration of the bar, resulted in a transient increase in fluorescence. The upper trace shows the synaptic current induced by the tetanus, recorded through a patch-pipette. The cell was internally dialysed and clamped at -35 mV to eliminate all voltage-gated Ca^{2+} channel activity and the slice was treated with thapsigargin to deplete intracellular Ca^{2+} stores. Under these conditions the fluorescence changes are caused by Ca^{2+} permeating through NMDA channels.

BOX 1 Classification of activity-dependent increases in synaptic efficiency in the hippocampus

SYNAPTIC potentiation can be divided into two principal categories on the basis of whether or not its induction is blocked by antagonists of the NMDA subtype of glutamate receptor. Several categories of NMDA receptor-dependent plasticity have been identified. A distinction can be made between short-term potentiation (STP), which decays within 1 h, and long-term potentiation (LTP), which is sustained for much longer periods. STP can be distinguished from LTP by the use of protein kinase inhibitors, in the presence of which potentiation usually persists for only 30–60 min^{81–85}. Potentiation of a similar duration can be produced by decreasing the number of stimuli in the tetanus or by other manoeuvres which reduce the level of NMDA receptor activation¹². Although it is convenient to make the distinction, the relationship between STP and LTP has not been clearly defined. LTP can be tentatively subdivided into several mechanistically distinct components: LTP1, with a duration of less than 3–6 h which is blocked by kinase inhibitors but not by protein synthesis inhibitors; LTP2, a component which is blocked by translational inhibitors but which appears to be independent of gene expression; and LTP3, with a time constant of several days, which is only obtained if the animal is anaesthetised at the time of induction¹⁵⁹ and which may require gene expression (see text).

Another form of NMDA receptor-dependent plasticity is E–S potentiation. This takes its name from the shift to the left of the curve relating the slope of the population e.p.s.p. (E) to the amplitude of the population spike (S) which is commonly observed following a tetanus¹⁶⁰. It appears not to be input-specific¹⁶¹ but may provide a generalized boost to impulse traffic. A further type of presumed NMDA receptor-dependent LTP has been described in which potentiation occurs not only at those synapses where there is coincident pre- and postsynaptic activity, but extends to synapses made by concurrently active terminals onto neighbouring cells, whether or not these are active¹⁶². This is interesting both because it suggests that non-Hebbian forms of potentiation occur in the hippocampus, and because it provides implicit evidence for the existence of a diffusible extracellular messenger (see text).

NMDA receptor-independent processes include paired-pulse facilitation and post-tetanic potentiation (PTP), which are general features of excitatory synaptic transmission. With the stimulus parameters usually employed to produce LTP, the duration of PTP is at most a few minutes. Both paired-pulse facilitation and PTP are additive with LTP, and can be produced repeatedly even when LTP has reached asymptotic levels. It follows that LTP cannot achieve the maximum strength of which a synapse is capable:



the potential for a further short-term increase is always held in reserve. Mossy fibres terminate in the stratum lucidum of area CA3, a subfield devoid of NMDA receptors. Consistent with this observation, LTP in mossy fibres is not blocked by the NMDA antagonist AP5 (ref. 163); moreover, it appears to be nonassociative. The projection is technically difficult to study, and the locus and cellular mechanisms of mossy fibre LTP remain controversial¹⁶⁴. Finally, an input-specific AP5-resistant component of LTP has been described in area CA1¹⁶⁵. The effect is small, develops gradually, is blocked by Ca^{2+} channel antagonists, and requires stronger tetanic stimulation for its induction than is needed for NMDA receptor-dependent LTP.

Long-lasting potentiation can also be induced by transient exposure of hippocampal synapses to a variety of chemical agents, including Ca^{2+} ¹⁶⁶, arachidonic acid⁶⁶, the metabotropic glutamate receptor (mGluR) agonist aminocyclopentane-1S,3R-dicarboxylate (1S,3R-ACPD)³⁶, the K^+ channel blocker, tetraethylammonium (TEA)¹⁶⁷ and the G-protein activator NaF/AlCl_3 ¹⁶⁸. Chemically-induced potentiation usually occludes with tetanically-induced LTP (that is, saturation of one prevents induction of the other), suggesting a convergence of mechanisms; in general, chemically induced LTP is not blocked by NMDA antagonists, presumably because the components of the LTP cascade activated by the various agents lie downstream from the NMDA receptor.

release of Ca^{2+} from intracellular stores can substitute for the NMDA receptor-mediated Ca^{2+} signal. Other routes by which Ca^{2+} could enter the cell to contribute to the induction of LTP include voltage-dependent Ca^{2+} channels and Ca^{2+} -permeable AMPA channels (that is, those lacking the GluR-2 subunit⁴⁷). At present, though, there is little evidence that either of these pathways plays a significant role in LTP.

Although clearly a necessary factor, it is unclear whether a rise in postsynaptic Ca^{2+} provides a sufficient trigger for the induction of LTP. Elevation of intracellular Ca^{2+} by the photolysis of caged Ca^{2+} induces a form of synaptic potentiation⁴⁸, but the relationship between this effect and LTP has not been determined (for example, occlusion experiments have not been done). Elevation of intracellular Ca^{2+} , either by evoking Ca^{2+} currents⁴⁹ or by slowly depleting intracellular Ca^{2+} stores⁴⁶, does not induce LTP. This could be due either to the failure of these methods to elevate Ca^{2+} in the appropriate manner (presumably what is needed is a large transient within spines) or to the need for additional pre- and/or postsynaptic signals.

In summary, the available evidence suggests that under normal conditions Ca^{2+} permeates NMDA channels to provide a transient signal which is necessary for the induction of LTP. It is probable that this signal is restricted to the vicinity of activated spines and is amplified by release from intracellular stores.

Expression of LTP

A major challenge is to identify the loci and nature of the alterations responsible for the expression of the potentiated state.

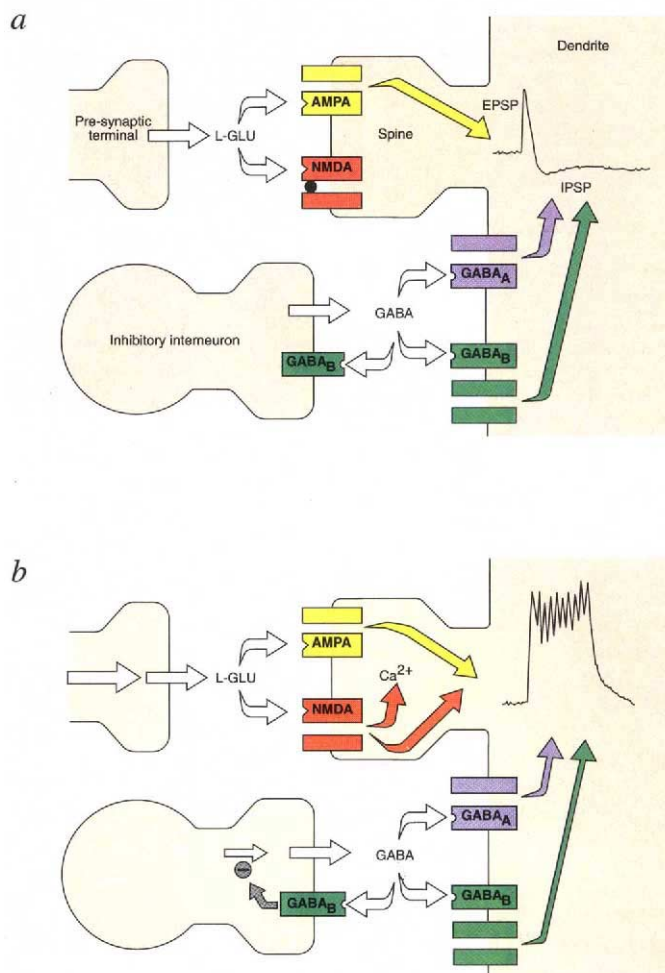
The locus of expression. Broadly speaking, the increase in the postsynaptic response generated at potentiated synapses could be due to (1) presynaptic modifications which result in an increase in the amount of L-glutamate released per impulse, (2) postsynaptic modifications, such as an increase in the number of receptors or a change in their functional characteristics, (3) an extrasynaptic change, such as a reduction in uptake of L-glutamate by glial cells leading to increased neurotransmitter availability at the receptors, or (4) morphological modifications. In reality, a combination of these changes, with different time courses, probably occurs.

