STRUCTURAL PLASTICITY AND MEMORY

Raphael Lamprecht and Joseph LeDoux

Much evidence indicates that, after learning, memories are created by alterations in glutamate-dependent excitatory synaptic transmission. These modifications are then actively stabilized, over hours or days, by structural changes at postsynaptic sites on dendritic spines. The mechanisms of this structural plasticity are poorly understood, but recent findings are beginning to provide clues. The changes in synaptic transmission are initiated by elevations in intracellular calcium and consequent activation of second messenger signalling pathways in the postsynaptic neuron. These pathways involve intracellular kinases and GTPases, downstream from glutamate receptors, that regulate and coordinate both cytoskeletal and adhesion remodelling, leading to new synaptic connections. Rapid changes in cytoskeletal and adhesion molecules after learning contribute to short-term plasticity and memory, whereas later changes, which depend on *de novo* protein synthesis as well as the early modifications, seem to be required for the persistence of long-term memory.

Center for Neural Science, New York University, 4 Washington Place, New York, New York 10003, USA.
Correspondence to J.L.
e-mail: ledoux@cns.nyu.edu
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It is widely accepted that, during learning, reversible physiological changes in synaptic transmission take place in the nervous system, and that these changes must be stabilized or consolidated in order for memory to persist[1,2]. The temporary, reversible changes are referred to as short-term memory (STM), and the persistent changes as long-term memory (LTM). The idea that the creation of stable, persistent LTM traces requires gene expression and the resultant synthesis of new proteins is supported by much evidence[3–5]. However, molecular changes are transient and so, on their own, are insufficient to explain LTM[6]. It is therefore generally believed that structural changes in synaptic morphology, occurring either consequent to protein synthesis or in parallel with it, are also necessary. This 'structural plasticity' and its relation to memory are the topics of this review.

The possibility that memory might involve structural changes in the nervous system was speculated on by the ancient Greeks and later philosophers. But the modern history of this idea began in the late nineteenth century, when Tanzi[7] proposed that repeated activation of a neuron leads to metabolic changes that cause the processes of the cell to move closer to other neurons, thereby forming an associative bond. These bonds, he argued, constitute the physical basis of memory. This idea was seized on by prominent neuroscience pioneers, Cajal[8] and Sherrington[9], and through their influence the notion that learning alters connections between neurons became a popular explanation for how memories are maintained. A related view by Holt[10] proposed that, during learning, structural changes take place that are similar to the neural growth processes that occur in embryological development.

These and other early speculations[11] were the background against which Hebb[12] developed his theory: when two neurons are repeatedly active at the same time, some growth occurs between them such that, at a later point in time, activity in one leads to activity in the other. Around the same time, Konorski proposed that neuronal plasticity, induced by repetitive association of stimuli, could be mediated by the transformation of a pre-existing set of potential synaptic connections into functional connections by morphological changes[13]. Today, the Hebb/Konorski theory, which equates learning with synaptic plasticity, is widely accepted[14–16].

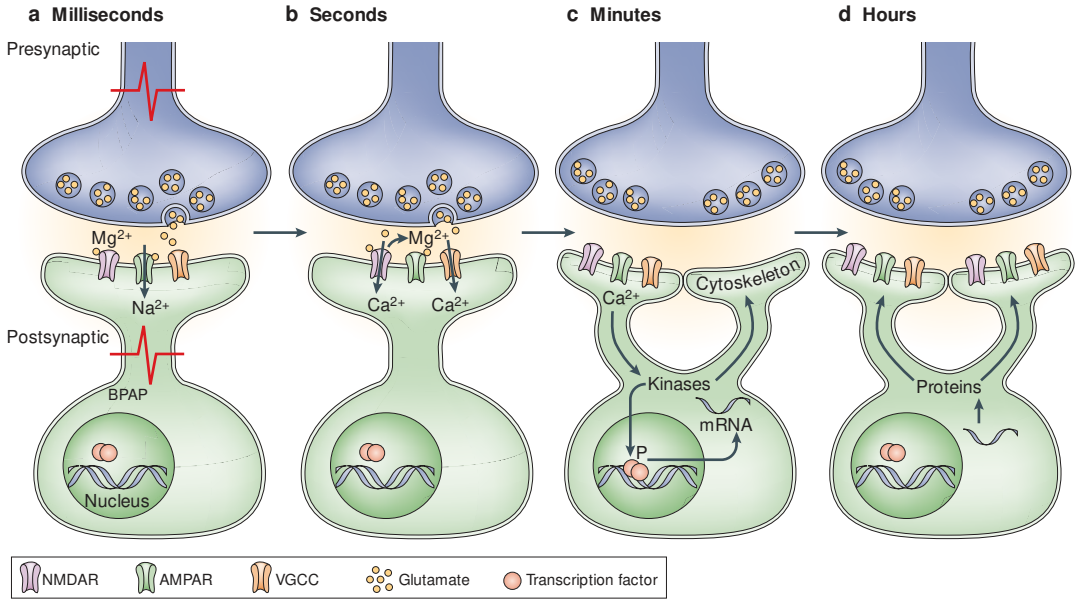

Figure 1 | **Molecular mechanisms involved in the initiation and maintenance of synaptic plasticity. a** | Activity-dependent release of glutamate from presynaptic neurons leads to the activation of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors (AMPARs) and to the depolarization of the postsynaptic neuron. Depolarization occurs locally at the synapse and/or by back-propagating action potentials (BPAP)[23,26,31,32]. **b** | Depolarization of the postsynaptic neuron leads to removal of NMDA (*N*-methyl-D-aspartate) receptor (NMDAR) inhibition, by Mg^{2+}, and to Ca^{2+} influx through the receptor[27]. Depolarization also activates voltage-gated calcium channels, another source of synaptic calcium[28–30]. **c** | Calcium influx into the synapse activates kinases which, in turn, modulate the activity of their substrates[33,34]. These substrates contribute to local changes at the synapse, such as morphological alteration through cytoskeletal regulation[65,86], or induce the transcription of RNA in the nucleus by regulating transcription factors (TFs)[36]. **d** | Transcribed mRNA is translated into proteins that are captured by activated synapses and contribute to stabilization of synaptic changes[5]. VGCC, voltage-gated calcium channel.

