Long-Term Potentiation

104

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Abstract

At many glutamatergic synapses in the brain, brief episodes of strong synaptic activity can lead to an enduring enhancement of synaptic strength, a property known as long-term potentiation (LTP). This chapter presents a concise overview of the cellular mechanisms responsible for LTP and of the role of LTP in learning

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and memory. Glutamate receptors of the NMDA subtype control the induction of LTP at many synapses, and both presynaptic and postsynaptic changes are involved in its maintenance. Recent studies employing optogenetic techniques have thrown light on how long-term synaptic changes are exploited to encode memories at the network level.

Keywords

As-PaRac1 · Channelrhodopsin · GluA1 · Immediate early genes · Long-term potentiation (LTP) · Associativity and cooperativity · Hippocampal pathways · Human hippocampus · Interneurons · Induction rules · Learning and memory · Mossy fibers · Optogenetic approach · Outside hippocampus · Postsynaptic mechanisms · Presynaptic mechanisms · Retrograde messengers · Synaptic tag hypothesis · Metaplasticity · Mossy fiber LTP · NMDA receptor · Presynaptic mechanisms · Retrograde messengers · Spike timing dependent plasticity (STDP) · Stimulus-selective response potentiation · Synaptic learning and memory (SPM) hypothesis · Synaptic tag hypothesis · Transmagnetic stimulation (TMS) · Zeta inhibitory peptide (ZIP)

Brief History

Initially described at synapses in the dentate gyrus of the hippocampal formation, LTP was the first example of a Hebbian synapse to be identified (Bliss and Lømo 1973). The Canadian psychologist Donald Hebb, building on ideas that originated with the Spanish anatomist Ramon y Cajal at the end of the nineteenth century, had postulated in his book *The Organization of Behavior*, published in 1949, that information is stored in the brain in modifiable synapses embedded in networks of interconnected neurons. Hebb's neurophysiological postulate asserts that if neuron A is connected to neuron B and takes part in firing it, then the synapse between A and B becomes strengthened. In the hippocampus, brief high-frequency trains of impulses to axons of cells in the entorhinal cortex (equivalent to cells A) produce an immediate and persistent increase in the strength of the monosynaptic excitatory connections made with granule cells in the hippocampus (equivalent to cells B). It is now clear that many, if not most, synapses in the mammalian CNS can display some form of activity-dependent synaptic plasticity. A vast body of research over the last 40 years has uncovered many of the molecular mechanisms that control the induction and expression of LTP. Among the active research topics that are discussed in this chapter are the molecular mechanisms responsible for the expression of the sustained changes in synaptic efficacy and the varied forms of plasticity that occur in different parts of the nervous system. Moreover, compelling evidence has been produced in the last few years to bolster the long-established view that LTP provides a neural mechanism for memory storage. This chapter presents an introduction to one of the most active areas in contemporary neuroscience.

Brief Tetanic Stimulation Induces LTP in Hippocampal Pathways

LTP was first revealed as a property of excitatory synapses in the dentate gyrus of the hippocampal formation, a cortical structure known to be essential for the formation of new episodic memories in humans. Many of the early experiments on LTP made use of the technique of field potential recording, and because it allows for indefinite recording in the intact animal, the technique is still widely used. Field potentials can be recorded from granule or pyramidal cell populations in the hippocampus by stimulating an appropriate monosynaptic excitatory input, for example, the perforant path input to granule cells, or the commissural/Schaffer collateral projections to CA1 pyramidal cells (Fig. 1).

In response to single stimuli, the simplified and laminated neural architecture of the hippocampus leads to large extracellular field potentials generated by the population of target cells. These comprise an early component reflecting the number of synapses activated and their average synaptic strength, and a later and briefer spike component, that reflects the number of postsynaptic cells discharging synchronous

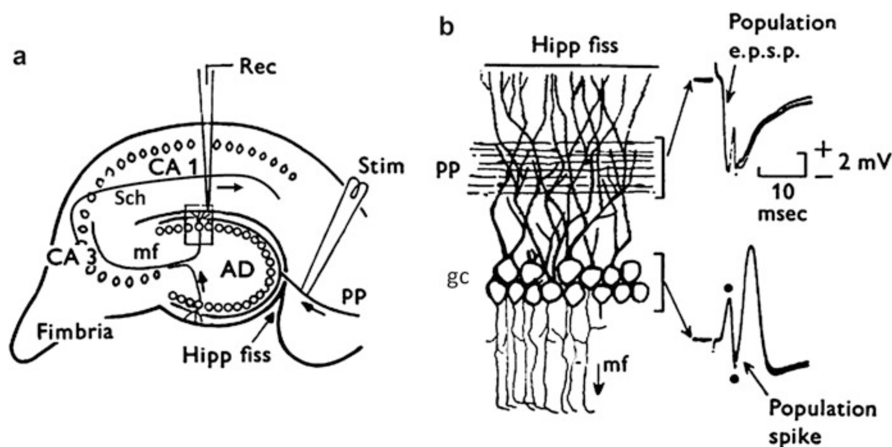

Fig. 1 Anatomy of the hippocampus (**a**) and field potentials evoked in the granule cells of the dentate gyrus by stimuli delivered to axons of the perforant path (**b**). Stimulation of the perforant path (*PP*) excites granule cells (*gc*) of the dentate gyrus (*AD*, area dentata). Mossy fibers (*mf*), the axons of granule cells, project to the proximal apical dendrites of CA3 pyramidal cells, whose axon collaterals (*Sch*, Schaffer collaterals) turn back to excite dendrites of CA1 pyramidal cells. Field potentials recorded in the dentate gyrus are shown in **b**. The early component of the evoked response (negative in the synaptic region and positive in the cell body region) reflects synaptically generated current at active synapses; this is the population EPSP (also known as the field EPSP). Superimposed on the population EPSP is the population spike, generated by the synchronous discharge of granule cells. Similar field potentials are generated in the CA1 field by joint stimulation of the Schaffer collaterals and commissural afferents arising from contralateral CA3 neurons. Identical responses can be recorded in the hippocampal slice and in anesthetized and unanesthetized mice. The field EPSP has been the primary measure in the analysis of LTP using field potential recording, since it directly reflects the strength of synaptic activation. (From Bliss and Lømo 1973)