Evidence for an increase in neurotransmitter release is derived from experiments that have measured the overflow of radiolabelled or endogenous L-glutamate from the hippocampus before and after the induction of LTP^{50–52}. Although not without difficulties of interpretation⁵³, these experiments establish a case for presynaptic changes lasting for at least several hours⁵⁴. Supporting evidence was obtained from experiments in which the ability of a depolarizing stimulus to release radiolabelled glutamate was shown to be elevated in potentiated hippocampal tissue⁵⁵.

Other studies have suggested purely postsynaptic modifications. The observation that paired-pulse facilitation is not altered after the induction of LTP has been interpreted as evidence for a postsynaptic modification in LTP⁵⁶ on the assumption that facilitation in the hippocampus is presynaptic, and that an interaction between facilitation and LTP would be expected if the expression of the latter were also presynaptically mediated. It is possible, however, to construct a model in which

BOX 2 The role of amino-acid receptors in the induction of LTP

a, Low frequency transmission: a single stimulus applied to the Schaffer collateral-commissural pathway evokes an e.p.s.p. which is mediated predominantly by the neurotransmitter (L-GLU) acting on ionotropic glutamate receptors of a non-NMDA type²⁴. This e.p.s.p. can be blocked by the quinoxalinedione antagonists, such as 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)^{169,170}, and is usually referred to as AMPA receptor-mediated after the selective ligand for these receptors α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA). This receptor corresponds to the cloned family GluR1-4⁴⁷. When the Schaffer collateral-commissural pathway is stimulated it also activates GABAergic interneurons (through glutamatergic synapses similar to those on pyramidal neurons¹⁶⁹) and this leads to the biphasic i.p.s.p. which curtails the e.p.s.p. The initial part of the i.p.s.p. is caused solely by the activation of GABA_A receptors (which contain integral Cl⁻ channels) and this is supplemented and followed by the activation of GABA_B receptors (which are indirectly coupled to K⁺ channels). NMDA receptors contribute little to the synaptic response because of their relatively slow activation kinetics^{22,171,172}. By the time that significant numbers of NMDA channels are in an open state the neuron has been hyperpolarized by the i.p.s.p. and this greatly enhances the block of NMDA channels by Mg²⁺ (ref. 22). Even so, there will still be a finite contribution of the NMDA receptor system to low frequency synaptic transmission; however, this is not sufficient (under normal circumstances) to initiate changes in the efficiency of synaptic transmission. **b**, High-frequency transmission. The contribution of NMDA receptors to synaptic transmission alters radically in response to a high-frequency input^{24,173}. This is because the tetanus maintains the neuron in a more depolarized state, which in turn reduces the extent of the Mg²⁺-induced block of NMDA channels, while at the same time providing the L-glutamate which promotes their opening. Several factors may contribute to the sustained depolarization during a tetanus; these include summation of AMPA receptor-mediated e.p.s.ps, depolarizing shifts in the Cl⁻ and K⁺ reversal potentials due to build up of intracellular Cl⁻ and extracellular K⁺. The primary mechanism (during primed or theta-burst LTP) is depression of GABA-mediated synaptic inhibition²³. This is an active process mediated by GABA_B autoreceptors. The effect takes more than 10 ms to develop and can last for up to a few seconds. As a result low frequency transmission is unaffected by this process; however, during high-frequency transmission there is considerably less GABA released per impulse which leads to a shift in the balance of excitation and inhibition. The reduction in inhibition allows greater expression of the NMDA receptor system which in turn contributes to the depolarization and thus further reduces the level of the Mg²⁺ block. The long duration of the synaptic conductance means that NMDA receptor-mediated e.p.s.ps summate very effectively during high-frequency transmission.



facilitation and LTP are both presynaptic and yet involve additive, non-interacting mechanisms; this could be the case, for example, if the initial probability of release were very low. Claims that LTP is associated with a specific^{57,58} increase in the AMPA receptor-mediated component of the synaptic response, have formed the basis of an argument for a purely postsynaptic change, on the assumption that a presynaptic change would result in a similar increase in both AMPA and NMDA receptor-mediated components. In support of this argument, an increase in both components was seen during PTP, whereas the isolated NMDA receptor-mediated component failed to exhibit LTP. But the argument has been undermined by subsequent reports that NMDA receptor-mediated synaptic transmission exhibits pronounced LTP⁵⁹⁻⁶².

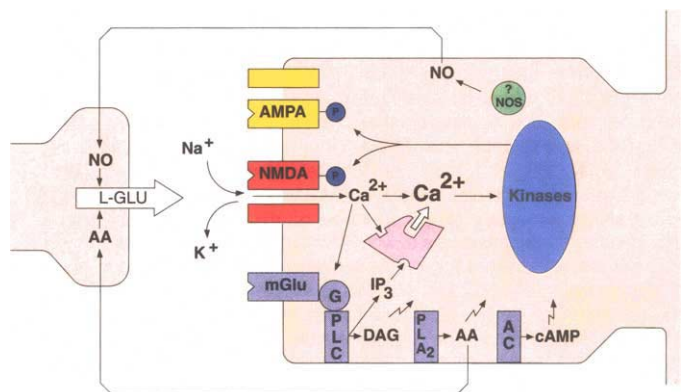
Another test for postsynaptic changes is to monitor the sensitivity of neurons to the application of agonists before and after the induction of LTP. Initial studies found no increase in the sensitivity to L-glutamate for up to 30 min post-tetanus^{63,64}. But in a more recent study, in which AMPA or quisqualate were used as agonists to avoid problems associated with the uptake and possible nonspecific actions of L-glutamate, a slow-onset increase in sensitivity was detected⁶⁵. The effect began within a few minutes but took an hour or more to reach a maximum. This time course parallels the slow-onset potentiation that can

be induced by the application of certain agents, such as arachidonic acid⁶⁶, and ACPD³⁶. It seems reasonable to assume that changes in the steady-state responses, as measured in the above experiments, reflect alterations that would also affect the response to synaptically released L-glutamate (for example, changes in the number, or conductance properties, of AMPA receptors). If this assumption is valid, then the results imply that the expression of STP is presynaptic whereas that of LTP is, at least in part, postsynaptic.