In spite of a long history of speculation, only recently has evidence accumulated that structural changes underlie synaptic plasticity and memory. This work has not only shown that structural plasticity exists, but it has also begun to identify the molecular mechanisms that underlie these changes. In surveying this literature, we will consider structural changes in synaptic organization that have been induced in three ways. One is by natural learning in behaving animals. Another is through induction of LONG-TERM POTENTIATION (LTP), an artificial form of plasticity that is produced in neurons by electrical stimulation of synaptic input pathways[16,17]. The third is by maturational processes that take place in early life. Although we concentrate on findings from memory and LTP studies, similar mechanisms have been found to underlie brain development[18–20]. Because the molecular basis of structural plasticity has been studied most thoroughly in developing nervous systems, findings from development might provide useful clues to mechanisms that are relevant for understanding structural plasticity associated with LTP induction and memory formation.

Ca^{2+} influx initiates synaptic plasticity

Before turning to the detailed mechanisms that underlie structural plasticity, we will briefly summarize the mechanisms that are known to be involved in the initiation and maintenance of synaptic plasticity, especially Hebbian or associative plasticity, which is believed to be a key mechanism in associative learning[14–16,21]. In Hebbian plasticity, coincident activation of pre- and postsynaptic cells leads to enduring modifications of synaptic efficacy between the two cells, thereby creating associative links between them. It is generally accepted that the influx of calcium into postsynaptic neurons, through excitatory amino-acid receptors, specifically NMDA (*N*-methyl-D-aspartate) receptors, and possibly L-type voltage-gated calcium channels (VGCCs), is the triggering event in Hebbian plasticity[22–26].

The NMDA receptor is uniquely suited to be involved in Hebbian plasticity[15,21,27] (FIG. 1). At resting membrane potentials, calcium flow through the receptor is blocked by magnesium. However, if presynaptically released glutamate binds to and activates NMDA receptors at a time when the postsynaptic cell membrane is depolarized by spike-triggering inputs at other synapses, thereby relieving the magnesium inhibition, then calcium entry will occur at that synapse. The NMDA receptor is therefore a coincidence detector — it only passes calcium when presynaptic activity and postsynaptic activity coincide.

Another source of calcium influx across the plasma membrane during depolarization is through VGCCs[28–30]. For example, BACK-PROPAGATING ACTION POTENTIALS open VGCCs widely in the cell's dendrites, but might contribute to plasticity at specific synapses by amplifying excitatory postsynaptic potentials (EPSPs) at synapses that were recently active — that is, that have glutamate bound to glutamate receptors, including AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), NMDA and metabotropic glutamate receptors[23,26,31,32].

LONG-TERM POTENTIATION (LTP). An enduring increase in the amplitude of excitatory postsynaptic potentials as a result of high-frequency (tetanic) stimulation of afferent pathways. LTP is considered to be a cellular model of learning and memory. It is measured both as the amplitude of excitatory postsynaptic potentials and as the magnitude of the postsynaptic cell population spike. LTP has also been used to study memory mechanisms in other brain regions, such as the amygdala and areas of the cerebral cortex.

BACK-PROPAGATING ACTION POTENTIALS
Although action potentials typically travel down the axon towards the presynaptic terminal, they can also be initiated at the cell body and propagate back into the dendrites, thereby shaping the integration of synaptic activity and influencing the induction of synaptic plasticity.