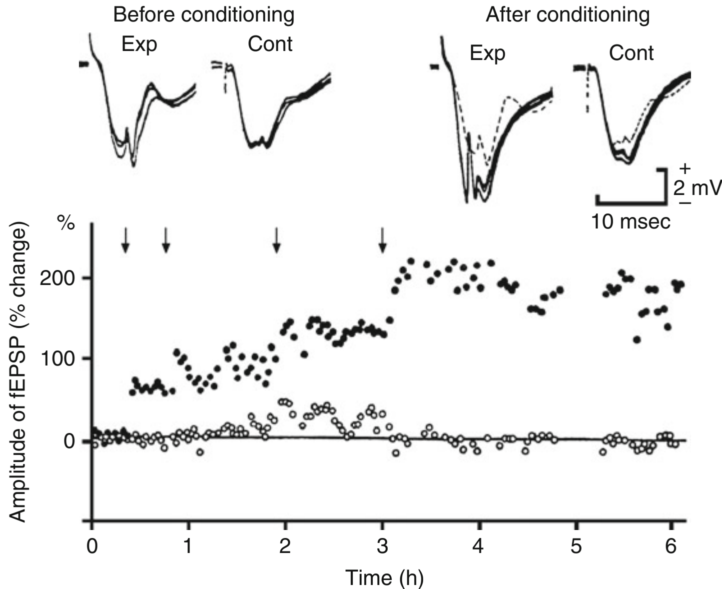

Fig. 2 An early experiment demonstrating LTP in the dentate gyrus of the anesthetized rabbit. By appropriate positioning of the stimulating and recording electrodes two pathways were selected, one of which received high-frequency stimulation. Both pathways received constant test stimuli delivered at 2–3 s intervals. Averaged responses are plotted. Each brief, high-frequency train (*arrows*) produced an abrupt and sustained increase in the field EPSP in the tetanized pathway, until after 4–5 trains saturation was reached. *Above*, sample field potentials from tetanized or experimental (exp) and control (cont) pathways. Superimposed responses before and after conditioning are shown at *top right* (From Bliss and Lømo 1973)

all-or-none action potentials. The early component is referred to as the field (or population) EPSP and the latter component as the population spike. A standard test shock given at frequency of less than around 0.2Hz evokes a relatively constant field response. However, following a high-frequency train, there is an immediate and sustained increase in the response evoked by the standard test shock; this is LTP (Fig. 2). Experimentally, LTP has been elicited in a wide range of mammalian species, including humans, and has been identified in many types of synapse throughout the central nervous system. It can be studied at the level of the single cell using intracellular recording techniques as well as at the population level using field potential recording. Its properties are similar in vitro (hippocampal slices) and in vivo (anesthetized or freely moving animals).

Temporal Components of LTP: STP, E-LTP, and L-LTP

The stability of field potential recording allows the time course of LTP to be studied in the intact animal. Strong and repeated trains can induce potentiation that may last indefinitely; in one heroic study, conducted by Cliff Abraham and colleagues at the

University of Otago, LTP was monitored for a year in the dentate gyrus of the adult rat. Three phases of LTP can be distinguished: a late phase (L-LTP) which can persist indefinitely but which in the presence of protein synthesis inhibitors declines back to baseline within 2–4 h. The potentiation which survives protein synthesis inhibitors is referred to as early phase or E-LTP and is itself blocked by broad-spectrum protein kinase inhibitors; the brief potentiation which survives both protein synthesis inhibitors and kinase inhibitors is known as STP. Pure L-LTP, that is to say a potentiation with a gradual onset to a plateau that is sustained indefinitely, can be induced in some synapses by application of the growth factor BDNF.

Associativity and Cooperativity

Experiments in which two separate pathways converge on the same cell or group of cells have revealed two important properties of LTP, input specificity and associativity (Fig. 3). Input specificity is implied by the observation that a tetanus to one pathway, provided it is strong enough, will induce LTP in that pathway, while the second unstimulated pathway remains unpotentiated. Consequently, it is the synapse which is the focus of activity-dependent plasticity and not the cell, thus greatly enhancing the mnemonic capacity of neural networks using LTP as an information storing device. If a weak tetanus, which does not by itself produce LTP, is paired with a strong tetanus that is above threshold for inducing LTP, then both pathways are potentiated. This is the property of associativity, which provides a direct cellular model of Pavlovian classical conditioning.

The conditions required for the induction of LTP became more precisely defined with intracellular studies in which two afferent pathways to the recorded cell could be activated independently. In the protocol of Wigstrom et al. (1986) no high frequency trains were employed; single test stimuli were given at 10 s intervals alternatively to two afferent pathways projecting to the recorded cell, and the EPSP evoked by each input was monitored (Fig. 4). The EPSPs evoked by each pathway remained stable until the input to one of the two pathways was combined with a depolarizing pulse. The EPSP evoked by the paired pathway grew steadily during pairing. After a few minutes, the depolarizing pulse was discontinued, but the EPSP evoked in the paired pathway remained at a potentiated level for the remainder of the experiment, while the control pathway was unaffected (Fig. 4). Experiments of this sort led to the conclusion that LTP occurred when a synapse was active at a time when the postsynaptic neuron was sufficiently depolarized.

Induction Rules for LTP

A fuller understanding of the induction criteria for LTP came in the early 1980s. By this time, it had become clear that glutamate was the transmitter at the great majority of excitatory synapse in the mammalian brain, and that glutamate receptors were comprised of several different ionotropic families, including AMPA receptors (GluA

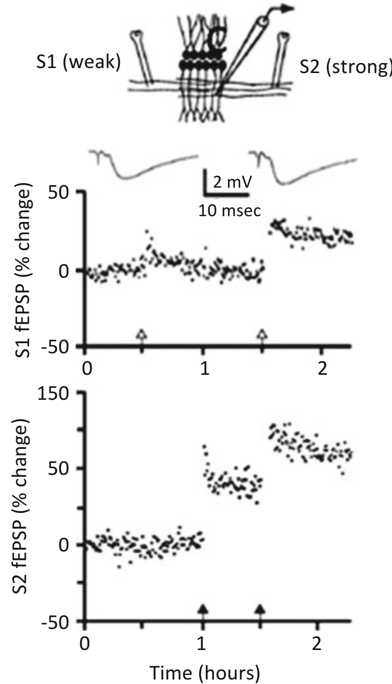

Fig. 3 Basic properties of LTP: input specificity and associativity. Diagram of a two pathway experiment in area CA1 in vitro. The two electrodes activated separate bundles of axons innervating a largely overlapping population of target cells. Field EPSPs were plotted for the weak and strong pathways. A weak tetanus failed to induce LTP (S1, first *open arrow*); a strong tetanus to S2 induced LTP in the S2 pathway (S2, first *solid arrow*) but not in the inactive S1 pathway: this illustrates the property of input specificity. However, when S1 and S2 were delivered together, both pathways were potentiated, demonstrating the property of associativity. Both input specificity and associativity can be explained by the voltage-dependence of the NMDA receptor (see text) (From Bliss and Collingridge 1993)

in current terminology), NMDA receptors (GluN), and Kainate (GluK) receptors, as well as a family of metabotropic glutamate receptors, mGluRs. In 1983, Graham Collingridge and his colleagues showed that the induction of LTP is blocked by a specific NMDA receptor antagonist, D-APV (Collingridge et al. 1983; Fig. 5). Shortly afterwards, it was established that the response of NMDA receptors to the binding of glutamate was voltage dependent. Activation of the receptor required not only binding of the transmitter, but in addition sufficient depolarization of the membrane in which the receptor was embedded to drive off Mg^{2+} ions which otherwise block the channel pore. These properties of the NMDA receptor led to its characterization as a "molecular coincidence detector," and they immediately explain two characteristics of LTP: input specificity (only stimulated inputs are potentiated; at nonstimulated synapses transmitter is not released to bind to the NMDA receptor) and associativity (a weakly stimulated input, that does not by itself

