Synaptic tagging: implications for late maintenance of hippocampal long-term potentiation

Uwe Frey and Richard G.M. Morris

A novel property of hippocampal LTP, 'variable persistence', has recently been described that is, we argue, relevant to the role of LTP in information storage. Specifically, new results indicate that a particular pattern of synaptic activation can give rise, either to a relatively short-lasting LTP, or to a longer-lasting LTP as a function of the history of activation of the neuron. This has led to the idea that the induction of LTP is associated with the setting of a 'synaptic tag' at activated synapses, whose role is to sequester plasticity-related proteins that then serve to stabilize temporary synaptic changes and so extend their persistence. In this article, we outline the synaptic tag hypothesis, compare predictions it makes with those of other theories about the persistence of LTP, and speculate about the cellular identity of the tag. In addition, we outline the requirement for aminergic activation to induce late LTP and consider the functional implications of the synaptic tag hypothesis with respect to long-term memory.

Trends Neurosci. (1998) 21, 181–188

THE HIPPOCAMPUS is one of a number of brain structures important for the formation of certain kinds of memory[1–3]. Although the information presented to the hippocampal network and that sent on from it to other brain structures remains uncertain, hippocampal neurons exhibit a number of intriguing biophysical properties that enable them to participate in aspects of memory formation. These include synaptic plasticity mechanisms that can respond to incoming information by detecting associative interactions between pre- and post-synaptic activity and register these conjunctions as changes in synaptic weights. Bliss and Lømo[4] gave the first detailed description of the physiological phenomenon now known as LTP that has since become the best studied model of the hypothetical cellular mechanisms of memory formation. Its input specificity, associativity, rapid induction and prolonged duration for hours, days or even weeks (in the intact animal) are all properties that render LTP an attractive model. Distinct forms of LTP have been found in various pathways of the hippocampus and in other structures of the CNS. Of these, associative NMDA-receptor-dependent LTP, which has the above properties, is the most widely studied. Although other forms of lasting synaptic plasticity, such as mossy fibre potentiation, neurotrophin-induced potentiation and LTD are important, we shall hereafter discuss only the NMDA-receptor-dependent form and refer to it, for simplicity, as 'LTP'.

Early LTP and late LTP

Much progress has been made in elucidating the cellular mechanisms that underly the induction and early expression of LTP (Ref. 5), but less is known about its maintenance. A key observation is that the extended persistence of NMDA-receptor-dependent LTP beyond about four hours is critically dependent on protein and mRNA synthesis[6–14]. LTP is, therefore, divided into an 'early' form whose induction is unaffected by protein-synthesis inhibitors, and a 'late' form that is apparently blocked by such inhibitors[15].

The mechanistic distinction between 'early' and 'late' LTP, however, can also be viewed as referring to either how long either type of synaptic change lasts or to the moment of onset. We now believe that the distinction between early and late LTP is best used in the former sense, primarily because our data[14] indicate that late LTP can, in certain circumstances, be induced very shortly after the expression of the enhanced synaptic change that constitutes early LTP. Thus, early LTP might be a staging post on the way to a longer-lasting change[16]; alternatively, it could be a separate or parallel phase of LTP expression that is not required for late LTP. Notably, it does not seem to be a phase of LTP that has to last some minimum time before late LTP can be induced.

Early LTP involves a number of cellular mechanisms. For example, it is well established that a specific concentration of intracellular Ca^{2+} must be reached for its induction, mainly by Ca^{2+} influx via the associative activation of the NMDA receptor. Additionally, possibly supplemented by a Ca^{2+}-dependent Ca^{2+} release from internal stores, this influx can activate several kinases that are responsible for both a short-lasting form of synaptic plasticity (STP) that decays within 60 min and a second stage of early LTP lasting about three to six hours (reviewed in Refs 5,8). In contrast to late LTP, early LTP precludes the additional induction of LTP at activated synapses, but only temporarily[17]. Therefore, it can be speculated that each synapse that is capable of responding with plastic changes contains certain receptors or channels that can be thought of as 'fast-acting plasticity processors' (FAPPs). These might, for example, transiently

Uwe Frey is at the Leibniz Institute for Neurobiology, Brenneckestrasse 6, D-39008 Magdeburg, Germany and Richard G.M. Morris is at the Centre for Neuroscience and Dept of Pharmacology, University of Edinburgh, Crichton Street, Edinburgh, UK EH8 9LE.

