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Structural plasticity upon learning: regulation and functions

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Abstract | Recent studies have provided long-sought evidence that behavioural learning involves specific synapse gain and elimination processes, which lead to memory traces that influence behaviour. The connectivity rearrangements are preceded by enhanced synapse turnover, which can be modulated through changes in inhibitory connectivity. Behaviourally related synapse rearrangement events tend to co-occur spatially within short stretches of dendrites, and involve signalling pathways partially overlapping with those controlling the functional plasticity of synapses. The new findings suggest that a mechanistic understanding of learning and memory processes will require monitoring ensembles of synapses in situ and the development of synaptic network models that combine changes in synaptic function and connectivity.

The contributions of brain networks to information processing and learning and memory are classically interpreted within the framework of Hebbian plasticity and the notion that synaptic weights can be modified by specific patterns of activity. However, accumulating evidence over the past decade indicates that synaptic networks are also structurally plastic, and that connectivity is remodelled throughout life, through mechanisms of synapse formation, stabilization and elimination. This has led to the concept of structural plasticity, which can encompass a variety of morphological changes that have functional consequences. These include on the one hand structural rearrangements at pre-existing synapses, and on the other hand the formation or loss of synapses, of neuronal processes that form synapses or of neurons. In this Review we focus on plasticity that involves gains and/or losses of synapses. Its key potential implication for learning and memory is to physically alter circuit connectivity, thus providing long-lasting memory traces that can be recruited at subsequent retrieval. Detecting this form of plasticity and relating it to its possible functions poses unique challenges, which are in part due to our still limited understanding of how structure relates to function in the nervous system.

We review recent studies that relate the structural plasticity of neuronal circuits to behavioural learning and memory and discuss conceptual and mechanistic advances, as well as future challenges. The studies establish a number of strong links between specific behavioural learning processes and the assembly and loss of specific synapses. Further areas of substantial progress include molecular and cellular mechanisms that regulate synapse dynamics in response to alterations in synaptic activity, the specific spatial distribution of the synaptic changes among identified neurons and dendrites and the relative roles of excitation and inhibition in regulating structural plasticity. The new findings provide exciting early vistas of how learning and memory may be implemented at the level of structural circuit plasticity. At the same time, they highlight major gaps in our understanding of plasticity regulation at the cellular, circuit and systems levels. Accordingly, achieving a better mechanistic understanding of learning and memory processes is likely to depend on the development of more effective techniques and models to investigate ensembles of identified synapses longitudinally, both functionally and structurally.

Molecular mechanisms of synapse remodelling

A remarkable feature of excitatory and inhibitory synapses is their high level of structural variability and the fact that their morphologies and stabilities change over time. This phenomenon is regulated by activity, and the size of spine heads correlates with synaptic strength, presynaptic properties and the long-term stability of the synapse. The morphological characteristics of synapses thus reveal important features of their function and stability. Most importantly, there is a continuity of regulatory processes relating synaptic activity to the strength, shape and long-term retention of existing synapses.

Synapse restructuring | Early electron microscopy studies provided the first evidence that the induction of synaptic plasticity could affect the size and shape of dendritic
Figure 1 | Molecular mechanisms regulating activity-mediated stabilization of dendritic spines. Induction of synaptic plasticity at individual synapses is associated with a rapid enlargement of the spine head, an increase in synaptic efficacy and a switch in the stability of the synapse that could make them persistent. Recent findings implicate an important role of protein kinases (such as PKC (protein kinase C) and CaMKII (calcium/calcmodulin protein kinase II)) contributing to long-term potentiation (LTP) maintenance, spine enlargement and in vivo spine stability (for PKC). In addition, local protein synthesis (for example of BDNF (brain-derived neurotrophic factor), TRKB (tyrosine kinase B), MAPK (mitogen-activated protein kinase), PI3K (phosphoinositide 3-kinase), PTEN (phosphatase and tensin homologue), AKT, TSC1 (tuberous sclerosis 1), TSC2, mTOR (mammalian target of rapamycin) and FMRP (fragile X mental retardation protein)) contributes to LTP maintenance, spine enlargement and spine stability. Proteins implicated in the regulation of the actin cytoskeleton (such as DISC1 (disrupted in schizophrenia 1), CDC42 (cell division control protein 42), RAC1 (Ras-related C3 botulinum toxin substrate 1), PAKs (p21-activated kinases) and adducin) contribute to LTP maintenance and spine enlargement (and spine stability for PAK3). The actin cytoskeleton is indicated as F-actin. Moreover, adhesion molecules and molecules of the postsynaptic density (including PSD95 (postsynaptic density protein of 95 kDa), SHANKs (SH3 and multiple ankyrin repeat domains proteins), neuroligins, N-cadherins, AMPA receptors (AMPARs) and NMDA receptors (NMDARs)) are implicated in LTP maintenance, spine enlargement and spine stability.