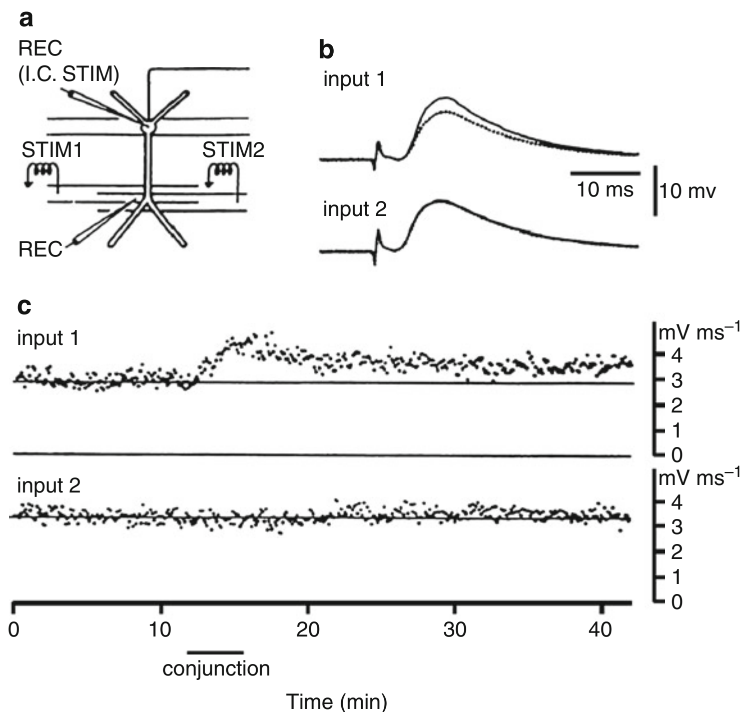

Fig. 4 The minimal requirements for inducing LTP in a single CA1 neuron is repeated synaptic activity at a time when the cell is depolarized. In this two-pathway experiment, single stimuli were given at a low frequency alternately to each pathway. The stimulus to one of the two pathways was then repeatedly paired with a depolarizing pulse delivered through the intracellular recording electrode in the soma. LTP was induced only in the pathway in which pairing occurred (From Wigstrom et al. 1986)

produce enough depolarization to allow activation of the NMDA receptor, may nevertheless be potentiated if it is combined with stimulation to a neighboring input; in effect, the first input takes advantage of the depolarization produced by the second).

While the induction of LTP at many synapses in the central nervous system requires the activation of the NMDA receptor, this is not universally the case. In the hippocampus itself, LTP at synapses made by the axons of granule cells (mossy fibers) onto CA3 pyramidal cells, LTP is not blocked by NMDA receptor antagonists, and several other exceptions have been documented.

A significant early finding from Gary Lynch's laboratory established the importance of Ca^{2+} in the induction process. Injection of BAPTA, a calcium chelator, into the postsynaptic cell blocks the expression of LTP in CA1 pyramidal cells. The NMDA receptor when activated is permeable to Ca^{2+} ions, and it is now generally accepted that for NMDA receptor-dependent LTP, the critical stages in the induction process are the activation of NMDA receptor channels on the subsynaptic membrane

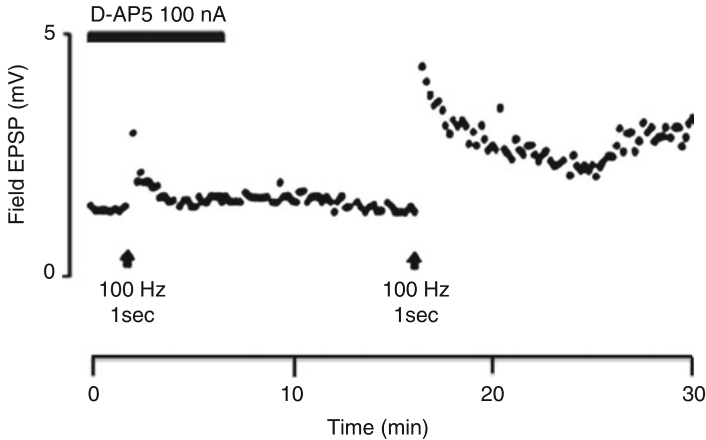

Fig. 5 The specific NMDA receptor antagonist APV blocks the induction of LTP in area CA1. Note that APV does not reduce the amplitude of the evoked response to single stimuli in the period before delivery of the high-frequency train; its action is limited to blocking the induction of LTP. Similarly, APV does not reduce the amplitude of the potentiated response once LTP has been induced (not shown here) (From Collingridge et al. 1983)

(the region of the postsynaptic membrane immediately opposite the presynaptic terminal) and the entry of Ca^{2+}, in part through the NMDA receptor channel itself, into the dendritic spine. How this leads to a sustained increase in synaptic efficacy is the subject of the next section.

Expression Mechanisms of LTP

In principle, an increase in synaptic efficacy could be achieved by: (i) presynaptic mechanisms leading to an increase in transmitter release, (ii) postsynaptic mechanisms leading to an increase in the synaptic current generated by a given amount of transmitter, or (iii) a combination of the two. It is now clear that both mechanisms can be harnessed in the expression of NMDA receptor-dependent LTP. Most evidence to date points to a dominant role for postsynaptic mechanisms, at least in the early stages of LTP, though this may reflect the fact that the search has been concentrated on the postsynaptic side of the synapse. As we have seen, LTP separates into two temporal components: early LTP (E-LTP) and late LTP (L-LTP) which requires protein synthesis and probably also gene expression for its maintenance. The great majority of experiments addressing expression mechanisms relate to E-LTP. At later times, potentiated synapses may become larger, with the presynaptic and postsynaptic specializations expanding together, implying that the enhanced efficacy is generated both by an increase in receptors and an increase in the size and/or number of release sites.