 0166 - 2236/98/$19.00 PII: S0166-2236(97)01189-2

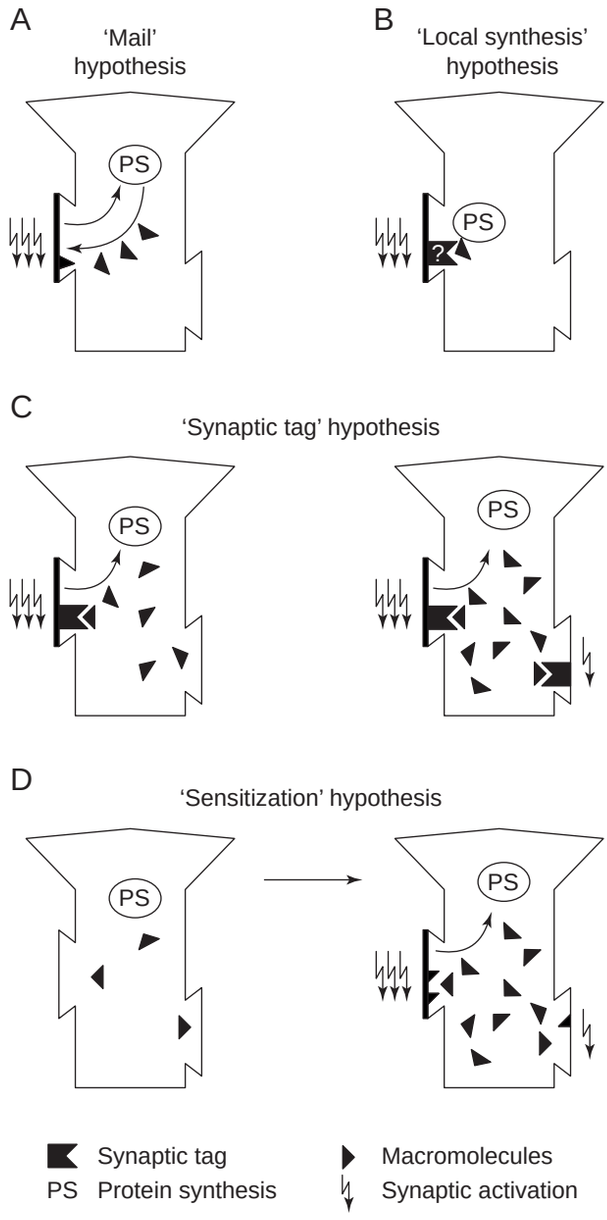

Fig. 1. ***Four ways in which the synapse specificity of late LTP could be achieved.*** **(A)** *The 'mail' hypothesis involves elaborate intracellular protein trafficking, where proteins, at the time of their synthesis, are given a 'synaptic address' to which they are then sent (indicated by curved arrows).* **(B)** *The 'local synthesis' hypothesis[26] asserts that the relevant protein synthetic machinery is present at, and only activated by, stimulation of nearby synapses. Input specificity is a straightforward consequence of this cellular architecture. However, as protein synthesis does not take place in single synaptic spines but in the nearby dendritic area[26–28], there might yet be a need for a supplementary tagging mechanism to guarantee input specificity illustrated by the 'tag' symbol. Recent findings by Kang and Schuman[29] support local dendritic protein synthesis by neurotrophin-induced potentiation, but this form of plasticity is input-nonspecific.* **(C)** *The 'synaptic tag' hypothesis involves setting, at activated synapses, a 'tag' whose job is to sequester selected proteins. This tag obviates the need for elaborate protein trafficking. Plasticity-related proteins can be synthesized in the soma (or in the dendrites) and then distributed throughout the dendritic tree of a cell relatively diffusely. In this view, the proteins have no address to go to, and thus are only used when captured.* **(D)** *The 'sensitization' hypothesis entails distribution of plasticity-related macromolecules to every synapse of the cell. These would have the effect of altering the threshold at which synaptic activation (or Ca^{2+} influx) gives rise to lasting synaptic changes. When few of these macromolecules are available, a high threshold prevails, and tetanization usually induces early LTP only; when many macromolecules are available, it is much easier for late LTP to be induced (R. Malinow, pers. commun.).*

transform silent AMPA receptors into active receptors[18,19] or contribute to enhanced transmitter release[5]. These processors guarantee its maintenance for the first two to four hours. Protein- and RNA-synthesis-dependent late LTP (Refs 6,7,9,11–13) could result in the creation of additional processors that take over the function of FAPPs after their inactivation, thus transforming early LTP into late LTP.

The requirement of late LTP for newly synthesized proteins is less often studied because most LTP experiments continue for less than two hours after tetanization. Its inhibition by the protein synthesis inhibitor anisomycin was first discovered *in vivo*[6] *a*nd subsequently revealed *in vitro*[7]. Inhibitors of protein synthesis prevent LTP lasting longer than about six hours, although two reports have shown effects at an earlier stage (after about one hour)[20,21]. In hippocampal slices, application of inhibitors of mRNA synthesis (actinomycin D) during LTP induction can prevent late LTP occurring after about three hours; this is similar to that seen after protein-synthesis inhibition[12]. However, if the side effects of this irreversible drug are limited by applying it transiently before tetanization (in either hippocampal slices or freely moving animals), an elevated level of maintained potentiation is seen at three hours, albeit at a decaying rate[13]. Proteins from newly synthesized mRNA might, therefore, only be effective or required at later time points. Support for this interpretation comes from experiments that show an increase in mRNA for ERK2 and Raf-1 24 h after LTP induction[22].

Protein trafficking and the synaptic tag hypothesis

Although the identity of the proteins responsible for stabilizing LTP has not been established, its occurrence raises the following fundamental question: given that macromolecule synthesis occurs mainly in the cell body[23], how do these proteins find their way through the dendrites to the appropriate synapses where the stabilization of early LTP is required? In general, there are considered to be four hypotheses about how the synapse specificity of late LTP could be achieved (see also Refs 11,24,25): the 'mail' hypothesis; the 'local synthesis' hypothesis; the 'synaptic tag' hypothesis; and the 'sensitization' hypothesis. The characteristics of each mechanism are illustrated in Fig. 1.

We believe the mail hypothesis is intrinsically unlikely. Nature is cleverer than to require proteins to travel from the soma to a specific synapse in a cell that, in the case of CA1 pyramidal cells, might have >10 000 synapses. The local-synthesis idea is supported by the presence of spine-associated polyribosomes[30], but it is expensive biochemically and, as described later, cannot explain our main experimental finding. The sensitization hypothesis is supported by recent findings of a *de novo* protein-synthesis-dependent formation of protein kinase Mζ (Ref. 21). It shares with the synaptic tag idea that the persistence of LTP can vary as a function of the recent history of activation of the neuron, but this variability would be strictly dependent on the past history of activation[31], and uninfluenced by the immediate future 'history' of activation. It also makes the false prediction that application of protein-synthesis inhibitors shortly after LTP induction should have no effect on LTP persistence[7] although effects later than eight hours

cannot yet be excluded. The synaptic tag hypothesis permits greater flexibility and more intracellular cooperativity than any of the other ideas.

Variable persistence: the induction of protein-synthesis-dependent LTP during the inhibition of protein synthesis

Frey and Morris[14] conducted the following test of the synaptic tag idea. Using hippocampal slices *in vitro*, two independent pathways were activated with low-frequency test pulses and each tetanized once (Fig. 2F). First, pathway S1 was tetanized with a strong tetanus to induce late LTP. Thirty-five minutes later, anisomycin was added to the test chamber to shut down protein synthesis, which happened quickly[9,32]. Then, 25 minutes after that, with protein synthesis arrested, pathway S2 was tetanized strongly. The key finding was that late LTP was also induced on this pathway (Fig. 2A). Late LTP has been shown repeatedly to require protein synthesis, and thus, this experiment reveals the paradoxical induction of protein-synthesis-dependent LTP on S2 during the inhibition of protein synthesis. It is important to appreciate that, as shown in control experiments, had S2 been tetanized in the presence of anisomycin without S1 having previously been tetanized, only early LTP, lasting less than four hours, would have been obtained. Within-slice control experiments also showed that, in the presence of anisomycin, strong tetanization of a pathway could give rise to early LTP at some terminals and late LTP at others. This was found to be determined by whether or not the neuronal population onto which the terminals were afferent had previously been tetanized using a separate pathway. Stimulation that normally leads to early LTP could also induce late LTP if a separate pathway had been strongly tetanized (Fig. 2C). Thus, tetanization of a pathway can induce an LTP with variable persistence as a function of the prior history of activation of the neuron.