Despite the formidable interpretational problems of applying quantal analysis to central synapses, there has been a resurgence of interest in the use of this technique to analyse the locus of expression of LTP. Early studies in area CA1 indicated a presynaptic locus⁶⁷. Results of the more recent studies of fluctuations in the amplitude of synaptic responses have produced conclusions ranging from purely presynaptic⁶⁸, to predominantly presynaptic^{19,69,70}, to purely postsynaptic⁷¹ and, finally, to a mixture of purely presynaptic, purely postsynaptic and both pre- and postsynaptic^{72,73}. This variability may reflect differences in the initial release probability which, in turn, will be influenced by experimental parameters such as the extracellular Ca²⁺ concentration⁷³. Analysis of spontaneous miniature synaptic currents, associated with NMDA- or L-glutamate-induced STP, has provided evidence for an increase in quantal

BOX 3 Ways in which L-glutamate through its action on postsynaptic receptors may affect signal transduction processes involved in LTP

THE initial induction signal is a Ca^{2+} transient which permeates NMDA channels. This signal is then amplified by the release of Ca^{2+} from Ca^{2+} /InsP₃-sensitive intracellular stores. A parallel pathway which may be important for the induction of LTP is provided by mGluRs. These receptors can couple, through G-proteins, to the phosphoinositide-specific phospholipase C (PLC), phospholipase A₂ (PLA₂) and adenylate cyclase (AC)¹⁵⁵, to produce diacylglycerol (DAG), arachidonic acid (AA), and to regulate the levels of cAMP, respectively. Note that the initial NMDA receptor-mediated Ca^{2+} transient may be necessary for the activation of these mGluR cascades by L-glutamate¹⁴⁸. The amplified Ca^{2+} signal, in association with the other activators of protein kinases (zig-zag arrows), then leads to the phosphorylation of substrate proteins including, probably, AMPA and NMDA receptors. Other enzymes, such as nitric oxide synthase (NOS), if present, may also be activated by the Ca^{2+} transient. Biochemical changes in the presynaptic terminal may be initiated by the action of retrograde messengers, such as arachidonic acid (AA), nitric oxide (NO) and K^{+} , perhaps in conjunction with the action of L-glutamate on presynaptic mGluRs¹⁷⁴.



size in the hippocampal slice, implying a postsynaptic locus⁷⁴, and an increase in miniature frequency in cultured hippocampal neurons, implying a presynaptic locus⁷⁵. Evidently, the hoped-for resolution of the locus of expression of LTP by the application of quantal analysis has not yet been achieved. Note that if STP and the several temporal phases of LTP (see Box 1) are expressed at different loci, then changes in quantal parameters may alter progressively with time^{69,70}.

Signal transduction mechanisms. Several different Ca^{2+} -sensitive enzymes have been proposed to play a part in converting the probable induction signal, the entry of Ca^{2+} through the NMDA channel, into persistent modifications of synaptic strength. These include the protease calpain⁷⁶, phosphatases such as calcineurin⁷⁷, phospholipases and protein kinases. Most interest has focused on phosphorylation cascades and, in particular, the role of protein kinases. The first kinase to be implicated in LTP was the Ca^{2+} /phospholipid-dependent protein kinase (PKC)^{78–80}. Inhibitors of the enzyme invariably block the induction of LTP; in most studies, STP is unaffected by PKC inhibitors^{81–85}, though with the use of high doses or the combined application of inhibitors STP may also be blocked⁸⁶. There is general agreement that PKC inhibitors will block LTP if they are applied after the tetanus, indicating that kinase activity outlasts the initial induction signal. But the duration of the time-window during which kinase inhibitors are effective and the manner in which the activation of kinases is maintained are both matters of debate. For example, it has been suggested that constitutively activated PKC is involved because H-7, which inhibits the activity of the catalytic subunit, but not sphingosine, which prevents the initial activation of PKC, can depotentiate synapses in a reversible manner even when applied up to 3 h after induction⁸³. But the selectivity of H-7 for potentiated pathways has been challenged⁸⁷, and other PKC inhibitors that act on the catalytic subunit, including K-252b (ref. 85), are not able to depotentiate fully established LTP. There is also disagreement as to whether the sustained kinase activity that might be necessary for LTP is located within the postsynaptic cell⁸⁶ or not⁸⁸. A recent view⁸⁹ is that a postsynaptic kinase is activated transiently (for less than a few minutes following the tetanus) and a presynaptic kinase is activated for longer periods (but for less than 1 hour). These kinases might be the γ and β isoforms of PKC, respectively. Intracellular injection of the catalytic subunit of PKC induces synaptic potentiation⁹⁰ as does the extracellular application of activators of PKC, such as certain phorbol esters⁹¹. But the enhanced response does not survive washout of phorbol ester, and occlusion experiments indicate that LTP and phorbol ester-induced potentiation use different mechanisms^{92,93}. Overall, it seems that activation of PKC is not

sufficient to induce LTP but is a necessary factor and may be specifically involved in the conversion of STP to LTP1 (that is in the consolidation or stabilization of LTP). The development of more selective PKC inhibitors and, in particular, subtype-specific inhibitors are needed to confirm and extend these ideas.

Several inhibitor studies have also indicated a role for calmodulin and the Ca^{2+} /calmodulin-dependent protein kinase CaMKII in LTP^{84,88,94–96}. Knockout of the gene encoding α CaMKII, an isoform which is heavily enriched in postsynaptic densities, severely impairs, though it does not always completely block, the ability of slices to exhibit LTP⁹⁷. The autophosphorylated form of this enzyme does not require Ca^{2+} and as a result becomes constitutively active. This has led to the proposal that CaMKII can act as a form of molecular memory, recording the occurrence of a previous Ca^{2+} transient⁹⁸. But contrary to the predictions of this model, NMDA does not alter the proportion of Ca^{2+} -independent CaMKII in organotypic hippocampal cultures⁹⁹. Less is known about the role of other kinases in LTP. The level of cAMP is elevated in an NMDA receptor-dependent manner in LTP and this may indicate an involvement of cAMP-dependent protein kinase (PKA)¹⁰⁰. It has been suggested, on the basis of inhibitor studies, that protein tyrosine kinases (PTKs) are involved in LTP¹⁰¹, and it may be relevant that NMDA receptor activation leads to tyrosine phosphorylation of MAP-2 kinase¹⁰².