Postsynaptic Mechanisms

The rise in intracellular Ca^{2+} that follows intense synaptic stimulation leads to the activation of Ca^{2+}-dependent kinases such as CaMKII, resulting in posttranslational increase in the conductance of individual AMPA receptors by a CaMKII-dependent phosphorylation of a serine residue on the cytoplasmic C-tail of the receptor (Malenka and Bear 2004; Bliss and Collingridge 2013). In addition to posttranslational modifications to existing AMPA receptors, there is now overwhelming evidence that LTP is associated with an increase in the number of GluA1 AMPA receptor subunits targeted to the postsynaptic density where they form new GluR1/GluR2 heteromeric receptors. The factors which control how receptors are transported to and removed from the subsynaptic membrane are not well understood. Many of the auxiliary proteins which assist in guiding receptor subunits to the synaptic membrane have been identified; for example, stargazin binds to AMPA receptor subunits and provides a link to the postsynaptic protein PSD-95. Imaging of single molecules using quantum dot technology has provided further real-time information, enabling a picture to be built up of how newly synthesized receptor subunits are transported along microtubules in the dendrites to sites on dendritic spines, where they may diffuse laterally through the plasma membrane to the subsynaptic site. Removal of receptors by endocytosis may lead to degradation or to recycling in endosomes (Kneussel et al. 2014).

Another powerful argument for a postsynaptic role in LTP has come from the work of Todd Sacktor and his group on the role of an atypical form of PKC called PKMζ which is involved in stabilizing AMPA receptor subunits in the subsynaptic membrane (Fig. 5). This work has led to the development of a peptide inhibitor of PKMζ called ZIP (zeta inhibitory peptide), currently the only agent able to reverse or depotentiate LTP at arbitrary times after induction (Pastalkova et al. 2006; Fig. 6b).

Presynaptic Mechanisms

The strongest evidence for a presynaptic component in the expression of LTP comes from the imaging of synaptically evoked calcium transients in single dendritic spines. In this technique, a hippocampal neuron (in an acute slice or in a culture preparation) is filled with a calcium-sensitive dye and weak stimuli are applied to afferent fibers. Using confocal microscopy, a responsive spine is located. The response consists of a transient increase in fluorescence, reflecting the increase in Ca^{2+} concentration in the spine that follows synaptic activation. Here, the postsynaptic side of the synapse is used to monitor the success or failure of transmitter release from the unseen presynaptic terminal. Following the induction of LTP by tetanic stimulation in cultured or acute slices, a sustained increase in the probability of release is seen in these single, visualized hippocampal synapses (Enoki et al. 2009; Bliss and Collingridge 2013).

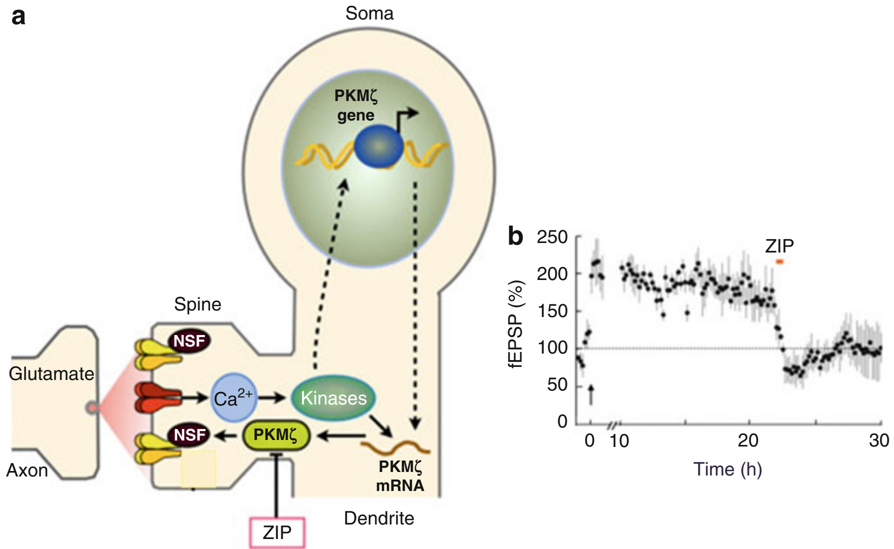

Fig. 6 (**a**) Postsynaptic trafficking of AMPA receptors contributes to the expression of LTP. In this scheme, NMDA receptor activation leads to the stabilization of GluA2-containing receptors through Ca^{2+}-mediated stimulation of local and (for L-LTP) nuclear synthesis of PKMζ. PKMζ is required for the stabilization of GluA2 in the membrane via an accessory protein NSF. The peptide inhibitor ZIP blocks the binding of PKMzeta to NSF, leading to internalization of GluA2-containing receptors. (**b**) ZIP injected into the dentate gyrus in vivo is able to reverse LTP many hours after its induction **a** (From Bliss and Collingridge 2013); **b** (From Pastalkova et al. 2006)

Retrograde Messengers

Given that the trigger for LTP is postsynaptic, a mechanism based on a sustained increase in transmitter release implies the need for a retrograde signal to carry an appropriate message to the presynaptic side of the synapse. This signal has not been definitively identified but the strongest candidate is nitric oxide, a gaseous signaling molecule for which a calcium-dependent synthesizing enzyme, nitric oxide synthase, is located in the postsynaptic terminal. A retrograde signal has been identified in the case of a form of mGluR-mediated LTD (see below), found in many parts of the brain, in which an endocannabinoid 2-AG (2-arachidonoylglycerol) is retrogradely released and binds to the cannabinoid CB1 receptor in the membrane of the presynaptic terminal to induce, by unknown mechanisms, a reduction in transmitter release (Kano 2014).

Transition from E-LTP to L-LTP: The Synaptic Tag Hypothesis

Early LTP does not involve protein synthesis or gene expression and thus does not require communication between the synapse and the nucleus. This is not the case with late LTP, where signals must pass not only from the synapse to the nucleus to

initiate gene transcription but the newly expressed transcripts must then be transported to the appropriate synapses, either as proteins newly translated in the nucleus or as mRNA destined for translation into protein by synthesizing machinery available at the base of the synapse. How is this achieved? It is difficult to conceive of a way in which newly synthesized "plasticity factors" could carry with them the "address" of recently active synapses. It is more likely that they are shipped out to dendrites indiscriminately and are captured by molecular tags established at active synapses (Fig. 7). Evidence that a synaptic tag is set at active synapses and persists for a period of a few hours was presented by Morris and Frey at the end of the 1990s (for a recent review see Takeuchi et al. 2014). The theory has gained support from the finding that after prolonged tetanization of the medial perforant path, mRNA for the immediate early gene Arc becomes concentrated in the middle of the dendritic tree, just where medial perforant path fibers terminate. However, the identity of the synaptic tag, if it exists, has not been established (Steward et al. 2014).

Mossy Fiber LTP

In the majority of cortical synapses where LTP has been characterized, it has turned out to be NMDA receptor-dependent. Yet this is notably not the case with the synapses made by the mossy fiber axons of the granule cells of the dentate gyrus. The mossy fibers terminate in giant boutons, containing 30 or more release sites, on the proximal dendrites of CA3 pyramidal cells. LTP at this highly unusual synapse is not blocked by NMDA receptor antagonists, and its expression appears to be entirely presynaptic. Depending on experimental conditions, induction of LTP at the giant mossy fiber synapse is blocked by kainate receptor antagonists or by mGluR1 and mGluR5 antagonists. Similar mGluR-dependent forms of LTP are found in corticothalamic synapses and at parallel fibers synapsing on cerebellar Purkinje cells (Malenka and Bear 2004).