These findings are incompatible with the 'mail' and 'local-synthesis' hypotheses because late LTP was induced during the inhibition of protein synthesis. Our interpretation involves the dual concept of a protein-synthesis-independent setting of a synaptic tag, and a protein-synthesis-dependent production of plasticity-related macromolecules; once plasticity proteins have been synthesized and distributed, they can be captured only at synapses that exhibit a tag (Fig. 3). Importantly, although tetanic activation of a synapse can give rise to the events that set a synaptic tag and can trigger the synthesis of macromolecules, a tag has no way of knowing whether the macromolecules it eventually captures were synthesized in response to these same events. A tag will presumably identify a specific subset of proteins, but beyond that, the tag–macromolecule relationship is promiscuous. A tag at one synapse can 'hijack' proteins synthesized in response to activity at another synapse.

The synaptic-tag hypothesis also makes a number of other predictions. One is that the successful induction of late LTP at a synapse will depend on the intersection of two parameters: the decay time course of the tag and the intracellular kinetics of relevant protein synthesis and distribution; however, which of these is initiated first is unimportant. In contrast to the sensitization hypothesis, which requires protein synthesis and distribution to occur before the thresholds for inducing late LTP can be lowered, the synaptic tag hypothesis allows for the possibility that early LTP might be stabilized by subsequent late LTP. Our most recent experiments (Fig. 2D,E) suggest that early LTP on one pathway induced five minutes or one hour before late LTP on another can be stabilized and thus 'transformed' into late LTP. However, the tag is short-lasting – one to two hours at most. Frey and Morris[14] also showed that early LTP induced three hours before late LTP cannot be 'rescued', a result we have since confirmed.

The synaptic-tag hypothesis can also help explain the observation that the induction of early LTP on one pathway precludes the induction of further early LTP on that same pathway for a period thereafter, but that early LTP can be induced on a pathway displaying late LTP (Ref. 17). The reason for this might be that tag–protein interactions result in the stabilization of a temporarily expressed synaptic change, freeing up FAPPs to be responsive to new inputs.

The identity of the tag

What is the molecular identity of the putative synaptic tag? One possibility is a change in spine-neck diameter. If synapses displaying early LTP had wider neck diameters than synapses that had not recently been potentiated, access to the synaptic apposition zone might then be easier for the large macromolecules that we assume are responsible for stabilizing LTP. Simulation studies have revealed that although changes in spine shape are not responsible for changes of synaptic efficacy, branching of spines and changes in their geometry could be significant[33]. A second possibility is the phosphorylated state of an early-LTP-associated kinase with a duration of about four to six hours[21]. This would explain our observation that, following late LTP on one pathway, the induction of STP on another does not result in a stable long-lasting potentiation. An economical arrangement would be one in which the biochemical cascade responsible for early LTP included phosphorylation of a protein that, in addition to its immediate effects on synaptic transmission, was also responsible for sequestering proteins later[11,16].

Experiments to identify the tag could profitably use the same two-pathway design, but using drugs that act on specific receptors or kinases. It would be valuable to know if the tag is downstream of the NMDA receptor, involves any interaction with metabotropic glutamate receptors or requires kinase activation and, if so, which one. Once identified, molecular techniques such as the yeast two-hybrid system could be employed to help identify proteins with which the tag interacts.

Plasticity-related proteins and the role of aminergic innervation

LTP experiments typically have a three-phase design: a low-frequency test-pulse baseline, a brief period of high-frequency stimulation (or, with intracellular recording, the pairing of pre- and postsynaptic activation) followed by a second period of test pulses. We have all got so used to this arrangement that it is easy to overlook the more likely situation, *in vivo*, where LTP of individual synapses is probably happening frequently. The synaptic population of an individual CA1 cell is likely to be changing dynamically, with