spines. Later, two-photon glutamate uncaging and imaging experiments demonstrated a close association between increased synaptic strength and an enlargement of the spine head. The significance of this enlargement could reflect several important functional modifications of the synapse. It could be linked to the changes in receptor expression that are thought to account for the increase in synaptic strength at many synapses. It could also result from the mobilization of subcellular resources to potentiated synapses, such as ribosomes or additional cytoskeleton-associated proteins. In addition, this restructuring could be part of a more global set of changes that promote the stabilization of the synapse. Several recent studies have indeed highlighted the importance of synapse stabilization as a defined feature associated with behavioural learning. Novel sensory experience was shown to promote the stabilization of a new set of persistent spines in the somatosensory cortex in vivo. Similarly, in motor skill learning experiments, new spines that grow on selective populations of neurons are preferentially stabilized during subsequent training, with the spines persisting long after training has stopped. In birds, song learning by imitation during a juvenile sensitive period leads to a rapid stabilization and enlargement of dendritic spines that is correlated with an enhancement of synaptic activity. These different studies support the idea that the stabilization of selective subpopulations of spines could represent a structural basis for memory storage. Although this stabilization process is often associated with the induction of plasticity, several important issues remain to be addressed. How does this stabilization relate to changes in synaptic strength or spine size? Are changes in synaptic strength required for the stabilization of a synapse? How stable is this mechanism? A recent study suggests that reconditioning following a procedure of conditioning and extinction preferentially eliminates dendritic spines formed and stabilized by extinction. Accordingly, stabilization may be considered as a key reversible property of individual synapses that is linked to the induction of plasticity.

Molecular mechanisms of synapse stabilization. The molecular mechanisms accounting for synapse stabilization are likely to implicate a variety of factors, which have often been inferred from indirect analyses of either mechanisms contributing to long-term potentiation (LTP) maintenance or mechanisms implicated in activity-mediated spine enlargement. Relatively few studies have examined molecular mechanisms contributing to spine stabilization by directly measuring the persistence of dendritic spines in vivo. Current evidence, however, suggests that there is a significant overlap between the molecular pathways implicated in these different aspects of stability (Figure 1), emphasizing the close link existing between induction of plasticity and synapse stability.

First, an important part is likely to be played by phosphorylation mechanisms. Both calcium/calcmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC) have been directly implicated in LTP maintenance and behavioural learning. CaMKII activity is required for activity-mediated spine enlargement, and PKC contributes to in vivo spine stabilization. Another central mechanism for spine stabilization involves the local regulation of protein synthesis, which includes the signalling cascades (such as the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways) downstream of receptor tyrosine kinase B (TRKB; also known as NTRK2) activation, the mammalian target of rapamycin (mTOR)
An important feature of appearance events.

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REVIEWS

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signalling complex and the translation of mRNAs that encode proteins such as ARC or CaMKII. Interference with this signalling, with protein synthesis or with ARC translation have been strongly implicated in LTP maintenance and in spine enlargement19–23, whereas in vivo blockade of protein synthesis results in synapse destabilization14. A third set of molecular factors critical for spine stabilization includes the various signalling pathways and actin-regulatory proteins that control the spine actin cytoskeleton. Interference with actin polymerization impairs LTP maintenance and changes in spine size23–25. Furthermore, phosphorylation of the cytoskeleton-stabilizing protein β-adducin through PKC is required for the stabilization of populations of synapses induced by environmental enrichment18. Additional evidence supporting a role of the cytoskeleton in spine stabilization comes from the implications of Rho GTPases and several upstream or downstream modulators of this pathway, such as kalirin 7, DISC1 (disrupted in schizophrenia 1) or PAKs (p21-activated kinases). Interference with this signalling affects LTP mechanisms and the capacity of spines to enlarge26,27. Finally, one important mechanism through which synapse stability could be improved is by changes in the organization of the postsynaptic density (PSD) that promote trans-synaptic adhesion and contact. Expression of PSD95 and/or AMPA receptors enhances synaptic strength and synapse stability28,29. Several adhesion molecule systems have also been linked to spine stability, including neuraligins 1 (REFS 29,30) and N-cadherin. Activity-mediated expression of N-cadherin correlates with, and is required for, the long-term stabilization of spines activated by theta-burst stimulation31. Secreted members of the C1q family have also been shown to rapidly induce changes in synapse numbers by, for example, stabilizing synapses in the mature cerebellum in vivo through the formation of trans-synaptic complexes32. Taken together, these data highlight how spine stabilization is regulated by a multiplicity of molecular mechanisms, probably reflecting the importance and complexity of the phenomenon.