LTP Outside the Hippocampus

So far in this chapter we have concentrated on LTP in the excitatory pathways of the hippocampus. It is becoming increasingly clear, however, that synaptic plasticity is a property exhibited in one form or another by most excitatory synapses in the central nervous system. In this section we will take a brief look at LTP-like phenomena in other structures in the brain and spinal cord.

Pain pathways: Chronic pain is one of humanity's major scourges, causing misery to millions of people worldwide. Unlike physiological pain which has an obvious protective function, chronic pain has no survival value and appears to be a consequence of maladaptive synaptic plasticity in sensory pathways subserving pain. LTP has been found at every synaptic relay in the pathway from pain receptors to target neurons in the dorsal spinal cord and from spinal cord to projection cells in the thalamus, as well as in target neurons in sensory cortex. Following peripheral injury, C fibers activated by pain receptors show a burst of firing, followed by a

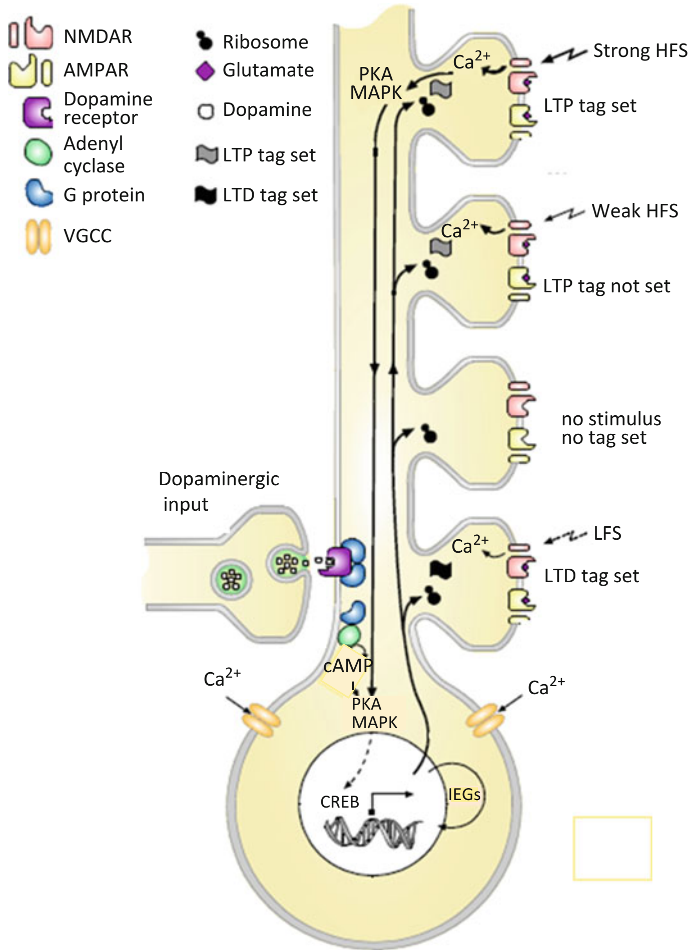

Fig. 7 Late LTP requires a two-way communication with the nucleus. Strong synaptic activity recruits the translocation to the soma of calcium-dependent kinases leading to the transcription of immediate early genes and the synthesis of plasticity-related mRNA and proteins which are transported into dendrites via the microtubule transport system. How these retrograde plasticity factors are targeted to active synapses, to convert locally mediated early LTP to the longer-lasting late LTP, is not known. According to the "synaptic tag" hypothesis of Frey and Morris, LTP-inducing synaptic activity sets a tag which captures passing plasticity factors. Both weak and strong synaptic activation (leading to E-LTP and L-LTP, respectively) set a tag, but only strong activation recruits the synapse-to-soma pathway. Frey and Morris showed how E-LTP induced in one pathway can be converted to L-LTP by intercepting the plasticity factors generated by L-LTP inducing stimuli to another pathway. Additional signals to boost the synapse-to-soma pathway are supplied by neuromodulatory inputs and by somatic Ca^{2+} channels (From Bliss et al. 2007)

prolonged discharge at 1 Hz. In striking contrast to the hippocampus, 1Hz is the frequency which is optimal for inducing LTP at C fiber synapses in the dorsal spinal cord (Sandkuhler and Gruber-Schoffnegger 2012). An important cortical destination

in the pathway subserving pain is the anterior cingulate cortex (ACC), and here too aberrant LTP contributes to chronic pain (Zhuo 2014). Detailed knowledge of the receptors and second messengers that underlie synaptic plasticity in these pain pathways may lead to specific treatments for chronic pain by allowing LTP to be reversed in affected pathways.

Reward pathways: The dopaminergic cells of the ventral tegmental area in the floor of the midbrain are key components of the brain's reward system, and there is evidence that LTP in the glutamatergic fibers that innervate these neurons may underlie the actions of addictive drugs. A recent review on the dopaminergic reward system and addiction reaches the conclusion that "while in most cases these early changes are not sufficient to induce the disease, with repetitive drug exposure, they may add up and contribute to addictive behavior"(Luscher and Malenka 2011).

Visual cortex: When a mouse is exposed daily to a moving bar of light at a particular orientation, the amplitude of the response evoked in the visual cortex grows steadily larger. This effect, called stimulus-selective response potentiation, bears all the hallmarks of LTP: it is evoked by repetitive activity, is blocked by NMDA receptor antagonists, is occluded by, and itself occludes, tetanus-induced LTP, and is reversed by the PKMζ inhibitor ZIP which in the hippocampus and elsewhere depotentiates LTP (see below). It has recently been found that this effect is linked, presumably via an inhibitory pathway, to the behavioral habituation that occurs to a visual stimulus repeatedly presented at a given orientation (Cooke et al. 2015).

These are just a few examples of LTP in nonhippocampal pathways, and many more examples exist in the literature. Are there excitatory or inhibitory synapses in the CNS that are not modifiable in an activity-dependent manner? In the absence of a survey of all the projections of every neuronal type in the brain, this question can't be answered definitively, though there appear to be no reports of long-term plasticity in the relay synapses of the spinothalamic tract.

LTP in interneurons: Perhaps surprisingly, given the absence of specialized postsynaptic structures on inhibitory interneurons comparable to dendritic spines on excitatory cells, there is no shortage of evidence that LTP can be induced at excitatory synapses onto interneurons (Laezza and Dingledine 2011). In general, though, the induction rules are different, requiring an anti-Hebbian coincidence of membrane hyperpolarization and afferent activity. The reason for this is that plasticity is mediated by Ca^{2+}-permeable AMPA receptors, leading to a greater Ca^{2+} influx when the cell is hyperpolarized. LTP has also been described at GABAergic synapses made by inhibitory interneurons on their target cells (Barberis and Bacci 2015).