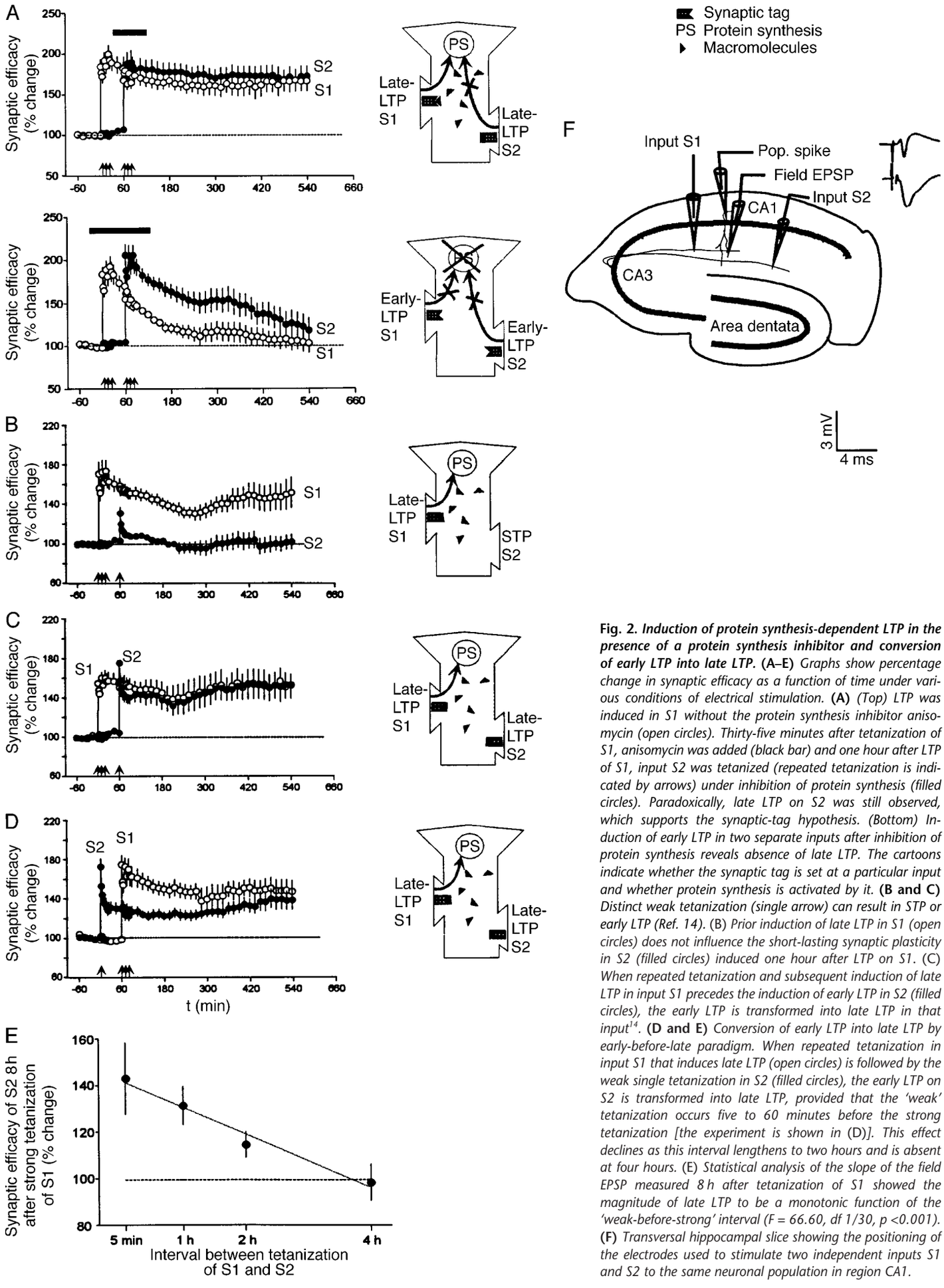

Fig. 2. ***Induction of protein synthesis-dependent LTP in the presence of a protein synthesis inhibitor and conversion of early LTP into late LTP.*** **(A–E)** *Graphs show percentage change in synaptic efficacy as a function of time under various conditions of electrical stimulation.* **(A)** *(Top) LTP was induced in S1 without the protein synthesis inhibitor anisomycin (open circles). Thirty-five minutes after tetanization of S1, anisomycin was added (black bar) and one hour after LTP of S1, input S2 was tetanized (repeated tetanization is indicated by arrows) under inhibition of protein synthesis (filled circles). Paradoxically, late LTP on S2 was still observed, which supports the synaptic-tag hypothesis. (Bottom) Induction of early LTP in two separate inputs after inhibition of protein synthesis reveals absence of late LTP. The cartoons indicate whether the synaptic tag is set at a particular input and whether protein synthesis is activated by it.* **(B and C)** *Distinct weak tetanization (single arrow) can result in STP or early LTP (Ref. 14).* (B) *Prior induction of late LTP in S1 (open circles) does not influence the short-lasting synaptic plasticity in S2 (filled circles) induced one hour after LTP on S1.* (C) *When repeated tetanization and subsequent induction of late LTP in input S1 precedes the induction of early LTP in S2 (filled circles), the early LTP is transformed into late LTP in that input[14].* **(D and E)** *Conversion of early LTP into late LTP by early-before-late paradigm. When repeated tetanization in input S1 that induces late LTP (open circles) is followed by the weak single tetanization in S2 (filled circles), the early LTP on S2 is transformed into late LTP, provided that the 'weak' tetanization occurs five to 60 minutes before the strong tetanization [the experiment is shown in* (D)*]. This effect declines as this interval lengthens to two hours and is absent at four hours.* (E) *Statistical analysis of the slope of the field EPSP measured 8 h after tetanization of S1 showed the magnitude of late LTP to be a monotonic function of the 'weak-before-strong' interval ($F = 66.60$, df 1/30, $p < 0.001$).* **(F)** *Transversal hippocampal slice showing the positioning of the electrodes used to stimulate two independent inputs S1 and S2 to the same neuronal population in region CA1.*

potentiation at some sites matched by heterosynaptic and homosynaptic decreases in synaptic efficacy elsewhere.

The implication of this more 'natural' way of looking at things is that the synthesis of plasticity-related proteins will be continuous rather than phasic, with varying rates of synthesis as a function of recent neuronal activation. In many experiments on late LTP, a strong tetanus is used to upregulate protein synthesis discretely at a specific time point. This leads to the concept of a 'time window' during which the expression of late LTP can be disrupted[12]. The synaptic-tag hypothesis, however, supposes that the time window arises from the interaction of the decay time course of the tag and kinetics of protein availability, with the moment of tetanization determining only the onset of the tag. Strong tetanization might be only one of a number of ways in which protein synthesis can be modulated.

A number of transmitters, including noradrenaline, ACh and opioids, are known to modulate LTP (Refs 34–45). These transmitters interact with LTP in different ways. However, given the effects of dopamine on learning[46], we decided to investigate its role during LTP induction in the CA1 region of the hippocampus. We found that dopamine levels increased during conventional LTP induction[47] (Fig. 4C), raising the question of what this dopamine might be doing. Is it required for late LTP? The hippocampus is innervated by dopaminergic fibres that course through the mesolimbic pathway[48,50], and there is evidence for the expression of D5 receptors in CA1 pyramidal cells. The D5 receptor is related to the D1 dopamine receptor, which is known to be positively coupled to adenylyl cyclase[51]. Our studies reveal that specific inhibitors of D1 (and D2, but not yet D5) receptors block the expression of late LTP without effect on early LTP (Fig. 4A)[47,49]. Furthermore, our unpublished observations and those of others have revealed that transient application of dopamine alone, or D1-receptor agonists, initiates a delayed increase in both the population spike[38] and the synaptic response, that is, the field EPSP (Fig. 4B); this potentiation is protein-synthesis dependent[52]. Similar results have been obtained investigating the role of β-adrenergic receptors during LTP in the dentate gyrus[40,41,45].

Activation of dopaminergic D1 receptors increases intracellular cAMP, which, in turn, activates protein kinase A (PKA)[10]. Tetanization that establishes late LTP causes a short-lasting increase in cAMP that can be blocked by D1 inhibitors and by NMDA-receptor antagonists, suggesting that the synergistic action of both inputs might be involved (Fig. 4C). In juvenile animals, strong activation of the NMDA receptor alone seems to be sufficient to induce changes in cAMP levels via a Ca^{2+}/calmodulin-dependent pathway[53]. However, in adults, pharmacological stimulation of the NMDA receptor alone, without the additional elevation of extracellular Ca^{2+}, does not produce late LTP (Ref. 54). This also points to the necessity for synergistic activation of a further signal through another input. Because application of a membrane-permeable cAMP analogue and activators of PKA induces a delayed-onset potentiation[10,55–57] (Fig. 4D) that can be blocked by inhibitors of macromolecule synthesis[10] (Fig. 4E), the combined action of glutamatergic and dopaminergic inputs in CA1 pyramidal