In addition to post-translational modification of existing proteins there is evidence that protein synthesis is also necessary for LTP. The extent to which protein synthesis inhibitors prevent LTP is variable, depending on the inhibitor used. Probably the clearest picture has emerged from the use of anisomycin, which inhibits translation of proteins from mRNAs. If present at the time of the tetanus anisomycin reduces the duration of LTP to 3–6 hours^{103–105}. A similar rate of decay is seen if LTP is induced in synapses that have been surgically isolated from the major site of protein synthesis in the cell body layer¹⁰⁵. In contrast, actinomycin, which prevents the transcription of mRNAs from DNA, has no effect on this anisomycin-sensitive phase¹⁰⁴. Taken together, these results suggest that proteins synthesized from pre-existing mRNA are required for the maintenance of LTP during the first few hours (corresponding to LTP2 in the classification shown in Box 1). The identity of proteins which are up- or downregulated during this period are not known, but several have been separated on two-dimensional gels¹⁰⁶. It is also intriguing that an increase in protease activity has been detected in perfusates from the dentate gyrus following potentiation¹⁰⁷, raising the possibility that cleavage of proteins with extracellular domains, such as neural cell adhesion molecules (NCAMs), may contribute to synaptic remodelling in LTP.

The experiment with actinomycin suggests that for the first 3 hours or so LTP does not depend on gene transcription. This does not preclude the possibility that genes are normally transcribed at or shortly after the time of induction but exert their effects at later times. An example of gene transcription induced by tetanic stimulation is the NMDA receptor-dependent increase in mRNA for the immediate early gene *zif/268* (refs 108–110). There is also a transient expression of *c-fos*, but only if the animal is unanaesthetised at the time of induction^{111,112}, suggesting that the *c-fos* protein is necessary for the generation of the most persistent form of LTP (LTP3; see Box 1). Changes in the abundance of mRNAs for a number of proteins have recently been identified in single CA1 cells 30 min to 3 hours after tetanization¹¹³. The reported changes in message for protein kinases (CaMKII is upregulated and the β -isoform of PKC is downregulated) suggests that protein kinases may play a role in the late stages of LTP, in addition to their presumptive action during the early phases.

Postsynaptic modifications. It is likely that the postsynaptic component of the expression of LTP involves alterations in the number and/or properties of the ion channels that mediate synaptic transmission. In view of the evidence that protein kinases are involved in LTP, the simplest scheme is that the kinases directly phosphorylate these ion channels. Consistent with this possibility, the gradual increase in AMPA sensitivity following the induction of LTP is prevented by K-252b, a potent kinase inhibitor⁸⁵. In addition, cloned AMPA receptors have several consensus sequences for phosphorylation by various kinases⁴⁷. Finally, the catalytic subunit of PKA can directly increase AMPA receptor function^{114,115}.

The finding that each of the AMPA receptor subunits can exist in two alternatively spliced variants, termed flip and flop, with different conductance properties, raises the possibility that LTP reflects a change in the relative expression of the flip and flop variants¹¹⁶. Alternatively, it could involve a change in the relative expression of the different subtypes of AMPA receptor, GluR 1–4 (ref. 47). A third possibility is regulation of RNA editing¹¹⁷.

The drug aniracetam, which potentiates responses to AMPA¹¹⁸ by preventing desensitization^{119,120}, has been used to explore how AMPA receptors may be modulated in LTP. The underlying idea is that if LTP and aniracetam share common mechanisms then their effects should interact. The weight of evidence suggests little interaction^{118,119,121,122} indicating that aniracetam and LTP do not regulate AMPA receptor function in the same manner.

So far, studies have concentrated on how the AMPA receptor-mediated component of synaptic transmission may be modified in LTP. But the NMDA receptor-mediated component also exhibits robust LTP^{59–62}. Alterations in this component could provide a means by which synapses increase their plasticity, as well as their efficiency. As with the AMPA receptor-mediated component, LTP of the NMDA receptor-mediated component of synaptic transmission could involve increases in L-glutamate release and/or postsynaptic modifications. A mechanism for the latter possibility is suggested by the observation that NMDA receptor function can be increased by the activation of PKC¹²³. This may involve phosphorylation of NMDA channels to alter the extent of the Mg^{2+} block of these channels¹²⁴. Another possibility is an upregulation of endogenous promoters of NMDA receptor function, such as arachidonic acid¹²⁵ and $InsP_3$ (ref. 126).

The nature of the retrograde messenger. The probable trigger for the induction of LTP is the entry of Ca^{2+} through NMDA channels located on the postsynaptic cell. But as we have seen, it is very likely that the potentiated response is maintained in part by presynaptic mechanisms. To reconcile these two observations, it was proposed that an intercellular signal is released from the postsynaptic site of induction to initiate increased transmitter release from the presynaptic terminal^{52,127}.

The first candidates to be considered were proteins. In addition to a tetanus-induced efflux of newly synthesised proteins from hippocampal slices¹²⁸ LTP is associated with an NMDA receptor-dependent increase in the protein content of hippocampal perfusates^{129,130}. The increases were slow to develop, apparently ruling out proteins as immediate retrograde messengers.

The next candidate to be examined was arachidonic acid. This unsaturated fatty acid satisfies several of the requirements for a retrograde messenger: (1) it is released from cultured neurons into the extracellular medium by the activation of NMDA receptors¹³¹, (2) there is an increase in its efflux¹³² and postsynaptic availability¹³³ following the induction of LTP, (3) inhibitors of phospholipase A_2 , an enzyme that liberates arachidonic acid from phospholipids, block the induction of LTP^{134–135}, and (4) the transient application of arachidonic acid to hippocampal synapses causes a slow-onset potentiation^{66,136}. Potential targets for arachidonic acid include not only the presynaptic terminal, where it may act to increase L-glutamate release⁶⁶, but also glial cells where it depresses L-glutamate uptake¹³⁷ and the postsynaptic cell, where, for example, it can potentiate NMDA receptor-mediated currents¹²⁵. Another phospholipase A_2 -derived lipid, platelet-activating factor, also has some of the properties expected of a retrograde messenger^{137,138}.

The possibility that nitric oxide (NO) may be a retrograde messenger in LTP has excited considerable interest. Like arachidonic acid, NO is released from cultured neurons exposed to NMDA¹³⁹. NO is derived from arginine in a reaction catalysed by NO synthase, and inhibitors of the enzyme have been reported to block the induction of LTP^{140–143}. Haemoglobin, a scavenger of NO which is presumably confined to the extracellular space, also blocks the induction of LTP^{141–143}, implying that NO (or another haem-binding molecule, such as CO) is released into the extracellular compartment. In addition, NO increases the frequency of miniature excitatory postsynaptic potentials (e.p.s.ps) in hippocampal cultures¹⁴². But although there is immunocytochemical evidence for NO synthase in hippocampal interneurons, there has been difficulty in obtaining evidence for its expression in pyramidal or granule cells¹⁴⁴. Furthermore, other laboratories¹⁴⁵, including our own, have not found a consistent block of LTP with NO synthase inhibitors. The story has been further complicated by the observation that under conditions where previous activation of the NMDA receptor system has disabled the induction mechanism^{28,29}, NO synthase inhibitors may promote the induction of LTP³⁰. Thus, the effect of NO synthase inhibitors may depend on the recent history of activity in the hippocampus. In summary, although NO remains an intriguing candidate, the evidence that it is a retrograde messenger is far from conclusive.