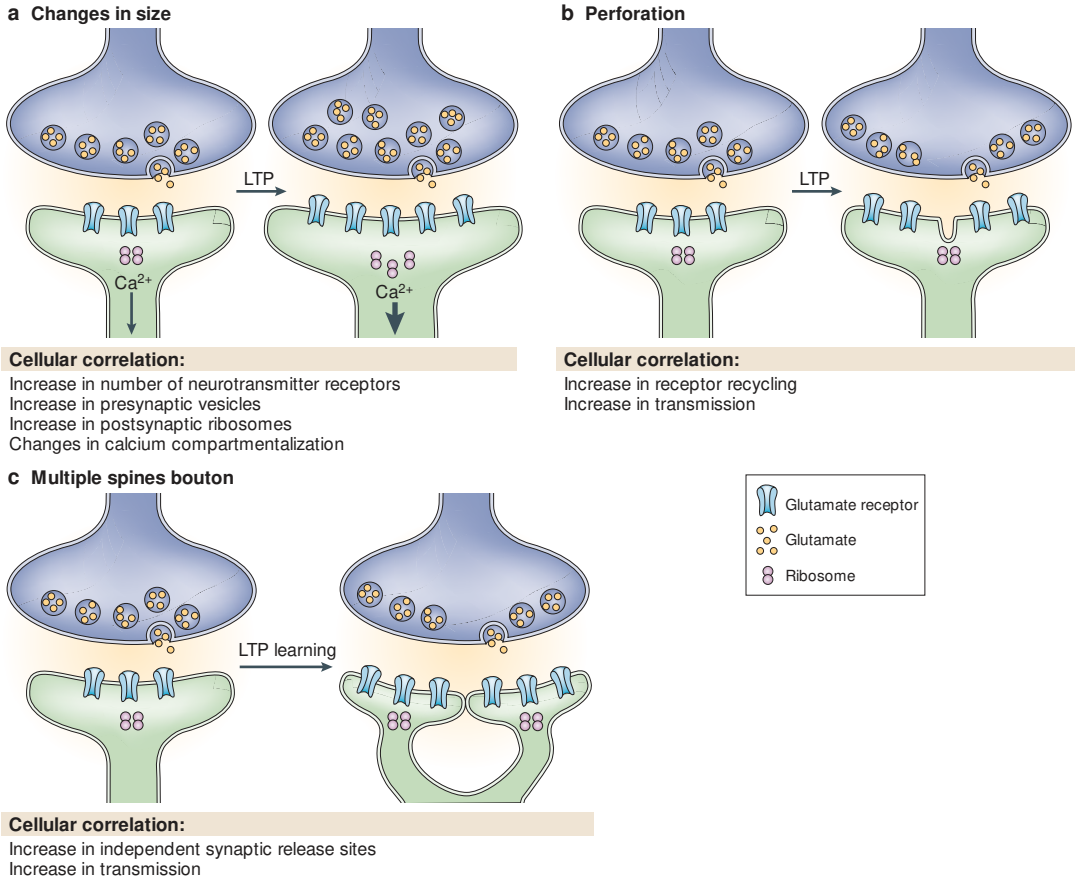

Figure 2 | **Long-term potentiation (LTP) or learning induces morphological changes in dendritic spines**. **a** | Increase in spine head volume and widening and shortening of spine neck[48,66,67]. **b** | Spine perforation[68]. **c** | Increase in the number of spines[65,71] and in the number of multiple-synapse boutons (where multiple spines contact the same presynaptic bouton)[68,72]. These changes in spine morphology and spine number are accompanied by alterations in the number and distribution of glutamate receptors[77], modulation of calcium compartmentalization in spines[75,76], and/or increases in ribosomes within spines[82]. These changes contribute to modifications in postsynaptic responsiveness to presynaptic stimulation.

In some situations, calcium entry through NMDA receptors is sufficient to initiate the subsequent cascade of events that lead to synaptic plasticity, whereas in others the combined calcium signal from NMDA receptors and VGCCs participates[24–26]. In either case, the net result is that additional signalling pathways are activated when intracellular calcium is sufficiently elevated. For example, phosphorylation of constitutively present protein kinases, such as calcium/calmodulin-dependent protein kinases (CaMKs) and protein kinase C[33,34], results in an increase in synaptic efficacy, initially by phosphorylation of membrane receptors such as AMPA receptors[35] and subsequently, over several hours, by activation of gene transcription and protein synthesis[36]. Some of the proteins that are synthesized, such as neurotrophins, can also lead to structural changes in synapses[37]. An alternative scenario involves calcium-regulated protein synthesis near the active synapses where certain RNA molecules, such as CaMKII mRNA, are constitutively present[38].

Recent findings show that elevation of calcium leads to rearrangement of the cytoskeleton at the synapse. Actin, for example, can be rapidly polymerized and induce cytoskeletal rearrangements that culminate in new synaptic structures[39]. These structural changes occur too quickly to be accounted for by nuclear or even dendritic protein synthesis. They should be considered to take place separately from the slower structural changes that result from protein synthesis. Such changes might participate in both STM and LTM.

In summary, the molecular and structural bases of synaptic plasticity are beginning to be understood. Much of the research in this area has focused on the contribution of gene expression and protein synthesis to the persistence of memory. It has often been assumed that structural changes that underlie memory persistence are consequences of gene expression and protein synthesis. However, recent findings showing rapidly induced and persistent synaptic changes mediated by cytoskeletal molecules indicate that these alterations might occur in parallel with the changes that are produced as a result of protein synthesis.

Morphological changes in dendritic spines

We now turn to evidence for the existence of structural plasticity. Early studies showed alterations in synaptic architecture (such as changes in size or shape) and in the number of synapses after non-associative learning and long-term facilitation in *Aplysia*[40–44] and in

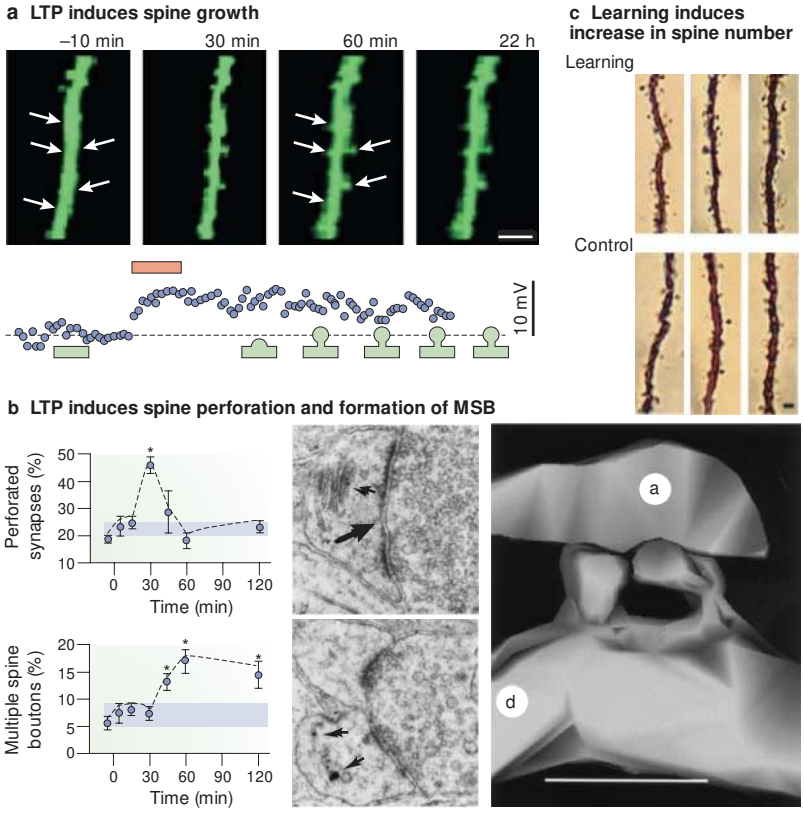

Figure 3 | **Methods used to monitor changes in dendritic spines following long-term potentiation (LTP) or learning. a** | Visualization of new spine growth (upper panel) after LTP (lower panel) of postsynaptic neurons using two-photon microscopy[65]. **b** | Detection of perforated spines and multiple spine boutons (MSB) after LTP using electron microscopy (left). A three-dimensional reconstruction of MSBs is also shown (right)[68]. a, axon; d, dendrite. Scale bar, 1μm. **c** | Detection of changes in spines 24 h after learning (trace eyeblink conditioning) using Golgi impregnation[71]. **a** modified, with permission, from *Nature* REF. 65 © (1999) Macmillan Magazines Ltd; **b** modified, with permission, from *Nature* REF. 68 © (1999) Macmillan Magazines Ltd; **c** modified, with permission, from REF. 71 © (2003) The Society for Neuroscience.

the mammalian hippocampus in response to injury, stimulation[45,46] or induction of LTP[47–50]. Similar changes were observed in the neocortex in response to environmental enrichment[51,52]. Stress also induces structural changes in the hippocampus and amygdala[53,54].