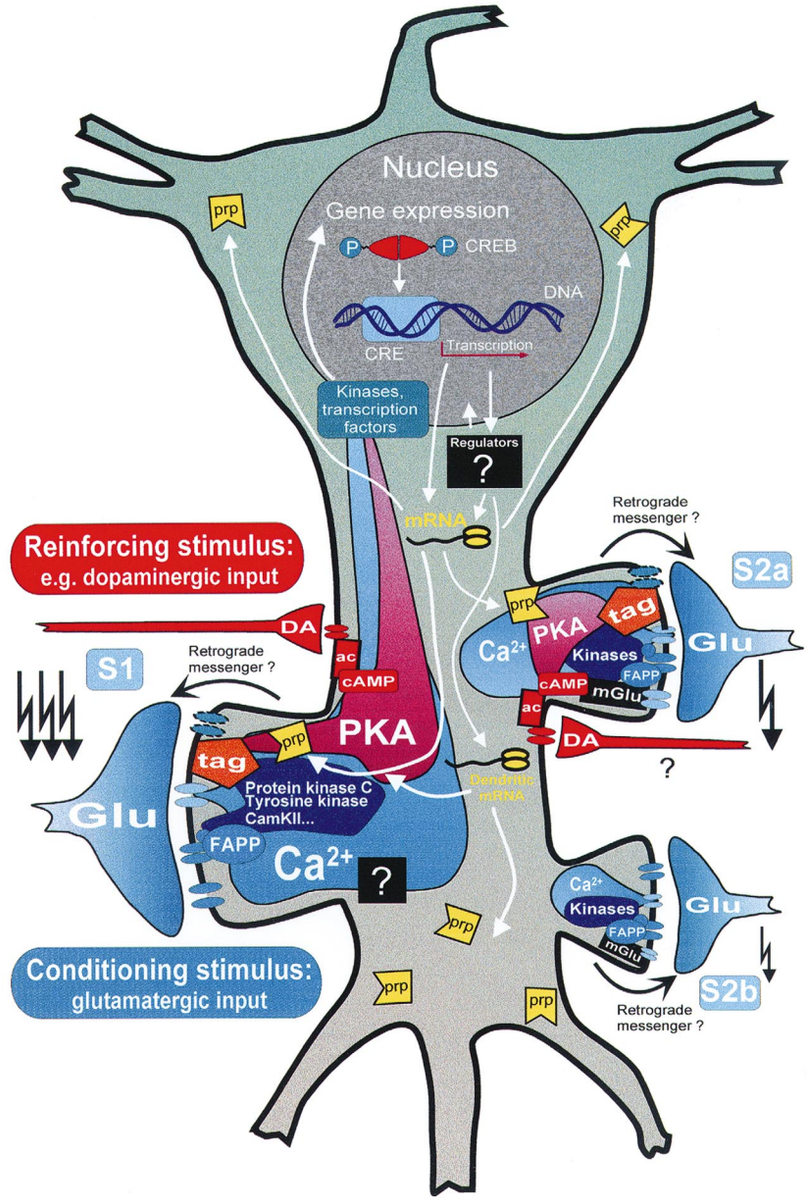

Fig. 3. ***A model of the cellular processes specifically required for the induction of late LTP in the hippocampal CA1 region.*** *Late LTP involves mRNA and protein synthesis at extrasynaptic but intracellular sites. Induction of early LTP involves mechanisms restricted to the activated synapse. High-frequency stimulation of fibres in the stratum radiatum leads to the associative and co-operative activation of the glutamatergic NMDA receptor. Influx of Ca^{2+} activates intracellular processes necessary for early LTP [activation of 'fast-acting plasticity processors' (FAPPs)]. During the early period immediately after LTP induction, a synaptic tag is set. Anatomical changes and phosphorylation of receptors and kinases are possible tag candidates. In addition to glutamatergic activation, a separate signal, activation of dopaminergic receptors, must arrive to activate the cAMP/PKA-pathway. This leads to gene activation and, in turn, to the synthesis and distribution of plasticity-related-proteins (prp) that can be captured by synaptic tags to reveal or stabilize new effector mechanisms (such as new receptors or ion channels). At this stage of LTP consolidation, the FAPPs will be reset, allowing additional change at these synapses. Local dendritic protein synthesis[26–28] may contribute to the persistence of late LTP for the first few hours. This could explain the different effects of blocking late LTP using protein or mRNA-synthesis inhibitors seen in our experiments. If a brief tetanus is applied through a second independent synaptic input, which alone elicits only a short-lasting potentiation, its persistence will be determined as follows: if the tetanization is very weak, only short-lasting synaptic plasticity is ever observed; previous induction of late LTP in another input does not affect this potentiation. If the brief tetanus is stronger and produces early LTP with a 'normal' duration of about four hours, it can be transferred into late LTP by prior (or future) stimulation that up-regulates the distribution of plasticity-related proteins within a time window of approximately 90 min. Abbreviations: CaMkII, Ca^{2+}/calmodulin-dependent protein kinase II; CRE, cAMP-responsive element; CREB, CRE-binding protein; ac, adenylyl cyclase; PKA, protein kinase A; mGlu, metabotropic glutamatergic receptor; Glu, glutamatergic input; DA, dopaminergic input; P, phosphate group; S1, strong, repeated tetanized input; S2a, induction of early LTP using a single weak tetanus; S2b, induction of STP using a very weak tetanus.*