A general problem with the candidates discussed above is the time course of their action. The evidence for increased transmitter release is strongest for STP, that is from a few seconds to an hour or so after the inductive event. But inhibitors of arachidonic acid and NO synthesis both spare STP. Moreover, the potentiation produced by arachidonic acid is comparably slow to develop. Thus none of the proposed candidates has the properties expected of a *rapid* retrograde messenger. An alternative means of relaying postsynaptic activity is through alterations in activities of extracellular ions. One possibility is K^+ , which will be released from the postsynaptic cell during a tetanus to a degree that will, in part, reflect the level of activation of NMDA receptors. As discussed elsewhere¹⁴⁶, this could provide a signal to the presynaptic terminal through an interaction with presynaptic mGluRs¹⁴⁷, because the coupling of these receptors to PLC is strongly potentiated by extracellular K^+ (ref. 148).

Presynaptic modifications. Regulation of transmitter release could occur at any of the sequence of events leading from Ca^{2+} entry to exocytosis, through the mobilization, docking and fusion of vesicles at release sites in the presynaptic terminal.

BOX 4 LTP: Some unresolved issues

- (1) What is the physiological significance of LTP? Specifically, is it a central component in the synaptic machinery of memory?
- (2) What percentage of excitatory synapses can be potentiated? Is LTP at an individual synapse a graded or an all-or-none event?
- (3) What are (1) the presynaptic, and (2) the postsynaptic mechanisms underlying expression of LTP? What is the relative contribution of these two components and how does this change with time?
- (4) How do changes in the number or structure of synapses contribute to LTP?
- (5) Do retrograde messengers exist? If so, what are they and how do they regulate neurotransmitter release?
- (6) How prevalent is NMDA receptor-independent LTP, and to what extent

- do the two forms of LTP share common mechanisms?
- (7) How do other neurotransmitter and neuromodulators, such as acetylcholine, monoamines and peptides, regulate the induction and expression of LTP?
- (8) Does LTP always decay or is there a non-decremental form in the brain? Can LTP be reversed (depotentiated)?
- (9) What is the extent and significance of long-term depression (LTD) in the hippocampus?
- (10) Can knowledge about the mechanisms of LTP be exploited to devise rational therapies for neurological disorders such as Alzheimer's disease?

LTP-related changes in Ca^{2+} homeostasis could in principle account for persistent changes in transmitter release. Ca^{2+} levels were found to be elevated in synaptosomes prepared from potentiated dentate gyrus 45 min after the induction of LTP¹⁴⁹, and this may explain the enhanced ability of potentiated synaptosomes to release preloaded transmitter. Another possibility is an increase in the size of the Ca^{2+} transient associated with each action potential, following the induction of LTP. The measurement of Ca^{2+} transients associated with single action potentials in hippocampal afferent terminals has not yet been reported; however, the Ca^{2+} signal produced in mossy fibre terminals by trains of stimuli is not changed following the induction of LTP in this NMDA receptor-independent pathway¹⁵⁰. Alternatively, LTP may be associated with an increase in the sensitivity to Ca^{2+} to one or more components of the release mechanism¹⁵¹. Because LTP is expressed as an enhanced response to single stimuli, it is processes controlling the rapid fusion of synaptic vesicles with release sites, and/or the formation of fusion pores, which are the most likely targets for regulation. Processes which govern the ability of the terminal to respond during sustained activity, such as the synthesis of transmitter, the transport and filling of vesicles and their release from the cytoskeletal cage, will contribute to LTP only to the extent that they influence either the probability of fusion, or the amount of transmitter packed into vesicles.

The nature of the retrograde messenger may give clues to the processes responsible for the sustained increase in transmitter release. Arachidonic acid stimulates basal phosphoinositide turnover in synaptosomes prepared from the dentate gyrus¹⁵², and, consistent with this finding, there is an increase in presynaptic phosphoinositide turnover in LTP¹⁴⁹. Arachidonic acid therefore could lead to an activation of presynaptic PKC both directly and as a consequence of the increased production of diacylglycerol. Among presynaptic substrates for PKC is the calmodulin-binding protein gap43, phosphorylation of which is increased in LTP^{153,154}. Because phosphorylated gap43 cannot bind calmodulin, it is possible that through the resulting increased availability of calmodulin, the phosphorylation of synaptic vesicle proteins such as CaMKII substrates synaptophysin and synapsin could be affected, leading to modulation of vesicle fusion and hence of transmitter release. The identity of presynaptic targets for NO, which could include guanylate cyclase and ADP ribosyltransferase¹⁴², have not been determined. Finally, a presynaptic mGluR could be coupled to transmitter release in a number of ways, as suggested by the coupling of mGluR1 to PI hydrolysis, arachidonic acid production, and cAMP levels¹⁵⁵.

Conclusion

The associative characteristics that define the induction criteria for NMDA receptor-dependent LTP have found an elegant and satisfying explanation in the voltage-dependent properties of the NMDA receptor/channel complex. In contrast, little is known about the biochemical cascades that are triggered by the permeation of Ca^{2+} through open NMDA channels and which

lead to the persistent enhancement of synaptic efficiency. The evidence considered here suggests that tetanus-induced potentiation proceeds in stages, beginning with a protein kinase-independent phase (STP), lasting less than ~1 hour, followed by three stages of LTP (LTP1–3), requiring protein phosphorylation, protein synthesis from existing mRNAs, and gene transcription, respectively. The expression of synaptic potentiation probably involves both pre- and postsynaptic mechanisms, not necessarily in the same proportion at each stage, the one leading to an increase in transmitter release and the other to an increase in the number or change in the properties of the ion channels which mediate synaptic transmission. Activity-induced changes in the morphology or number of spines may also contribute to changes in synaptic efficiency, as suggested by a number of electron-microscopic studies^{156,157}. Advances in microscopy may soon allow the real-time visualization of any such changes¹⁵⁸.

In this review we have charted the substantial progress which has been made in understanding the cellular and molecular basis of NMDA receptor-dependent LTP in the hippocampus. It is part of the fascination of LTP that it can be studied experimentally at many levels, from the molecular to the behavioural; at the same time, knowledge about properties of LTP feeds directly into theoretical investigations of information storage in distributed neural networks. This catholicity of interest is reflected in the scope of the many questions that remain (Box 4). In the end, the overriding motivation for studying synaptic plasticity in the brain is the hope of gaining an understanding of the physical basis of memory in health and disease, and it is the nature of the link between LTP and memory that is likely to provide a major focus for research in the future. □