Most excitatory synapses in the brain terminate on dendritic spines, which have been the focus of recent work in the mammalian brain[55–57]. Spines are specialized perturbations on dendrites that contain a postsynaptic density (PSD). The PSD includes receptors, channels and signalling molecules that couple synaptic activity with postsynaptic biochemistry[58,59]. Spines provide a closed compartment that allows rapid changes in the concentrations of signalling molecules, such as calcium, and therefore make possible efficient responses to inputs[56].

Modulation of the number of dendritic spines and/or their morphology has been proposed to contribute to alterations in excitatory synaptic transmission during learning[44,56] (FIG. 2). Indeed, there is evidence that induction of synaptic plasticity (LTP induction or memory formation) leads to changes in the number or shape of spines[60–64].

TWO-PHOTON MICROSCOPY
A form of microscopy in which a fluorochrome that would normally be excited by a single photon is stimulated quasi-simultaneously by two photons of lower energy. Under these conditions, fluorescence increases as a function of the square of the light intensity, and decreases as the fourth power of the distance from the focus. Because of this behaviour, only fluorochrome molecules near the plane of focus are excited, greatly reducing light scattering and photodamage of the sample.

For example, using TWO-PHOTON MICROSCOPY it has been shown that induction of LTP in hippocampal slice cultures leads to the formation of new spines (FIG. 3a), and that inhibition of LTP with an NMDA receptor antagonist (D(-)-2-amino-5-phosphonovaleric acid; AP5) prevents this structural change[65]. Changes in spine morphology have also been seen after LTP induction[48,66,67]. An increase in the size of the spine head, as well as a widening and shortening of the spine neck, has been reported to follow LTP[48,66,67]. These effects begin as soon as 2 min after stimulation and last up to 23 h. In addition, using a combination of electron microscopy and calcium precipitation, more pairs or triplets of spines making contact with the same presynaptic terminal were found, indicating that LTP induces the multiplication of existing axonal-dendritic contacts[68] (FIG. 3b). Other studies have shown that these multiple spine contacts with the presynaptic axon are not formed by the splitting of existing spines, indicating that an alternative mechanism, such as the growth of new spines, must be involved[69,70].

Changes in spine morphology and number after learning have also been found in several brain areas using histological methods, such as Golgi impregnation and electron microscopy[44] (FIG. 3). For example, an increase in spine number (density) was detected in the hippocampus 24 h after trace eyeblink conditioning, and these changes were blocked by NMDA antagonists[71]. Also, an increase in the number of multiple synaptic boutons that formed synapses on spines was found in the hippocampus 24 h after trace eyeblink conditioning[72]. The number of synapses also increases in the cerebellum after eyeblink conditioning[73] and in the piriform cortex after olfactory learning[74].

Long-term changes in spine morphology could contribute to the modulation of synaptic transmission that occurs after learning or LTP. Shortening or widening the neck of a spine affects calcium influx into the dendrite[75,76] and therefore might affect biochemical events in spines. By measuring glutamate-induced currents, it has been shown that glutamate sensitivity correlates with spine shape — sensitivity is highest at spines with larger heads[77,78]. In Schaffer collateral commissural synapses, the ratio of AMPA to NMDA receptors increases linearly with the diameter of the PSD[79]. Moreover, large spines receive input from large presynaptic terminals with more vesicles[80,81]. In addition, polyribosomes are preferentially translocated to large spines during synaptic plasticity — an event that might facilitate the incorporation of local protein synthesis machinery[82]. Finally, increases in the number of spines could potentially contribute to enhanced transmission, as more connections would be made with the presynaptic neuron.

In summary, modulations in spine morphology correlate with synaptic plasticity and memory formation. These alterations last for many hours and could contribute to enduring changes in synaptic transmission. Modulation of spine shape is correlative, and no causal relationship with synaptic plasticity has been established. However, as described later, the fact that