Evidence for LTP in the Human Hippocampus

Direct evidence that LTP exists in the human brain has come from experiments carried out on tissue excised from patients undergoing surgery. Tetanic stimulation of the perforant path induces an NMDA receptor-dependent potentiation that

continues undiminished for an hour (Beck et al. 2000; Fig. 8). LTP-like synaptic plasticity has also been recorded in human visual cortex, using checkerboard stimuli to elicit evoked potentials recorded by scalp electrodes placed over visual cortex, or by auditory stimuli eliciting evoked potentials over auditory cortex, with responses measured by fMRI. In both cases, LTP-like increases in response amplitude were obtained following episodes of higher frequency stimulation; moreover, these changes were specific to the orientations or frequencies used to produce LTP. A noninvasive simulation technique that has been used to investigate plasticity in human motor cortex is transmagnetic stimulation (TMS), in which cortical cells are stimulated by applied magnetic fields; pairing of electrical stimuli to afferent fibers in the median nerve with appropriately timed TMS to contralateral motor cortex can produce a long-lasting increase in the TMS-evoked response in a corresponding muscle in the thumb. This is reminiscent of pairing induced potentiation at hippocampal and cortical synapses, but the cellular mechanisms remain unknown (Cooke and Bliss 2006).

Fig. 8 LTP in excised, nonepileptic human dentate gyrus. Tetanic stimulation (*arrows*) to one of two pathways had no effect in the presence of APV, but produced a persistent potentiation when repeated after APV had been washed out (From Beck et al. 2000)

Other Forms of Synaptic Plasticity: LTD, STDP, and Metaplasticity

While the focus of this chapter is on LTP, it is important to note that there are several other forms of activity-dependent synaptic plasticity in the brain, notably long-term depression (LTD). In 1982, Masao Ito and his colleagues reported that the excitatory synapses made by parallel fibers on the dendrites of Purkinje cells in the cerebellum exhibit LTD when paired with climbing fiber activation (Ito et al. 1982). This was an important experimental verification of predictions derived from influential models of cerebellar learning developed independently around 1970 by David Marr and James Albus. Induction of LTD at these synapses requires activation of metabotropic glutamate receptors, and its expression is postsynaptic, involving the internalization of AMPA receptors. Some years later, it was found that long trains of low frequency stimulation (typically 1 Hz for 15 min) can evoke, particularly in young mice, an NMDA receptor-dependent form of LTD at excitatory synapses in area CA1 of the hippocampus (Dudek and Bear 1993). An mGluR-dependent form of LTD can also be induced at these synapses, either by application of the mGluR agonist DHPG or, in vivo, by a modified form of low frequency stimulation using pairs of pulses. Another form of synaptic plasticity, spike timing dependent plasticity (STDP), can be invoked by repeated pairing of presynaptic and postsynaptic spiking. In general, if the presynaptic spike occurs a few msec before the postsynaptic spike, NMDA receptor-dependent LTP will result; in some conditions LTD is produced if the timing is reversed (Feldman 2012).

The effects of a given LTP-inducing protocol depend on the previous history of the synapse; in the simplest case, if a synapse has already been maximally potentiated, further trains will have little if any effect. This is an example of metaplasticity, which adds a further layer of complexity to the study of synaptic plasticity. Another process which should be mentioned, though it is outside the scope of this chapter, is homeostatic plasticity, which operates at a network level to scale up or down the level of synaptic strength so as to keep the overall excitability of the network within appropriate limits (Turrigiano 2012).

LTP and Its Relation to Learning and Memory

Since its discovery, LTP has been regarded as a synaptic model of learning and memory. It is obvious enough why this should be the case. For over a century, it has been a central tenet of neuroscience that memory is encoded as activity-driven changes in the strength of synaptic connections within the network of neurons encoding the memory. As we have seen, this idea takes its origins from Cajal and was formalized 50 years later by Donald Hebb in his famous “neurophysiological postulate” which formulates a simple plasticity rule for two connected neurons A and B, where A is one of the neurons that project to B:

> When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.

It is difficult to conceive a mechanism by which brains could store information which does not involve an LTP-like process. This of course is very far from proving that LTP is the mechanism by which learning occurs and memories are stored. The difficulty arises from the fact that LTP is a physiological phenomenon, studied with the tools of cellular neuroscience, while learning is a network phenomenon, involving the coordinated activity of large numbers of cells and even larger numbers of synapses. Bridging this gap has only just begun, but with the advent of optogenetics, multiple electrode recording, and multiphoton microscopy, the techniques are becoming available which may finally allow a definitive answer to be reached.

The Synaptic Plasticity and Memory Hypothesis

It is helpful to formalize the problem by setting up an appropriate hypothesis and deriving some predictions, as suggested by Richard Morris and colleagues (Takeuchi et al. 2014). Most of the relevant work addressing the link between LTP and learning has been carried out in the context of hippocampus-dependent learning. Here, the synaptic learning and memory (SPM) hypothesis states:

> Activity-dependent synaptic plasticity is induced at hippocampal synapses during the formation of hippocampus-dependent memories and is both necessary and sufficient for the information storage underlying hippocampus-dependent memory.

Here are some predictions that follow from the hippocampal version of the SPM hypothesis.

1. Blocking the induction of LTP in the hippocampus will block acquisition of hippocampus-dependent learning.
2. Learning will be accompanied by LTP at some synapses.
3. Reversal of LTP after learning will lead to forgetting of the learned behavior.
4. It should be possible to install memories by manipulating the efficacy of individual synapses in an appropriate network.

While prediction 4 is still in the realm of science fiction, there have now been many tests of the other three predictions, which by and large have been confirmed. The first test of Prediction 1 was made by Morris and colleagues who examined the effect of blocking the induction of LTP on performance in the water maze. (In this hippocampus-dependent spatial learning task, the rodent has to find and remember the location, relative to fixed external visual cues, of a submerged platform in a circular swimming pool filled with an opaque liquid such as milky water. Over a

number of daily trials, the time taken to reach the platform from randomized release points is reduced as animals learn the location of the platform and are able to swim directly to it. Over subsequent days, recall trials are carried out with the platform removed, and the proportion of time spent swimming over the platform's location is taken as a measure of the animal's memory of its location). When LTP was blocked by infusing the NMDA receptor antagonist APV directly into the hippocampus of rats being trained in the water maze, learning was slower and memory of the location of the platform was abolished (see (Morris and Frey 1997) for a review of this work, and a discussion of some of the difficulties associated with a straightforward interpretation of the results in terms of the hypothesis).