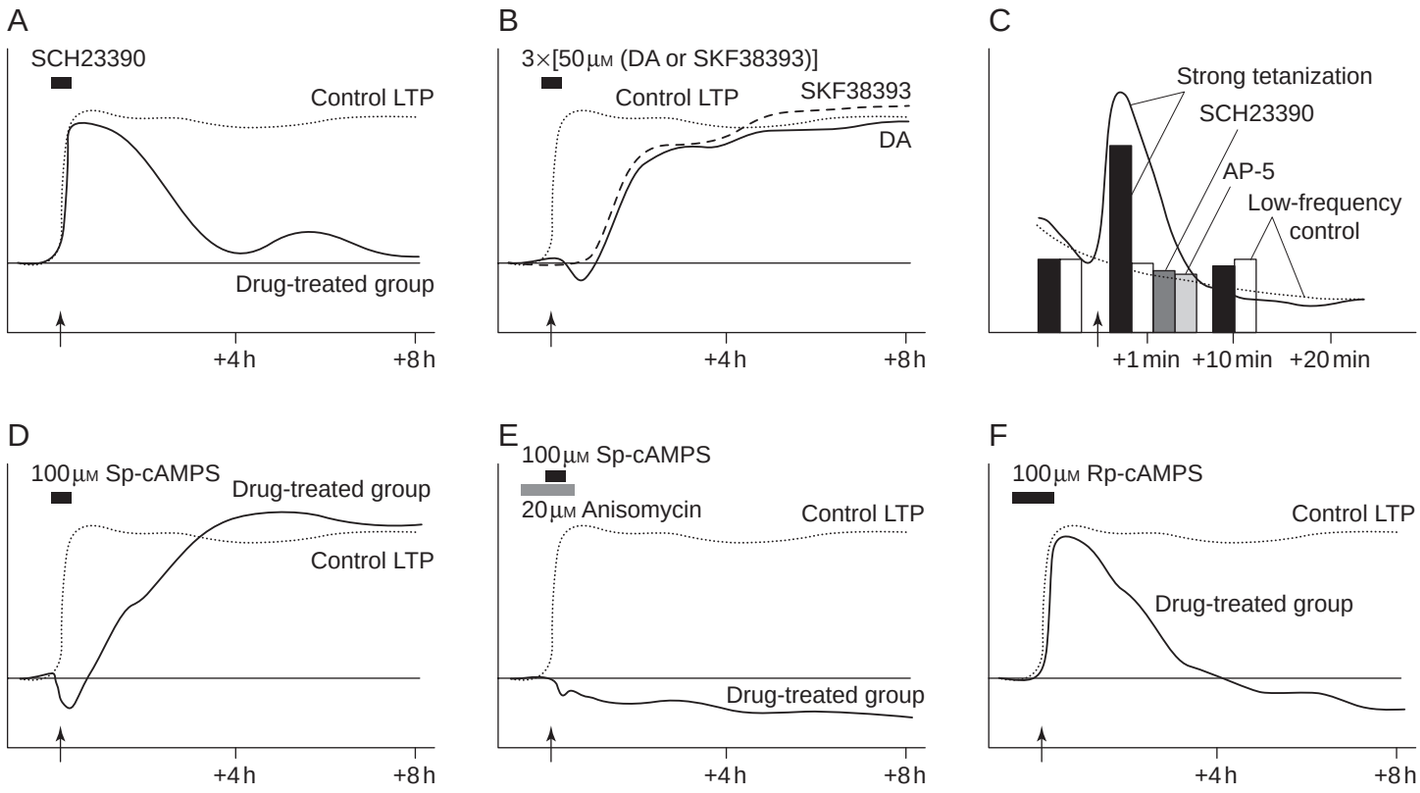

Fig. 4. ***Dopaminergic processes during late LTP in hippocampal CA1 neurones.*** *Illustration of mechanisms activated by dopaminergic receptors during or immediately after repeated tetanization required for late LTP, or both.* **(A)** *Application of a D1-receptor antagonist (SCH23390) prevents late LTP, despite use of conventional repeated tetanization. Arrow on x-axis indicates time of LTP induction.* **(B)** *Threefold application of dopamine or of the D1-receptor agonist SKF38393 induces a late-onset potentiation. Arrow on x-axis indicates LTP induction in the control group.* **(C)** *Cyclic AMP levels are shown during control stimulation (white bars) and high-frequency stimulation (black and shaded bars). Repeated tetanization produces a transient enhancement of cAMP levels which is blocked by antagonists of NMDA and D1 receptors. Lines indicate the release of [^{14}C]-dopamine after low- (broken line) and high-frequency (unbroken line) stimulation. Tetanization enhances the release of [^{14}C]-dopamine in hippocampal slices, in a manner similar to the increase of cAMP levels after tetanization. Arrow on x-axis indicates tetanization or low-frequency stimulation.* **(D)** *The effect of transient application of the PKA activator Sp-cAMPS. The time course of Sp-cAMPS-induced potentiation resembles the time course of potentiation induced by dopamine or by the D1-receptor agonist SKF38393 in* (B). *Arrow on x-axis indicates LTP induction in the control group.* **(E)** *The potentiation induced by Sp-cAMPS can be prevented by the protein-synthesis inhibitor anisomycin, which parallels the blockade of late LTP by anisomycin during electrically induced LTP. Arrow on x-axis indicates LTP induction in the control group.* **(F)** *The action of the PKA inhibitor Rp-cAMPS on electrically induced LTP, illustrating the role of the cAMP/PKA complex during hippocampal LTP in the CA1 region. Arrow on x-axis indicates LTP induction.* Adapted from Refs 10,47,49.

neurons is a possibility. Consistent with this emerging picture, electrically-induced late LTP can be prevented when a PKA inhibitor is applied during its induction[10] (Fig. 4F), supporting results obtained in other labs showing a specific role of PKA in the late phase of LTP, as well as hippocampus-dependent learning using genetic approaches and learning in invertebrates[57,58].

One of the targets of the cAMP/PKA pathway is the phosphorylation of transcription factors such as the cAMP response element-binding protein (CREB), which directly affects gene expression required for late LTP. It has been demonstrated repeatedly that after high-frequency stimulation there is a direct activation of CREB; this might also be a requirement for certain types of long-term learning[59–63]. Deisseroth *et al.*[63] have shown that Ca^{2+} entry after high-frequency stimulation is insufficient to trigger CREB phosphorylation. They argue that a 'submembranous Ca^{2+} sensor, just beneath sites of Ca^{2+}' is crucial for triggering these events. However, we suggest that synergistic activation of the D1 receptor might also be necessary (see also Ref. 64).

Summarizing the effect of aminergic innervation on late LTP, we suggest that strong, artificial stimulation (which probably does not occur in nature, but which is often used to induce late LTP), simultaneously activates glutamatergic and aminergic receptors. The cooperative action of these two inputs is necessary to induce late LTP.

Functional implications

Our concept of synaptic tagging has been developed in hippocampal slices *in vitro*. Future experiments should explore whether or not the concept is applicable to the intact animal and of any relevance to memory formation and learning. Two-pathway experiments of the kind we have used[14] need to be conducted *in vivo*, preferably in freely-moving animals. However, an indication that synaptic tagging might occur in the intact organism has already come from experiments investigating the influence of appetitive and aversive stimulation on early LTP. Induction of early LTP in the dentate gyrus of water-deprived rats can be extended (or 'reinforced') if water is made available at distinct time points during or after its induction[65]. Similar results have been obtained when aversive stimulation was administered in combination with induction of early LTP. The time window (about 30 min after tetanization) might be different from that in area CA1 of hippocampal slices *in vitro*, but it must be recognized that stimulation via reinforcing structures requires its own extra-hippocampal processing in separate neuronal circuits. Seidenbecher *et al.*[65] also found that application of

β-adrenergic inhibitors can prevent the prolongation of LTP. This result is intriguing when considered with the findings on aminergic requirement for late LTP in the hippocampus described earlier.