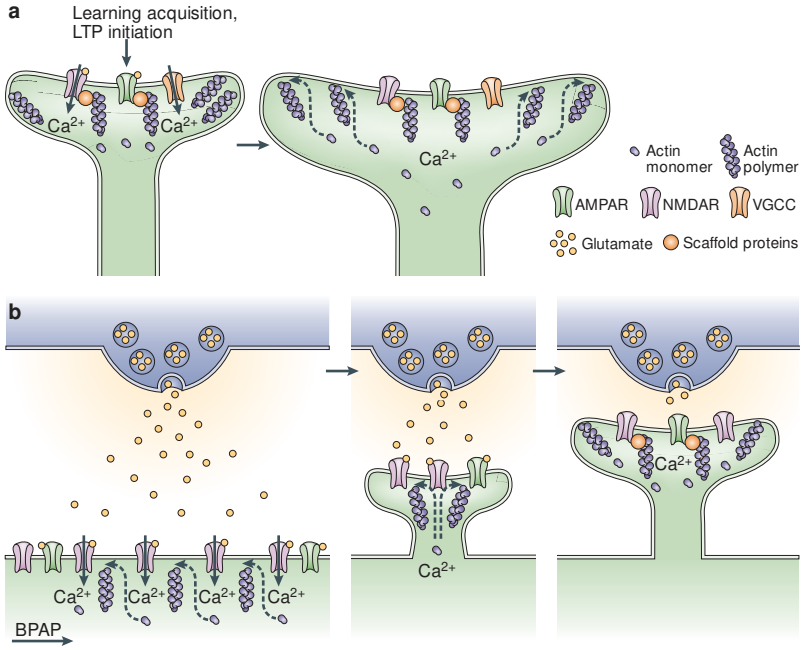

Figure 4 | **Actin cytoskeleton is involved in spine morphogenesis**. Extracellular stimulation induces rapid actin polymerization (indicated by addition of actin monomers into actin polymers) leading to changes in spine shape (**a**) or the formation of new spines (**b**)[39]. Blockade of actin polymerization or inhibition of extracellular stimulation (for example, by *N*-methyl-D-aspartate receptor (NMDAR) antagonists) interferes with spine morphogenesis[39,83,90]. AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; BPAP, back-propagating action potential; LTP, long-term potentiation; VGCC, voltage-gated calcium channels.

molecules that are essential for spine formation during development are involved in LTP induction and memory formation indicates that morphological changes might have a key role in the maintenance of plastic changes in synaptic transmission.

The cytoskeleton and structural changes

The architecture of spines, and therefore their ability to change, depends on the specialized underlying structure of cytoskeletal filaments[39]. These microfilaments are composed of actin, which is present throughout the spine cytoplasm in close interaction with the PSD. Developmental studies have shown that changes in spine stability and motility depend on actin polymerization[83,84]. Reorganization of actin could therefore contribute to the structural plasticity of spines after LTP induction and memory acquisition. Subsequently, LTP and memory consolidation could be promoted by a reduction in actin-based spine motility, leading to spine stabilization[39]. Consistent with this hypothesis is the involvement of actin in synaptic plasticity (FIG. 4). Drugs that block actin polymerization suppress LTP in the rodent hippocampus[85]. LTP induction in the dentate gyrus of freely moving adult animals also increases the content of polymerized actin (F-actin) in dendritic spines in the hippocampus[86]. The elevated level of F-actin persists for at least five weeks after stimulation. The orientation, kinetics of assembly and stability of F-actin filaments are known to contribute to spine shape and are regulated by extracellular stimulation that could contribute to spine formation after LTP (for example, NMDA receptor activation)[39,87]. The rapid formation and persistence in spines of F-actin after LTP indicate that it contributes to spine morphogenesis and stability. Inhibition of NMDA receptors blocks LTP and active polymerization of actin, and blockade of actin polymerization in urethane-anesthetized adult rats by latrunculin A prevents the development of late-phase LTP (8 h), leaving the initial amplitude and early phase (30–50 min) of LTP intact. Together, these results indicate that NMDA-dependent actin polymerization is important for the consolidation of the early phase of LTP into the late phase in adult rats *in vivo*.

In addition to the role of actin in postsynaptic structural plasticity, studies have shown that actin is involved in the presynaptic structural changes that are induced by tetanic stimulation in rat hippocampal neurons and in serotonin-induced synaptic facilitation in *Aplysia* cell culture[88,89].

Changes in spine shape, presumably mediated by underlying cytoskeleton rearrangements, can last for hours or days, and could therefore make it possible for changes in synaptic transmission during learning to persist. This raises questions about the molecular events that lead to the stabilization of cytoskeletal rearrangements.

AMPA receptors stabilize structural changes

The AMPA class of glutamate receptors has been found to have a stabilizing effect on spine morphology[90]. Actin-based spine motility is suppressed when AMPA is applied to hippocampal neurons, and this suppression is completely blocked by the AMPA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). As discussed earlier, NMDA receptors might be important in the initial phase of spine motility, followed by a stabilization phase that is mediated by AMPA receptors. Enduring changes in AMPA receptor transmission could therefore contribute to long-lasting spine stability.

Two observations support this hypothesis. First, there is a decrease in spine density after deafferentation of CA1 pyramidal cells in hippocampal slice cultures. This effect is prevented by the application of small amounts of AMPA. Blocking activity-dependent release of glutamate with tetrodotoxin had no effect on spine density, whereas blocking both activity-dependent glutamate release and activity-independent glutamate release, by blocking vesicular glutamate release using botulinum toxin A or C, resulted in marked loss of spines[91]. So, AMPA receptor activation by spontaneous glutamate release in synapses is sufficient to maintain dendritic spines. Second, AMPA receptor levels in spines increase after LTP induction or learning experiences[35] (FIG. 5). It is therefore possible that an increase in AMPA receptors in spines could contribute to enduring spine stability and memory persistence. This hypothesis is strengthened by the fact that AMPA receptor insertion into synapses is essential for maintenance of LTP and possibly for memory consolidation (see later in text).

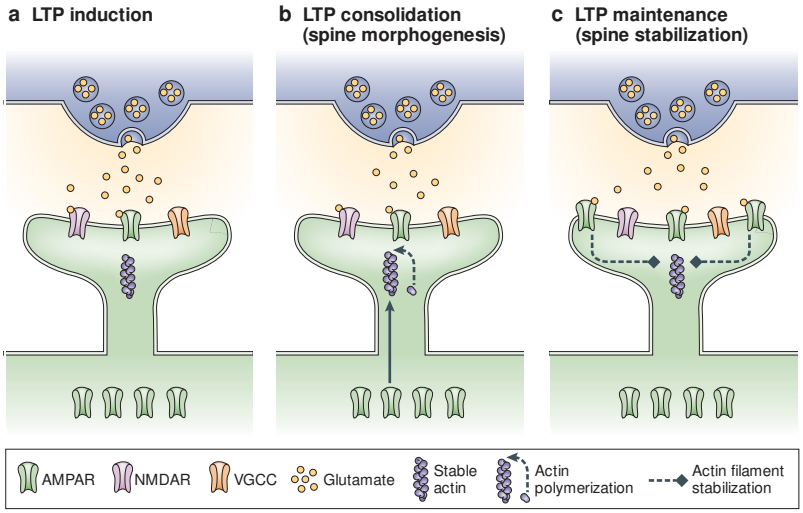

Figure 5 | **Long-term potentiation (LTP) and behavioural experience induce glutamate receptor trafficking into spines. a** | LTP induction leads to activation of *N*-methyl-D-aspartate receptors (NMDARs). **b** | Ca^{2+} influx through NMDA receptors leads to trafficking of glutamate receptors into spines[35,93]. **c** | Increase in AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors (AMPARs) in dendritic spines could enhance the responsiveness to glutamate and could contribute to glutamate-induced maintenance of spines by stabilizing actin filaments[39]. VGCC, voltage-gated calcium channels.