This result appeared to confirm directly that LTP is necessary for spatial learning and memory. It was a confirmation of prediction 1 certainly, but, as the authors realized, the result did not itself constitute a proof that hippocampal LTP was necessary for learning. For a start, the site of action of the drug was not accurately known – it may have spread outside its injection site to other cortical areas. Secondly, although AP5 blocked LTP, it was not known what other possible effects it could exert which might affect learning. The advent of transgenic technology made it possible to test the effects of knocking out particular LTP-related proteins, and eventually the technology was developed to do this in a regionally specific and time-dependent manner. Even here, however, it is rarely possible to reach definitive conclusions. A good example is provided by a physiological and behavioral study of the GluA1 knockout mouse by Sakmann and colleagues (Zamanillo et al. 1999). As we have seen, GluA1 is a glutamate receptor subunit that is necessary for the expression of many forms of postsynaptically expressed LTP. It was thus unsurprising that LTP could not be elicited in hippocampal slices from GluA1 knockout animals. What was unexpected was that these animals showed normal performance in the watermaze. This result created a storm of interest as it seems to violate one of the predictions of the SPM hypothesis. Partial clarification came from subsequent work by the same group which revealed that although tetanus-induced LTP was blocked in area CA1 of these animals in hippocampal slices, other induction protocols, such as pairing pre- and postsynaptic activity, were more successful (Jensen et al. 2003). Moreover, tetanus-induced LTP in the dentate gyrus of mutant animals is less severely affected. These findings suggest that mutant animals make use of whatever residual plasticity is available to solve hippocampus-dependent learning tasks and that the original result is not necessarily inconsistent with the SPM.

In testing for prediction 2, experimenters have had to face the problem of not knowing what proportion of synapses in a network are required to be potentiated in order to encode a memory. If the proportion is very small, or if LTD is involved in addition to LTP, then conventional field potential recording may be too coarse a technique to detect changes. Despite this, some success has been achieved, particularly in the analysis of LTP in eyeblink trace conditioning in mice, in which a delay is inserted between the conditioned stimulus, CS (a tone) and the unconditioned stimulus, US (a shock to the intraorbital nerve). Trace conditioning is a hippocampus-dependent task, and in a series of studies, Gruart and her colleagues

have demonstrated that training is associated with a long-lasting potentiation of transmission in the Schaffer collateral projection from CA3 to CA1, and that this occludes with tetanically induced LTP. These results provide the most convincing evidence to date in support of prediction 2 (Gruart et al. 2015).

Prediction 3 was difficult to test before the introduction by the PKMζ inhibitor ZIP (Pastalkova et al. 2006). As we saw above, ZIP has the striking property of reversing LTP (that is, restoring synaptic transmission to its baseline strength) at arbitrary times after induction (Fig. 5b). Until the advent of ZIP, there was no reliable method of reversing LTP once it had been become firmly established by 30 min or so after induction (at shorter periods LTP is amenable to reversal by anoxia, low-frequency stimulation, and a number of other procedures). Both spatial learning and trace eyeblink conditioning are reversed when ZIP is injected into the hippocampus, as required by Prediction 3. Other forms of long-term memory, such as the avoidance of food associated with sickness (bait memory) can also be reversed by injection of ZIP into the relevant cortical area.

The experiments of the kind outlined above validate the predictions made by the SPM hypothesis and strengthen the evidence that LTP is central to hippocampus-dependent learning. The next step is to provide a map of the network of cells that use LTP to encode a particular memory – in other words, to move to a network description of the encoding and retrieval of memories. Recent advances in optogenetic technology have brought this dream close to reality.

From Pathway to Network: Optogenetic Approaches to the Cellular Basis of Learning

How might we label those cells in the hippocampus which are activated during hippocampus-dependent learning? The answer to this goes back to the discovery that a class of genes called immediate early genes (which are often transcription factors) are activated during the induction of LTP. In the dentate gyrus, the constitutive expression of IEGs is at very low levels. They are strongly activated by LTP-inducing stimulation via calcium signaling from activated synapses to the nucleus (Fig. 6), and mRNA for IEGs can be detected by in situ hybridization within 30 min of the high-frequency stimulation that induces LTP. A similar result is seen in CA1, although in that region there is greater on-going constitutive expression. Hippocampus-dependent learning induces the expression of IEGs (for example, *cfos* and *zif268*) in a subset of granule and pyramidal cells. This population of activated cells can be visualized by in situ hybridization, or by driving a reporter gene such as green fluorescent protein, or luciferase off the promoter of an IEG. This approach allows an estimate to be made of the proportion of neurons in the hippocampus that are activated during hippocampus-dependent learning (about a third). The next step, achieved within the last 5 years, was to make this subpopulation accessible to electrical excitation or suppression, thus allowing the function of the network to be probed in behavioral tasks.

The approach is based on the properties of a class of light-gated ion channels found in certain algae. Channelrhodopsin is a membrane protein which when illuminated by blue light changes its confirmation to allow the passage of monovalent ions, leading to membrane depolarization. Another is halorhodopsin, which when illuminated by green/yellow light pumps chloride ions into the cell, leading to hyperpolarization. The strategy for controlling the activity of cells encoding a learned response is as follows: Make a transgenic mouse in which either channelrhodopsin or halorhodopsin is driven off the promoter for an IEG such as cfos. Then train the animals in, say, a hippocampus-dependent task. Cells in which LTP was induced, and which by hypothesis encode the memory for that task, will now express the light-gated ion channel. A light-emitting electrode (optrode) inserted into CA1 or DG will allow the excitation (in the case of channel-rhodopsin) or suppression (in the case of halorhodopsin) of cell firing in just those cells which together constitute the engram for the particular learned behavior. In context-dependent fear learning, the learned behavior is for the animal to stop moving ("freeze") when placed in a chamber in which a shock had previously been administered. Freezing can then be induced at will by activating the channelrhodpsin-expressing cells, irrespective of the animal's location (Fig. 7). Further refinements can be added to this outline. The expression can be made conditional using the tet system (in which expression is controlled by adding or withdrawing doxycycline from the diet), allowing expression of the IEG and light-gated ion channels only at a time of the experimenter's choosing. In a series of studies on context-dependent fear memory (Tonegawa et al. 2015), Susumo Tonegawa and his lab have been able to:

(i) Cause animals to freeze by activating the subset of neurons in the hippocampus that were activated during acquisition of a fear memory (Fig. 9).
(ii) Induce a false memory, causing animals to freeze in a location where no shock had been received.
(iii) Override a fear memory with a pleasant memory.