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1. Hebb, D. O. *The Organization of Behaviour* (Wiley, New York, 1949).
2. Konorski, J. *Conditioned Reflexes and Neuron Organisation* (Cambridge Univ. Press, Cambridge, 1948).
3. Bliss, T. V. P. & Lømo, T. *J. Physiol., Lond.* **232**, 331–356 (1973).
4. Bliss, T. V. P. & Gardner-Medwin, A. R. *J. Physiol., Lond.* **232**, 357–374 (1973).
5. Morris, R. G. M., Davis, S. & Butcher, S. P. *Phil. Trans. R. Soc.* **329**, 187–204 (1990).
6. Doyere, V. & Laroche, S. *Hippocampus* **2**, 39–48 (1992).
7. Larson, J., Wong, D. & Lynch, G. *Brain Res.* **368**, 347–350 (1986).
8. Rose, G. M. & Dunwiddie, T. V. *Neurosci. Lett.* **69**, 244–248 (1986).
9. Otto, T., Eichenbaum, H., Wiener, S. I. & Wible, C. G. *Hippocampus* **1**, 181–192 (1991).
10. McNaughton, B. L., Douglas, R. M. & Goddard, G. V. *Brain Res.* **157**, 277–294 (1978).
11. Lovinger, D. M. & Routtenberg, A. *J. Physiol., Lond.* **400**, 321–334 (1988).
12. Malenka, R. C. *Neuron* **6**, 53–60 (1991).
13. Levy, W. B. & Steward, O. *Brain Res.* **175**, 233–245 (1979).
14. Andersen, P., Sundberg, S. H., Sveen, O. & Wigström, H. *Nature* **266**, 736–737 (1977).
15. Lynch, G., Dunwiddie, T. & Gribkoff, V. *Nature* **266**, 737–739 (1977).
16. Kelso, S. R., Ganong, A. H. & Brown, T. H. *Proc. natn. Acad. Sci. U.S.A.* **83**, 5326–5330 (1986).
17. Wigström, H., Gustafsson, B., Huang, Y.-Y. & Abrahams, W. C. *Acta physiol. scand.* **126**, 317–319 (1986).
18. Sastry, B. R., Goh, J. W. & Auyeung, A. *Science* **232**, 988–990 (1986).
19. Malinow, R. *Science* **252**, 722–724 (1991).
20. Malinow, R. & Miller, J. P. *Nature* **320**, 529–530 (1986).
21. Ascher, P. & Nowak, L. *J. Physiol., Lond.* **399**, 247–266 (1988).
22. Collingridge, G. L., Herron, C. E. & Lester, R. A. J. *J. Physiol., Lond.* **399**, 283–300 (1988).
23. Davies, C. H., Starkey, S. J., Pozza, M. F. & Collingridge, G. L. *Nature* **349**, 609–611 (1991).
24. Collingridge, G. L., Kehl, S. J. & McLennan, H. *J. Physiol., Lond.* **334**, 33–46 (1983).