Induction of LTP in the hippocampus or sensory experience in the barrel cortex induces the insertion of AMPA receptors containing the GluR1 and GluR4 subunits into the activated postsynaptic sites, thereby increasing the concentration of AMPA receptors and altering synaptic transmission[92–94]. The increase in GluR1 in spines after LTP is dependent on NMDA receptor activation. Furthermore, a mutation in the GluR1 subunit, which interferes with its LTP-dependent synaptic trafficking, impairs LTP formation and experience-driven synaptic potentiation. Knock-in mice with mutations in the GluR1 S831 and S845 phosphorylation sites are deficient in NMDA-dependent trafficking of AMPA receptors[95]. These mice have impaired LTP and long-term depression (LTD), and impaired spatial water maze learning. Interestingly, the knock-in mice could learn as well as the wild-type mice when tested 2 h after training, but were not able to retain the memory when tested 8–24 h after training. So, phosphorylation sites on AMPA receptors that control AMPA receptor trafficking might be important for consolidation of LTM. Other studies have indicated that the elevated level of AMPA receptors in the synapse is maintained by an ongoing mechanism that is responsible for the enduring replacement of the additional GluR1/4 receptors with GluR2/3-containing receptors, thereby outlasting protein degradation[96]. The level of NMDA receptors is also elevated for at least 3 h after LTP induction[97]. So, enduring changes in neurotransmission in specific synapses that are activated during periods of information acquisition might be maintained by the active insertion of AMPA receptors into these synapses. This increase could contribute to spine stability, underlying enduring synaptic plasticity and leading to memory consolidation.

FEAR CONDITIONING
A form of Pavlovian (classical) conditioning in which the animal learns that an innocuous stimulus (for example, an auditory tone — the conditioned stimulus or CS), comes to reliably predict the occurrence of a noxious stimulus (for example, foot shock — the unconditioned stimulus or US) following their repeated paired presentation. As a result of this procedure, presentation of the CS alone elicits conditioned fear responses.

Consistent with this hypothesis is the observation that the insertion of AMPA receptors into the synapse is governed by protein kinases that have been shown to be required for memory consolidation. For example, phosphorylation of GluR1 at S845 by protein kinase A (PKA) is necessary (although not sufficient) for the delivery of GluR1 into synapses[98]. PKA activity during a crucial time window is essential for several forms of learning and memory, including fear memory consolidation in the lateral amygdala and spatial memory consolidation in the hippocampus[99–101]. Furthermore, the A-kinase anchoring proteins (AKAPs), which couple PKA (among their other targets) to GluR1 through synapse-associated protein 97 kDa (SAP97)[102], are essential for fear memory formation in the lateral amygdala[103]. Injection of an inhibitory peptide that competes with the binding of PKA to AKAP into the lateral amygdala 1 h before FEAR CONDITIONING impaired memory formation 4 h and 24 h later, but not 1 h after training. Injection of the peptide several hours after training had no effect. The specific contribution of AKAP and PKA with phosphorylation of GluR1 and its insertion into specific synapses in the lateral amygdala during fear memory formation is a subject for further study. Together, these findings indicate that increases in glutamate transmission by AMPA receptors can lead to stabilization of spine morphology, LTP and memory.

Rho GTPases and cytoskeletal rearrangements

How does extracellular stimulation culminate in the cytoskeletal rearrangements and spine morphogenesis that follow LTP initiation and memory acquisition? Rho GTPases and their downstream effectors have an important role in regulating the cytoskeleton, and consequently in regulating spine and dendritic morphology, in response to extracellular stimulation[104] (FIG. 6). Recently, several studies have implicated these molecules in synaptic plasticity and consolidation of LTP and memory (see later in text).

Rho GTPases are intracellular signalling molecules that serve as a signalling switch and that can be an activated molecule bound to GTP or an inactive molecule bound to GDP[105–107]. In their active form, Rho GTPases bind downstream effectors that regulate the actin cytoskeleton. The best studied Rho GTPases (RhoA (Ras homologous member A), Rac (Ras-related C3 botulinum toxin substrate 1) and Cdc42 (cell division cycle 42)) are essential for regulating axonal morphology and dendritic and spine formation during development. Expression of dominant-negative Rac in young rat hippocampal slices reduces the number of spines on the pyramidal dendrites, and constitutively activated RhoA leads to a simplification of the dendritic tree[108]. In mice, overexpression of constitutively activated RhoA reduces the number and length of spines on cortical neurons[109]. Rho GTPases also mediate the effects of neurotransmitters and adhesion molecules on neuronal morphology. For instance, during development and after visual stimulation, NMDA receptor-mediated regulation of dendritic arbour elaboration in