These and similar studies do not directly show that LTP has been induced in the projections to the neurons expressing the IEG, or in the interconnections between them; that conclusion is based on the not fully validated assumption that IEG expression is an accurate and exclusive reporter of the induction of LTP. Nevertheless, for the first time it is possible to identify a group of cells which when synchronously activated reproduce a learned behavior. Ultimately, a full description of the engram has to be specified at the level of the individual synapses which together encode the memory. Within a given assembly of neurons encoding a memory, only a small subset of synapses will be potentiated. We will conclude this summary of contemporary approaches to identifying the engram with a recent study which has outlined a way of tagging and destroying a population of potentiated synapses.

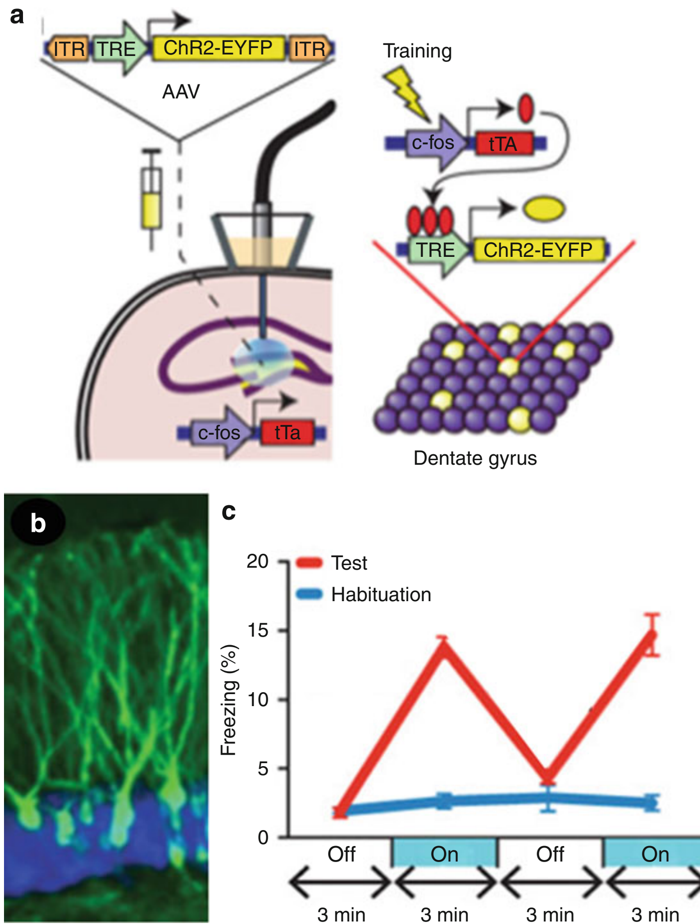

Fig. 9 Network analysis of contextual fear learning in transgenic mice. Mice are trained to associate a novel chamber with an electric shock. When subsequently placed in this chamber animals freeze, indicating the persistence of a fear memory. This is a hippocampus- and amygdala-dependent task. (**a**) viral construct carrying the tet promoter driving channelrhodopsin and a fluorescent protein, EYFP, is injected into the dentate gyrus of hippocampus. The construct allows channelrhodpsin and EYFP to be expressed in those cells and only those cells in which the promoter for cfos is active (by implication, those cells in which LTP has been induced). Moreover, the system can be suppressed by feeding animals doxycyclin, which sequesters tTA and prevents its binding to tet and driving expression of channelrhodopsin. Animals were taken off doxycycline and exposed to shocks in a novel chamber and then replaced on doxycyclin. This leads to expression of cfos in a subset of granule cells (**b**). Those cells, and those cells only, can subsequently be driven by blue light. When the animal is placed in a neutral chamber, and blue light is delivered via an optrode inserted into the hippocampus, the animals freeze (**c**), indicating that the memory of the chamber in which they received the shock has been reactivated. Animals which have been habituated to the chamber in which the shock was delivered do not freeze when blue light is delivered (From Liu et al. 2012)

Identifying the Synaptic Engram in Motor Cortex

By injecting a fluorescent dye into neurons in motor cortex, it is possible to image repeatedly the same length of dendrite, together with its dynamic population of dendritic spines, over a period of many days in intact mice. In 2009, two groups reported that when mice are trained in a motor task there is a net increase in the number of dendritic spines in motor cortex, with new spines tending to occur in clusters. Moreover, learning of a second motor task leads to the emergence of new clusters of spines on different dendritic branches (Chen et al. 2014). A significant recent advance from Haruo Kasai's laboratory in Tokyo has enabled synapses that have emerged or been enlarged during the learning of a motor task to be specifically targeted and degraded. The new approach exploits a DNA construct encoding a light-activated version of the signaling protein Rac1. Prolonged activation of Rac1 leads to degradation and shrinkage of spines. The construct also contains a signaling sequence derived from the immediate early gene Arc, which directs Arc mRNA to dendrites and specifically to regions of the dendrite which have been subject to strong activation of an intensity which induces LTP. The transgene thus encodes an "optoprobe," called As-PaRac1 (Active synapse targeting photo-activatable Rac1), which when activated by light causes retraction specifically of those spines which were recently active. Activation of the optoprobe led to impaired performance in a recently learned motor task (running on a rotating rod), while performance was not affected by similar manipulation of cortical synapses encoding a different motor task. This powerful technique provides for the first time a method of identifying the specific synaptic network recruited to establish a memory for a particular learned task (Hayashi-Takagi et al. 2015).

How do these insights into the synaptic basis of motor learning relate to LTP and the SPM hypothesis? LTP is traditionally considered to be a property of preexisting synapses, although there is evidence that LTP-inducing patterns of stimulation can lead to production of new spines and/or the enlargement of existing spines (in the hippocampus, synaptic growth of dendritic spines in vitro can be stimulated by an LTP-inducing protocol involving rapid photolytic release of caged glutamate onto individual synapses). Because mRNA for the immediate early gene Arc is directed to strongly activated synapses, the As-PaRac1 study is consistent with a model in which during learning the Arc-derived signal sequence directs the As-PaRac1 mRNA to the base of potentiated spines. Prediction 2 of the Synaptic Plasticity and Memory hypothesis asserts that reversal or block of LTP will abolish learning; here there is a degree of overkill, in that the affected synapses are shrunk to an extent where they are likely to be less effective than before their engagement in the training task. Nevertheless, the resulting loss of memory is clearly consistent with the SPM hypothesis as applied to motor cortex.

Conclusion

LTP has enjoyed a remarkable journey over the last four decades, during which it has travelled from a laboratory curiosity to a place at the high table of contemporary neuroscience. There are several reasons for this; first, it is simple to obtain, both

in vivo and in vitro, and the enhancement of synaptic strength elicited by a brief tetanus is gratifyingly immediate and dramatic; second, it can be studied at many levels, from genes to cell signaling, from synaptic function to the behavior of neuronal ensembles in behaving animals. Finally, and most importantly, there is every indication that LTP lies at the heart of memory, a precious faculty of mind that we may, in time, come to understand in complete mechanistic detail.

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