25. Coan, E. J., Saywood, W. & Collingridge, G. L. *Neurosci. Lett.* **80**, 111–114 (1987).
26. Bashir, Z. I., Tam, B. & Collingridge, G. L. *Neurosci. Lett.* **108**, 261–266 (1990).
27. Kauer, J. A., Malenka, R. C. & Nicoll, R. A. *Nature* **334**, 250–252 (1988).
28. Coan, E. J., Irving, A. J. & Collingridge, G. L. *Neurosci. Lett.* **105**, 205–210 (1989).
29. Huang, Y.-Y., Colino, A., Selig, D. K. & Malenka, R. C. *Science* **255**, 730–733 (1992).
30. Izumi, Y., Clifford, D. B. & Zorumski, C. F. *Science* **257**, 1273–1276 (1992).
31. Reymann, K. G. & Matthies, H. *Neurosci. Lett.* **98**, 166–171 (1989).
32. Izumi, Y., Clifford, D. B. & Zorumski, C. F. *Neurosci. Lett.* **122**, 187–190 (1991).
33. McGuinness, N., Anwyl, R. & Rowan, M. *Eur. J. Pharmacol.* **197**, 231–232 (1991).
34. Collingridge, G. L. *et al. Int. Acad. Biomed. Drug Res.* **2**, 41–49 (1991).
35. Radpour, S. & Thomson, A. M. *Neurosci. Lett.* **138**, 119–122 (1992).
36. Bortolotto, Z. A. & Collingridge, G. L. *Neuropharmacology* **32**, 1–9 (1993).
37. Lynch, G., Larson, J., Kelso, S., Barriounevo, G. & Schottler, F. *Nature* **305**, 719–721 (1983).
38. MacDermott, A. B., Mayer, M. L., Westbrook, G. L., Smith, S. J. & Barker, J. L. *Nature* **321**, 519–522 (1986).
39. Jahr, C. E. & Stevens, C. F. *Nature* **325**, 522–525 (1987).
40. Guthrie, P. B., Segal, M. & Kater, S. B. *Nature* **354**, 76–80 (1991).
41. Regehr, W. G. & Tank, D. W. *Nature* **345**, 807–810 (1990).
42. Müller, W. & Connor, J. A. *Nature* **354**, 73–76 (1991).
43. Malenka, R. C., Lancaster, B. & Zucker, R. S. *Neuron* **9**, 121–128 (1992).
44. Alford, S. & Collingridge, G. L. in *Excitatory Amino Acids and Second Messenger Systems* (eds Teichberg, Turski, V. I.) 43–53 (Springer, Berlin, 1992).
45. Obenaus, A., Mody, I. & Baimbridge, K. G. *Neurosci. Lett.* **98**, 172–178 (1989).
46. Harvey, J. & Collingridge, G. L. *Neurosci. Lett.* **139**, 197–200 (1992).
47. Gasic, G. P. & Hollmann, M. A. *Rev. Physiol.* **54**, 507–536 (1992).
48. Malenka, R. C., Kauer, J. A., Zucker, R. S. & Nicoll, R. A. *Science* **242**, 81–84 (1988).
49. Malenka, R. C., Kauer, J. A., Perkel, D. J. & Nicoll, R. A. *Trends Neurosci.* **12**, 444–450 (1989).
50. Skrede, K. & Mølle-Sørensen, D. *Brain Res.* **208**, 436–441 (1981).
51. Dolphin, A. C., Errington, M. L. & Bliss, T. V. P. *Nature* **297**, 496–498 (1982).
52. Bliss, T. V. P., Douglas, R. M., Errington, M. L. & Lynch, M. A. *J. Physiol., Lond.* **377**, 391–408 (1986).
53. Aniksztejn, L., Roisin, M. P., Amsellem, R. & Ben-Ari, Y. *Neuroscience* **28**, 387–392 (1989).
54. Bliss, T. V. P., Errington, M. L. & Lynch, M. A. *Adv. exp. med. Biol.* **268**, 269–278 (1990).
55. Feasey, K. J., Lynch, M. A. & Bliss, T. V. P. *Brain Res.* **364**, 39–44 (1986).
56. McNaughton, B. L. *J. Physiol., Lond.* **324**, 249–262 (1982).
57. Kauer, J. A., Malenka, R. C. & Nicoll, R. A. *Neuron* **1**, 911–917 (1988).
58. Müller, D. & Lynch, G. *Proc. natn. Acad. Sci. U.S.A.* **85**, 9346–9350 (1988).
59. Bashir, Z. I., Alford, S., Davies, S. N., Randall, A. D. & Collingridge, G. L. *Nature* **349**, 156–158 (1991).
60. Berretta, N. *et al. Eur. J. Neurosci.* **3**, 850–854 (1991).
61. Xie, X., Berger, T. W. & Barriounevo, G. *J. Neurophysiol.* **67**, 1009–1013 (1992).
62. Asztely, F., Wigström, H. & Gustafsson, B. *Eur. J. Neurosci.* **4**, 681–690 (1992).
63. Lynch, G., Gribkoff, V. & Deadwyler, S. A. *Nature* **263**, 151–153 (1976).
64. Taube, J. S. & Schwartzkroin, P. A. *J. Neurosci.* **8**, 1632–1644 (1988).
65. Davies, S. N., Lester, R. A. J., Reymann, K. G. & Collingridge, G. L. *Nature* **338**, 500–503 (1989).
66. Williams, J. H., Errington, M. L., Lynch, M. A. & Bliss, T. V. P. *Nature* **341**, 739–742 (1989).
67. Voronin, L. L. *Neuroscience* **10**, 1051–1069 (1983).
68. Bekkers, J. M. & Stevens, C. F. *Nature* **346**, 724–729 (1990).
69. Malinow, R. & Tsien, R. W. *Nature* **346**, 177–180 (1990).
70. Voronin, L. L., Kuhn, U. & Gusev, A. G. *Exp. Brain Res.* **89**, 288–299 (1992).
71. Foster, T. C. & McNaughton, B. L. *Hippocampus* **1**, 79–91 (1991).
72. Kullmann, D. M. & Nicoll, R. A. *Nature* **357**, 240–244 (1992).
73. Larkman, A., Hannay, T., Stratford, K. & Jack, J. *Nature* **360**, 70–73 (1992).
74. Manabe, T., Renner, P. & Nicoll, R. A. *Nature* **355**, 50–55 (1992).
75. Malgaroli, A. & Tsien, R. W. *Nature* **357**, 134–139 (1992).
76. Oliver, M. W., Baudry, M. & Lynch, G. *Brain Res.* **505**, 233–238 (1989).
77. Halpain, S. & Greengard, P. *Neuron* **5**, 237–246 (1990).
78. Bär, P. R., Wiegant, F., Lopes da Silva, F. H. & Gispén, W. H. *Brain Res.* **321**, 381–385 (1984).
79. Akers, R., Lovinger, D., Colley, P., Linden, D. & Routtenberg, A. *Science* **231**, 587–589 (1986).
80. Klann, E., Chen, S.-J. & Sweatt, J. D. *J. Biol. Chem.* **266**, 24253–24256 (1991).
81. Lovinger, D. M., Wong, K. L., Murakami, K. & Routtenberg, A. *Brain Res.* **436**, 177–183 (1987).
82. Reymann, K. G., Frey, U., Jork, R. & Matthies, H. *Brain Res.* **440**, 305–314 (1988).
83. Malinow, R., Madison, D. V. & Tsien, R. W. *Nature* **335**, 821–824 (1988).
84. Malenka, R. C. *et al. Nature* **340**, 554–557 (1989).
85. Reymann, K. G., Davies, S. N., Matthies, H., Kase, H. & Collingridge, G. L. *Eur. J. Neurosci.* **2**, 481–486 (1990).
86. Wang, J. & Feng, D.-P. *Proc. natn. Acad. Sci. U.S.A.* **89**, 2576–2580 (1992).
87. Müller, D., Buchs, P.-A., Dunant, Y. & Lynch, G. *Proc. natn. Acad. Sci. U.S.A.* **87**, 4073–4077 (1990).
88. Malinow, R., Schulman, H. & Tsien, R. W. *Science* **245**, 862–866 (1989).
89. Huang, Y.-Y., Colley, P. A. & Routtenberg, A. *Neuroscience* **49**, 819–827 (1992).
90. Hu, G.-Y. *et al. Nature* **328**, 426–429 (1987).
91. Malenka, R. C., Madison, D. V. & Nicoll, R. A. *Nature* **321**, 175–177 (1986).
92. Müller, D., Turnbull, J., Baudry, M. & Lynch, G. *Proc. natn. Acad. Sci. U.S.A.* **85**, 6997–7000 (1988).
93. Gustafsson, B., Huang, Y.-Y. & Wigström, H. *Neurosci. Lett.* **85**, 77–81 (1988).
94. Mody, I., Baimbridge, K. G. & Miller, J. J. *Neuropharmacology* **23**, 625–631 (1984).
95. Reymann, K. G., Brodemann, R., Kase, H. & Matthies, H. *Brain Res.* **461**, 388–392 (1988).
96. Ito, I., Hidaka, H. & Sugiyama, H. *Neurosci. Lett.* **121**, 119–121 (1991).
97. Silva, J. J., Stevens, C. F., Tonegawa, S. & Wang, Y. *Science* **257**, 201–206 (1992).
98. Lisman, J. E. & Goldring, M. A. *Proc. natn. Acad. Sci. U.S.A.* **85**, 5320–5324 (1988).
99. Molloy, S. S. & Kennedy, M. B. *Proc. natn. Acad. Sci. U.S.A.* **88**, 4756–4760 (1991).
100. Chetkovich, D. M., Gray, R., Johnston, D. & Sweatt, J. D. *Proc. natn. Acad. Sci. U.S.A.* **88**, 6467–6471 (1991).