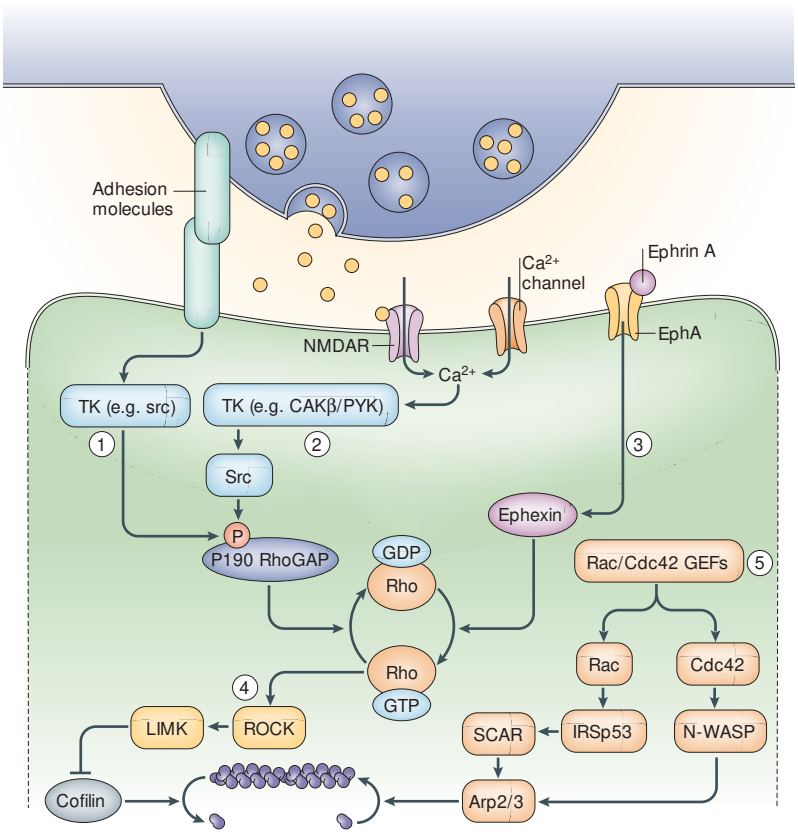

Figure 6 | **Rho GTPaseas mediate extracellular stimulation-induced actin cytoskeleton rearrangements.** Stimulation of the postsynaptic neuron leads to actin-dependent morphological changes mediated by Rho GTPases[105–108]. 1) Activation of adhesion molecules, such as integrin or cadherin, which have been shown to be involved in synaptic plasticity, regulates Rho GTPase inactivation by RhoGAPs. 2) Calcium influx through membrane channels can induce activation of tyrosine kinases (TKs), such as the cell adhesion kinase-β/proline-rich tyrosine kinase 2 (CAKβ/Pyk2), that in turn activate Src. The later modulates p190 RhoGAP activity and thereby controls Rho GTPase inactivation. 3) On the other hand, Rho GTPase activators, RhoGEFs, are also regulated by extracellular stimulation. Ephrin A activates, through the receptor tyrosine kinase EphA, a Rho GEF called ephexin. EphA has been implicated in memory formation[133]. 4) Rho GTPase controls actin polymerization through downstream effectors such as Rho-associated kinase (ROCK). ROCK activates LIM-domain-containing protein kinase (LIMK), which in turn inhibits the actin depolymerizing factor cofilin. This event can contribute to actin polymerization. ROCK, LIMK and cofilin have been shown to be involved in synaptic plasticity. 5) Cdc42 and Rac, other members of the Rho GTPase family, induce actin polymerization by regulating downstream effectors. GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; N-WASP, neuronal Wiskott-Aldrich syndrome; SCAR, suppressor of cAR.

Xenopus tadpoles might be mediated by a pathway that decreases RhoA activity[110,111].

Several studies have recently linked Rho GTPases to synaptic plasticity and memory formation. Fear conditioning leads to the formation of a molecular complex in the lateral amygdala. This complex includes the tyrosine-phosphorylated p190 RhoGAP (Rho GTPase-activating protein)[112]. p190 RhoGAP is also localized in dendritic spines in the lateral amygdala, indicating that this might be its site of action. Inhibition of the downstream Rho-associated kinase (ROCK) in the lateral amygdala before fear conditioning, by infusion of the specific inhibitor Y-27632, impaired long- but not short-term fear memory. This observation indicates that ROCK is involved in consolidating STM into LTM. Moreover, injection of a ROCK inhibitor ~22 h after fear conditioning training had no effect on memory retrieval, indicating that fear memory consolidation occurred and became resistant to blockade of the ROCK pathway. ROCK, which is regulated by Rho GTPase, is intimately involved in dendritic morphogenesis during development through regulation of the cytoskeleton[104,105,113], supporting the idea that cytoskeletal rearrangement might contribute to fear memory formation.

Other studies have found that the LIM-domain-containing protein kinase (LIMK) is also involved in spine morphogenesis, LTP and memory formation. LIMK can be activated by p21-activated kinase (PAK), an important effector of Rac GTPase, but also, although to a lesser extent, by ROCK[104]. Activation of LIMK by PAK induces actin polymerization through the phosphorylation and inhibition of cofilin, a protein that facilitates depolymerization of actin. Limk1-knockout mice have abnormal spine morphology and synaptic transmission. They also have enhanced hippocampal LTP and enhanced spatial and fear conditioning memory[114]. Moreover, LTP in the hippocampus induces the phosphorylation of cofilin, and injection of a cofilin-inhibitory peptide into the hippocampus impairs the development of late-phase LTP, leaving the early phase of LTP intact[86]. These results show that LIMK and cofilin, which are downstream from Rho GTPases, are involved in memory formation by regulating the actin cytoskeleton.

Together, these results indicate that the Rho GTPases and their downstream effectors — proteins that are intimately involved in cytoskeletal regulation and the formation of neuronal processes during brain development — have an important role in synaptic plasticity and memory formation. Rho GTPases have also been shown to mediate the activity of adhesion molecules and to regulate cellular interactions.