What are the implications of variable persistence for the possible role of LTP in memory? We have elsewhere speculated that it may contribute to aspects of 'flashbulb memory'[14]. Our view is that LTP is a lab phenomenon whose underlying mechanisms take part in information processing and storage within various neural networks (including the hippocampus). The rapid induction of early LTP [within 30 s (Ref. 66)] enables it to form neural circuits for the processing or retention of attended information. Glutamatergic STP and early LTP might underlie the automatic recording of attended experience[67], providing such circuits with information for a distinct time during which circuits elsewhere can evaluate its content, and, as appropriate, transform it via heterosynaptic stimulation into long-term memory traces. In this view, the variable persistence of LTP, and specifically the ability to transform early into late LTP, extends the window of time for the creation of lasting memory traces elsewhere in the brain from a few hours into something that might last days. An alternative, that we cannot rule out, is that late LTP constitutes the formation of lasting long-term memory traces within the hippocampus itself[68]. Variable persistence should also be thought of as including the resetting of synaptic enhancement prior to its stabilization by plasticity proteins. During STP synaptic enhancement can be destabilized, for example, by trains of low-frequency activity[69]. This, and other patterns of stimulation, might also reset tags.

Concluding remarks

The synaptic tag hypothesis allows us to think about the properties of LTP in a new way. In thinking about associativity, input specificity, rapid induction and persistence, we should now recognize that persistence can be variable. The usual way of thinking about associativity is in terms of the heterosynaptic interaction of two or more inputs, over a short time scale (less than one second), mediated via the voltage dependence of the NMDA receptor[70]. The synaptic tag idea points to a secondary form of associativity in which one input can influence another over a much longer time scale (about 90 min). Input specificity is usually considered in relation to the compartmentalization of Ca^{2+} transients within dendritic spines and thus local Ca^{2+}-dependent phosphorylation. However, the input specificity of late LTP is determined by local tags that sequester proteins manufactured a relatively long way away. Finally, persistence can be variable; whether or not early LTP is transformed into late LTP will depend on the history of activation of the neuron, during both the immediate past and the time that follows shortly after. This history includes heterosynaptic activation of aminergic as well as glutamatergic input pathways, the former being particularly important in freely moving animals. Synaptic tagging encourages us to think of LTP in the context of the entire neurone; it is a step towards a better understanding of the cellular and molecular basis of memory.

Selected references

1 **O'Keefe, J. and Nadel, L.** (1978) *The Hippocampus as a Cognitive Map*, Clarendon Press
2 **Schacter, D.L. and Tulving, E.** (1994) *Memory Systems*, MIT Press
3 **Squire, L.R., Knowlton, B. and Musen, G.** (1993) *Annu. Rev. Psychol.* 44, 453–495
4 **Bliss, T.V. and Lømo, T.** (1973) *J. Physiol.* 232, 331–356
5 **Bliss, T.V. and Collingridge, G.L.** (1993) *Nature* 361, 31–39
6 **Krug, M., Lössner, B. and Ott, T.** (1984) *Brain Res. Bull.* 13, 39–42
7 **Frey, U. *et al.*** (1988) *Brain Res.* 452, 57–65
8 **Matthies, H. *et al.*** (1990) *Adv. Exp. Med. Biol.* 268, 359–368
9 **Otani, S. *et al.*** (1989) *Neuroscience* 28, 519–526
10 **Frey, U., Huang, Y-Y. and Kandel, E.R.** (1993) *Science* 260, 1661–1664
11 **Matthies, H.** (1982) in *Neuronal Plasticity and Memory Formation* (Marsan, C.A. and Matthies, H., eds), pp. 1–15, Raven Press
12 **Nguyen, P.V., Abel, T. and Kandel, E.R.** (1994) *Science* 265, 1104–1107
13 **Frey, U. *et al.*** (1996) *J. Physiol.* 490, 703–711
14 **Frey, U. and Morris, R.G.M.** (1997) *Nature* 385, 533–536
15 **Matthies, H.** (1989) *Annu. Rev. Psychol.* 40, 381–404
16 **Goelet, P. *et al.*** (1986) *Nature* 322, 419–422
17 **Frey, U. *et al.*** (1995) *Neuroscience* 67, 799–807
18 **Liao, D., Hessler, N.A. and Malinow, R.** (1995) *Nature* 375, 400–404
19 **Isaac, J.T.R., Nicoll, R.A. and Malenka, R.C.** (1995) *Neuron* 15, 427–434
20 **Stanton, P.K. and Sarvey, J.M.** (1984) *J. Neurosci.* 4, 3080–3088
21 **Osten, P. *et al.*** (1996) *J. Neurosci.* 16, 2444–2451
22 **Thomas, K.L. *et al.*** (1994) *Neuron* 13, 737–746
23 **Wenzel, J., Desmond, N.L. and Levy, W.B.** (1993) *Brain Res.* 619, 331–333
24 **Sossin, W.S.** (1996) *Trends Neurosci.* 19, 215–218
25 **Schuman, E.M.** (1997) *Neuron* 18, 339–342
26 **Steward, O. and Wallace, C.S.** (1995) *J. Neurobiol.* 26, 447–458
27 **Lyford, G.L. *et al.*** (1995) *Neuron* 14, 433–445
28 **Link, W. *et al.*** (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 5734–5738
29 **Kang, H.J. and Schuman, E.M.** (1996) *Science* 273, 1402–1406
30 **Torre, E.R. and Steward, O.** (1996) *J. Neurosci.* 16, 5967–5978
31 **Abraham, W.C. and Bear, M.F.** (1996) *Trends Neurosci.* 19, 126–130
32 **Jork, R., Grecksch, G. and Matthies, H.** (1986) *Pharmacol. Biochem. Behav.* 25, 1137–1144
33 **Rusakov, D.A. *et al.*** (1995) *NeuroReport* 6, 1557–1561
34 **Dunwiddie, T.V., Roberson, N.L. and Worth, T.** (1982) *Pharmacol. Biochem. Behav.* 17, 1257–1264
35 **Bliss, T.V.P., Goddard, G.V. and Riives, M.** (1983) *J. Physiol.* 334, 475–491
36 **Krug, M. *et al.*** (1983) *Brain Res. Bull.* 11, 1–6
37 **Bramham, C.R., Errington, M.L. and Bliss, T.V.** (1988) *Brain Res.* 449, 352–356
38 **Gribkoff, V. and Ashe, J.** (1984) *Brain Res.* 292, 327–338
39 **Hopkins, W.F. and Johnston, D.** (1984) *Science* 226, 350–352
40 **Stanton, P.K. and Sarvey, J.M.** (1985) *Brain Res.* 358, 343–348
41 **Stanton, P.K. and Sarvey, J.M.** (1985) *J. Neurosci.* 5, 2169–2176
42 **Markram, H. and Segal, M.** (1990) *J. Physiol.* 427, 381–393
43 **Buzsaki, G. and Gage, F.H.** (1989) *Brain Res.* 484, 94–101
44 **Bramham, C.R. and Sarvey, J.M.** (1996) *J. Neurosci.* 16, 8123–8131
45 **Bramham, C.R., Bacher-Svendsen, K. and Sarvey, J.M.** (1997) *NeuroReport* 8, 719–724
46 **Flood, J.F., Smith, G.E. and Jarvik, M.E.** (1980) *Brain Res.* 197, 153–165
47 **Frey, U., Schroeder, H. and Matthies, H.** (1990) *Brain Res.* 522, 69–75
48 **Baulac, M., Verney, C. and Berger, B.** (1986) *Rev. Neurol.* 142, 895–905
49 **Frey, U., Matthies, H. and Reymann, K.G.** (1991) *Neurosci. Lett.* 129, 111–114
50 **Grace, A.A.** (1991) *Neuroscience* 41, 1–24
51 **Sunahara, R.K. *et al.*** (1991) *Nature* 350, 614–619
52 **Huang, Y-Y. and Kandel, E.R.** (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 2446–2450
53 **Chetkovich, D.M. and Sweatt, J.D.** (1993) *J. Neurochem.* 61, 1933–1942
54 **Kauer, J.A., Malenka, R.C. and Nicoll, R.A.** (1988) *Nature* 334, 250–252
55 **Slack, J.R. and Pockett, S.** (1991) *Neurosci. Lett.* 130, 69–72
56 **Pockett, S., Slack, J.R. and Peacock, S.** (1993) *Neuroscience* 52, 229–236
57 **Abel, T. *et al.*** (1997) *Cell* 88, 615–626