101. O'Dell, T. J., Kandel, E. R. & Grant, S. G. N. *Nature* **353**, 558–560 (1991).
102. Bading, H. & Greengard, M. E. *Science* **253**, 912–914 (1991).
103. Krug, M., Lossner, B. & Ott, T. *Brain Res. Bull.* **13**, 39–42 (1984).
104. Otani, S., Marshall, C. J., Tate, W. P., Goddard, G. V. & Abraham, W. C. *Neuroscience* **28**, 519–526 (1989).
105. Frey, U., Krug, M., Brödemann, R., Reymann, K. & Matthies, H. *Neurosci. Lett.* **97**, 135–139 (1989).
106. Fazeli, M. S., Corbett, J., Dunn, M. J., Dolphin, A. C. & Bliss, T. V. P. *J. Neurosci.* **13**(4) (in the press).
107. Fazeli, M. S., Errington, M. L., Dolphin, A. C. & Bliss, T. V. P. *Brain Res.* **521**, 247–253 (1990).
108. Abraham, W. C., Dragunow, M. & Tate, W. P. *Molec. Neurobiol.* **5**, 297–314 (1991).
109. Cole, I., Tanabe, S., Khoda, A. W., Baraban, J. M. & Worley, P. F. *Nature* **340**, 474–476 (1989).
110. Wisden, W. *et al. Neuron* **4**, 603–614 (1990).
111. Dragunow, M. *et al. Neurosci. Lett.* **101**, 274–280 (1989).
112. Nikolaev, E., Tischmeyer, W., Krug, M., Matthies, H. & Kaczmarek, L. *Brain Res.* **560**, 346–349 (1991).
113. Mackler, S. A., Brooks, B. P. & Eberwine, J. H. *Neuron* **9**, 539–548 (1992).
114. Greengard, P., Jen, J., Nairn, A. C. & Stevens, C. F. *Science* **253**, 1135–1138 (1991).
115. Wang, L.-Y., Salter, M. W. & MacDonald, J. F. *Science* **253**, 1132–1135 (1991).
116. Sommer, B. *et al. Science* **249**, 1580–1585 (1990).
117. Sommer, B., Kohler, M., Sprengel, R. & Seeburg, P. H. *Cell* **67**, 11–19 (1992).
118. Ito, I., Tanabe, S., Khoda, A. W., Sugiyama, H. *J. Physiol., Lond.* **424**, 533–543 (1990).
119. Isaacson, J. S. & Nicoll, R. A. *Proc. natn. Acad. Sci. U.S.A.* **88**, 10936–10940 (1991).
120. Tang, C. M., Shi, Q. Y., Katchman, A. & Lynch, G. *Science* **254**, 288–290 (1991).
121. Staubli, U., Kessler, M. & Lynch, G. *Psychobiology* **18**, 377–381 (1990).
122. Asztely, F., Hanse, E., Wigström, H. & Gustafsson, B. *Synapse* **11**, 342–345 (1992).
123. Kelso, S. R., Nelson, T. E. & Leonard, J. P. *J. Physiol., Lond.* **449**, 705–718 (1992).
124. Chen, L. & Huang, L.-Y. *M. Science* **356**, 521–523 (1992).
125. Miller, B., Sarantis, M., Traynelis, S. F. & Attwell, D. *Nature* **355**, 722–725 (1992).
126. Markram, H. & Segal, M. *J. Physiol., Lond.* **447**, 513–533 (1992).
127. Bliss, T. V. P. & Dolphin, A. C. in *The Neurobiology of Learning and Memory* (eds McGaugh, J. L. & Lynch, G.) (Guilford, New York, 1984).
128. Duffy, C., Teyler, T. J. & Shashoua, V. E. *Science* **212**, 1148–1151 (1981).
129. Fazeli, M. S., Errington, M. L., Dolphin, A. C. & Bliss, T. V. P. *Brain Res.* **473**, 51–59 (1988).
130. Otani, S., Roisin-Lallemant, M.-P. & Ben-Ari, Y. *Neuroscience* **47**, 265–272 (1992).
131. Dumuis, A., Sebben, M., Haynes, L., Pin, J.-P. & Bockaert, J. *Nature* **336**, 68–70 (1988).
132. Bliss, T. V. P., Errington, M. L., Lynch, M. A. & Williams, J. H. *Cold Spring Harb. Symp. quant. Biol.* **55**, 119–129 (1990).
133. Clements, M. P., Bliss, T. V. P. & Lynch, M. A. *Neuroscience* **45**, 379–389 (1991).
134. Okada, D., Yamagishi, S. & Sugiyama, H. *Neurosci. Lett.* **100**, 141–146 (1989).
135. Williams, J. H. & Bliss, T. V. P. *Neurosci. Lett.* **107**, 301–306 (1989).
136. Barbour, B., Szatkowski, M., Ingledew, N. & Attwell, D. *Nature* **342**, 918–920 (1989).
137. Arai, A. & Lynch, G. *Eur. J. Neurosci.* **4**, 411–419 (1992).
138. Clark, G. D., Happel, L. T., Zorumski, C. F. & Bazan, N. G. *Neuron* **9**, 1211–1216 (1992).
139. Garthwaite, J., Charles, S. L. & Chess-Williams, R. *Nature* **336**, 385–388 (1988).
140. Böhm, G. A., Bon, C., Stutzmann, J.-M., Doble, A. & Blanchard, J.-C. *Eur. J. Pharmacol.* **199**, 379–381 (1991).
141. Schuman, E. M. & Madison, D. V. *Science* **254**, 1503–1506 (1991).
142. O'Dell, T. J., Hawkins, R. D., Kandel, E. R. & Arancio, O. *Proc. natn. Acad. Sci. U.S.A.* **88**, 11285–11289 (1991).
143. Haley, J. E., Wilcox, G. L. & Chapman, P. F. *Neuron* **8**, 211–216 (1992).
144. Brett, S. B. & Snyder, S. H. *Neuron* **8**, 3–11 (1992).
145. Gribkoff, V. K. & Lum-Ragan, J. T. *J. Neurophysiol.* **68**, 639–642 (1992).
146. Collingridge, G. L. *Expl. Physiol.* **77**, 771–797 (1992).
147. Baskys, A. & Malenka, R. C. *J. Physiol., Lond.* **444**, 687–701 (1991).
148. Irving, A. J., Collingridge, G. L. & Schofield, J. G. *Cell Calcium* **13**, 293–301 (1992).
149. Lynch, M. A. & Voss, K. L. *J. Neurochem.* **56**, 113–118 (1991).
150. Regehr, W. G. & Tank, D. W. *Neuron* **7**, 451–459 (1991).
151. Lynch, M. A. & Bliss, T. V. P. *Brain Res.* **369**, 405–408 (1986).
152. Lynch, M. A. & Voss, K. L. *J. Neurochem.* **55**, 215–221 (1990).
153. Linden, D. J., Wong, K. L., Sheu, F.-S. & Routtenberg, A. *Brain Res.* **458**, 142–146 (1988).
154. Giannotti, C., Nunzi, M. G., Gispén, W. H. & Corradetti, R. *Neuron* **8**, 843–848 (1992).
155. Aramori, I. & Nakanishi, S. *Neuron* **8**, 757–765 (1992).
156. Desmond, N. L. & Levy, W. B. *Synapse* **5**, 139–143 (1990).
157. Geinisman, Y., de Toledo-Morell, L. & Morrell, F. *Brain Res.* **566**, 77–88 (1991).
158. Hosokawa, T., Bliss, T. V. P. & Fine, A. *Neuroreport* **3**, 477–480 (1992).
159. Jeffery, K. J., Abraham, W. C., Dragunow, M. & Mason, S. E. *Molec. Brain Res.* **8**, 267–274 (1990).
160. Andersen, P., Sundberg, S. H., Sveen, O., Swann, J. W. & Wigström, H. *J. Physiol., Lond.* **302**, 463–482 (1980).
161. Abraham, W. C., Bliss, T. V. P. & Goddard, G. V. *J. Physiol., Lond.* **363**, 335–349 (1985).
162. Bonhoeffer, T., Staiger, V. & Aertsen, A. *Proc. natn. Acad. Sci. U.S.A.* **86**, 8113–8117 (1989).
163. Harris, E. W. & Cotman, C. W. *Neurosci. Lett.* **70**, 132–137 (1986).
164. Johnston, D., Williams, S., Jaffe, D. & Gray, R. A. *Res. Physiol.* **54**, 489–505 (1992).
165. Grover, L. M. & Teyler, T. J. *Nature* **347**, 477–479 (1990).
166. Turner, R. W., Baimbridge, K. G. & Miller, J. J. *Neuroscience* **7**, 1411–1416 (1982).
167. Aniksztejn, L. & Ben-Ari, Y. *Nature* **349**, 67–69 (1991).
168. Publicover, S. J. *Expl. Brain Res.* **84**, 680–684 (1991).
169. Davies, S. N. & Collingridge, G. L. *Proc. R. Soc. B* **236**, 373–384 (1989).
170. Andreasen, M., Lambert, J. D. C. & Jensen, M. S. *J. Physiol., Lond.* **414**, 317–336 (1989).
171. Dale, N. & Roberts, A. *J. Physiol., Lond.* **363**, 35–59 (1985).
172. Lester, R. A. J., Clements, J. D., Westbrook, G. L. & Jahr, C. E. *Nature* **346**, 565–567 (1990).
173. Collingridge, G. L., Herron, C. E. & Lester, R. A. J. *J. Physiol., Lond.* **399**, 301–312 (1988).
174. Herrero, I., Miras-Portugal, M. T. & Sánchez-Prieto, J. *Nature* **360**, 163–166 (1992).

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