Adhesion molecules and synaptic plasticity

The formation of new synaptic contacts is a dynamic process that involves ongoing morphological alterations and modulation of adhesion between the pre- and postsynaptic neurons[115,116]. These processes require coordinated activity between molecules that regulate cytoskeletal rearrangements and morphology, and those that control adhesion between the pre- and postsynaptic membranes. Adhesion molecules, mostly integrins, CADHERINS, neurexin and the immunoglobulin superfamily, are membrane-bound molecules that have hetero- or homophilic interactions with proteins in the extracellular matrix and synaptic membranes to control the adhesion between the pre- and postsynaptic membranes. Adhesion molecules, which also have an intracellular component, can initiate signalling pathways that couple the dynamics of extracellular connectivity with intracellular events that control morphology. For example, cadherin regulates dendritic spine morphogenesis and function. Blockade of cadherin function leads to elongation of the spine, bifurcation of its head structure and alterations in the

CADHERINS
Calcium-dependent cell adhesion molecules that tend to engage in homophilic interactions.

Box 1 | Structural plasticity and reconsolidation

Recent findings have added a new piece to the memory persistence puzzle, showing that activation of a consolidated memory results in a new round of lability and stabilization that is also mediated by protein synthesis[125–132]. These observations raise questions about the cellular mechanisms of memory persistence and storage. For example, does the reconsolidation process involve alterations in cytoskeletal elements and adhesion properties at the synapse that are similar to the consolidation process, as has been reviewed here? As reconsolidation might involve the stabilization of a pre-existing neuronal circuit, rather than a remodelling of the network in the manner that occurs during consolidation, the molecular mechanisms might be different. One of the many mysteries of reconsolidation is how a stable consolidated memory, and the corresponding structural plasticity that sustains it, are rapidly destabilized during retrieval. The observation that some aspects of structural plasticity are rapidly initiated during experience, and occur in parallel with slower, protein-synthesis-dependent structural plasticity, indicates a possible means by which rapid changes in memory availability could be mediated.

distribution of postsynaptic proteins[117]. Moreover, neuronal activity induces the movement of β-catenin (which mediates the interaction of cadherin with the actin cytoskeleton) from dendritic shafts into spines to become associated with cadherin and to influence synaptic size and strength[118]. Adhesion molecules such as cadherin also associate with molecules that regulate cytoskeletal rearrangements, such as proteins that control the Rho GTPase pathway[119].

Adhesion molecules could therefore contribute to the morphological alteration and stabilization of connectivity between neurons, a process that is hypothesized to underlie memory consolidation. Consistent with this hypothesis is the role of adhesion molecules in the formation and stabilization of LTP and LTM. Integrin-mediated adhesion helps to stabilize early-phase LTP (E-LTP) into late-phase LTP (L-LTP). For example, inhibition of integrin with a peptide that contains the integrin recognition sequence 10 min before, immediately after and 10 min after LTP induction caused a gradual decay of synaptic strength over 40 min (REF. 120). The peptide had no effect when applied 25 min after LTP initiation, indicating that integrin has a role in stabilization of synaptic connectivity. Furthermore, N-cadherin is synthesized and internalized to new assembled synapses during the induction of L-LTP, and blocking N-cadherin adhesion prevents the induction of L-LTP but not E-LTP[121]. This event depends on glutamate receptor activity. In chicks, memory is impaired 24 h after a visual categorization task when antibodies against the cell adhesion molecule L1 are injected before, 5.5 h or 15–18 h after training (but not later)[122]. In addition, intraventricular injection of antibodies against neural cell adhesion molecule (NCAM) in rats 6–8 h after passive avoidance training, but not later, impaired retention of the avoidance response[123]. These observations indicate that adhesion molecules are essential for memory consolidation during a period of hours after acquisition.

The level and distribution of adhesion molecules is also correlated with synaptic plasticity and learning. In *Aplysia*, repeated application of 5-hydroxytryptamine (serotonin; 5-HT), which leads to long-term facilitation of the sensory–motor connection, induces the internalization of the adhesion molecule apCAM (*Aplysia* cell adhesion molecule)[43]. This could destabilize the interaction between sensory neurons, permitting the growth of new sensory axons. ApCAM could be redistributed to the area where new synapses are formed. In rats, N-cadherin is induced in the piriform cortex and hypothalamus 2 h after fear conditioning[124]. N-cadherin was not induced in control animals that were presented with the conditioned stimulus and unconditioned stimulus in a non-associative manner.

On the whole, these observations indicate that adhesion molecules have a central role in mediating neuronal connectivity and morphogenesis, and in the progressive stabilization of synaptic connectivity that leads to memory consolidation.

Concluding remarks

The formation of LTM involves several phases, including acquisition — during which inputs impinge on specific synapses to initiate molecular changes — and consolidation — when cellular alterations are progressively stabilized. The resulting modified neuronal circuit underlies the neural representation of memory in the brain. The circuit modifications, in turn, are mediated by molecular activity at the synapse during specific time windows after learning. The molecular activities are complex and require coordination within and between signalling pathways. After LTP induction or learning, modulation and stabilization of excitatory transmission — by insertion of glutamate receptors into synapses — is controlled in part by actin dynamics. The actin cytoskeleton, which mediates morphological changes that accompany memory formation and LTP induction, in turn, is regulated by glutamate receptors. So, NMDA receptor activity initiates actin dynamics, followed by AMPA-receptor-induced stabilization. During this process the adhesion and stabilization of the pre- and postsynaptic elements are controlled by adhesion molecules that also affect and are affected by both the cytoskeleton and glutamate receptors. The joint, coordinated activity of these molecules results in the modulation and stabilization of synaptic efficacy during memory consolidation.

Although considerable advances have been made in understanding the roles of synapses, spines and dendrites in plasticity and memory, many questions remain open. For example, what molecules are synthesized in the soma after learning, and how are they involved in mediating morphological changes in distal synapses that consolidate memory? Are there molecules that are involved in synaptic morphogenesis in adults that are not involved during early development? If so, how might they contribute exclusively to synaptogenesis in adults? How do changes in spines and dendritic morphology contribute to alterations in the input–output properties of the neurons, leading to the encoding of LTM? Does resconsolidation involve similar or distinct mechanisms of structural plasticity (BOX 1)? Elucidation of these issues will foster our understanding of the relationship between molecular activities and memory consolidation.

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Competing interests statement
The authors declare that they have no competing financial interests.

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