Acknowledgements
This work was supported by grants of German Bundesministerium für Bildung und Forschung and of Kultusministerium of Land Saxony-Anhalt held by UF, and an MRC Programme Grant and an HFSP Grant held by RGMM.

58 Schacher, S., Castellucci, V.F. and Kandel, E.R. (1988) *Science* 240, 1667–1669
59 Dash, P.K., Moore, A.N. and Dixon, C.E. (1995) *J. Neurosci.* 15, 2030–2039
60 Schulman, H. (1995) *Curr. Opin. Neurobiol.* 5, 375–381
61 Moore, A.N., Waxham, M.N. and Dash, P.K. (1996) *J. Biol. Chem.* 271, 14214–14220
62 Blendy, J.A. *et al.* (1995) *Brain Res.* 681, 8–14
63 Deisseroth, K., Bito, H. and Tsien, R.W. (1996) *Neuron* 16, 89–101
64 Das, S. *et al.* (1997) *Synapse* 25, 227–233
65 Seidenbecher, T., Reymann, K.G. and Balschun, D. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 1494–1499
66 Gustafsson, B. and Wigström, H. (1990) *Prog. Brain Res.* 83, 223–232
67 Morris, R.G.M. and Frey, U. (1997) *Philos. Trans. R. Soc. London* 352, 1489–1503
68 Nadel, L. and Moscovitch, M. (1997) *Curr. Opin. Neurobiol.* 7, 217–227
69 Stäubli, U. and Chun, D. (1996) *J. Neurosci.* 16, 853–860
70 Clark, K.A. and Collingridge, G.L. (1996) *J. Physiol.* 490, 455–462

Language within our grasp

Giacomo Rizzolatti and Michael A. Arbib

In monkeys, the rostral part of ventral premotor cortex (area F5) contains neurons that discharge, both when the monkey grasps or manipulates objects and when it observes the experimenter making similar actions. These neurons (mirror neurons) appear to represent a system that matches observed events to similar, internally generated actions, and in this way forms a link between the observer and the actor. Transcranial magnetic stimulation and positron emission tomography (PET) experiments suggest that a mirror system for gesture recognition also exists in humans and includes Broca's area. We propose here that such an observation/execution matching system provides a necessary bridge from 'doing' to 'communicating', as the link between actor and observer becomes a link between the sender and the receiver of each message.

Trends Neurosci. (1998) 21, 188–194

> 'In all communication, sender and receiver must be bound by a common understanding about what counts; what counts for the sender must count for the receiver, else communication does not occur. Moreover the processes of production and perception must somehow be linked; their representation must, at some point, be the same.'

WHAT IS SAID HERE by Alvin Liberman[1] for speech where individuals have an explicit intent to communicate, must apply also for 'communications' in which such an overt intention is absent. We understand when one individual is attacking another or when someone is peacefully eating an apple. How do we do it? What is shared by the (involuntary) sender and by the receiver? Is this mechanism the precursor of willed communications? The present review addresses these questions.

Giacomo Rizzolatti is at the Istituto di Fisiologia Umana, Università di Parma, Via Gramsci 14, 43100 Parma, Italy, and Michael A. Arbib is at the USC Brain Project, University of Southern California, Los Angeles, CA 90089-2520, USA.

The mirror system

Neurons located in the rostral part of monkey inferior area 6 (area F5) discharge during active movements of the hand or mouth, or both[2–4]. Some years ago we found that in most F5 neurons, the discharge correlates with an action, rather than with the individual movements that form it[3]. Accordingly, we classified F5 neurons into various categories corresponding to the action associated with their discharge. The most common are: 'grasping with the hand' neurons, 'holding' neurons and 'tearing' neurons[3,5]. Further study revealed something unexpected: a class of F5 neurons that discharge not only when the monkey grasped or manipulated the objects, but also when the monkey observed the experimenter making a similar gesture[6–8]. We called the neurons endowed with this property 'mirror neurons' (Fig. 1).

The response properties of mirror neurons to visual stimuli can be summarized as follows: mirror neurons do not discharge in response to object presentation; in order to be triggered they require a specific observed action. The majority of them respond selectively when the monkey observes one type of action (such as grasping). Some are highly specific, coding not only the action aim, but also how that action is executed. They fire, for example, during observation of grasping movements, but only when the object is grasped with the index finger and the thumb.

All mirror neurons show visual generalization: they discharge when the agent of the observed action (typically a hand) is far away from or close to the monkey. A few neurons respond even when the object is grasped by the mouth. The actions most represented are: grasp, manipulate, tear, and put an object on a plate. Mirror neurons also have motor properties that are indistinguishable from those of F5 neurons that do not respond to action observation. In this review, they will be referred to collectively and regardless of their other properties, as 'canonical neurons'. Typically, mirror neurons show congruence between the observed and executed action. This congruence can be extremely strict, that is, the effective motor action (for example, precision grip) corresponds with the action that, when seen, triggers the neuron (that is, precision grip). For other neurons the congruence is broader: the motor requirements (for example, precision grip) are usually stricter than the visual ones (for example, any type of hand grasping). An example of a highly congruent mirror neuron is shown in Fig. 2. What is

  0166 - 2236/98/$19.00 PII: S0166-2236(98)01260-0