Synapse turnover specificity in vivo

A comparatively small but significant fraction of synapses in the adult in vivo undergo a continuous turnover process, which may allow a continuous adaptation of synaptic networks to experience1. The magnitude of this turnover process varies strongly during development, decreasing significantly in adult brain33–34, but a substantial capacity for circuit rewiring is maintained throughout life and can be reactivated by lesions1. As discussed below, processes known to involve enhanced plasticity also enhance the fraction of synapses that undergo turnover in the adult.

Remodelling of connectivity. An important feature of synapse turnover is its regulation by activity and sensory experience35. Whereas initial in vitro experiments mainly focused on spine growth and synapse formation in response to neuronal activation34-36, more recent experiments have shown that activity also destabilizes existing synapses30,35. Under in vivo conditions, training in motor skill learning tasks results in a rapid rewiring through the formation and elimination of spines in the primary motor cortex, affecting different sets of synapses for different motor skills11,12. Spine elimination and formation caused by fear conditioning and extinction, respectively, occur in a cue- and location-specific manner14. Similarly, a major correlate of environmental enrichment is a marked increase in synapse remodelling, including synapse formation and destabilization36.

An interesting feature of activity-mediated spine dynamics is that it might be regulated locally: evidence suggests that induction of plasticity is facilitated in the vicinity of potentiated spines and that new spines preferentially form close to activated spines36,37. Two recent studies further support these results. Using a repetitive motor learning task, it has been shown that new spines formed during the acquisition of learning emerge in clusters as neighbouring spine pairs that are more likely to persist than non-clustered spines38. Another study carried out during development by monitoring synaptic activity through calcium imaging shows that neighbouring synapses are more likely to be co-active than synapses farther from each other39. Local regulation of spine dynamics may thus be an important mechanism to promote such clustering activity.

A different aspect of the regulation of spine turnover is that, in some cases, the effect may be more global and differentially affect spine formation and elimination, resulting in actual changes in spine density40. In the motor learning task experiments, the increase in spine formation and spine loss roughly cancelled each other out, resulting in no marked changes in spine density11,12. By contrast, the enriched environment protocols greatly promoted spine growth, leading to an increase in the absolute numbers of spines41. Regulation of spine dynamics thus not only promotes rewiring but also controls the level of connectivity of the network. Taken together, these observations suggest that the rewiring observed under behavioural learning conditions represents a structural correlate of learning (FIG. 2).

Regulation of spine and synapse turnover. One important factor controlling synapse turnover appears to be the balance between excitation and inhibition. Alterations of this balance during critical (or sensitive) periods — that is, developmental time windows of enhanced plasticity — strongly affect the capacity for structural plasticity42. Furthermore, several recent studies have shown that manipulations that reduce inhibition in adulthood are able to restore visual plasticity to levels comparable to those observed during development42,43. Although it remains unclear how exactly modulation of the excitatory–inhibitory balance can promote or reduce cortical plasticity, part of the effect could implicate changes in synapse dynamics. Consistent with this possibility, spine changes correlate with the capacity for visual plasticity in vivo44 and, during development, short-term anaesthesia or administration of drugs that enhance GABAergic inhibition results in rapid and marked changes in spine growth and synapse gain41.
Several additional molecular mechanisms have also been reported to modify spine numbers and dynamics. For example, spine turnover can rapidly shift the balance of spine growth towards an increased spine density and stabilization, leading to an increase in spine density and stabilization. The fact that structural plasticity may not be adequate to the extent of structural plasticity changes observed in dendritic spines and axon terminals suggests that both intracellular and extracellular factors play a role in modifying spine numbers and dynamics. These factors include focal adhesion kinase (FAK), which mediates spine growth by modulating the actin cytoskeleton, as well as protein kinase C (PKC), which regulates spine morphology and stability. Moreover, the role of FAK and PKC in regulating spine dynamics is further supported by studies showing that FAK knockdown decreases spine density and total volume, whereas PKC overexpression increases spine density and stability.

Figure 2 | Learning-induced structural rewiring of synaptic networks. a–c: Schematic showing a characteristic spine turnover sequence under baseline activity. a: Baseline activity. b: Under conditions of learning-induced structural plasticity, synapses are modified repeatedly during an initial period of learning. c: After sufficient learning, a small subpopulation of persistent spines are unaffected. d: Under conditions of de novo structural plasticity, learning events do not lead to the formation of additional new spines. Small dark spines are not modified, and the diminished pre-existing spines (dashed lines) are weakened and lost in the process of memory consolidation. e: An area of interest in the learning process may be associated with the different stages of structural plasticity. f: Learning-induced structural plasticity may be associated with the transition between the different stages of structural plasticity, with the potential substrates of plasticity serving as potential substrates for specific learning tasks.
of learning. Nevertheless, the detection of synapse remodelling events did not reflect a lack of specificity in the circuit elements involved in the structural plasticity. For example, in agreement with behavioural observations, structural plasticity in the motor cortex upon learning of a grasping movement was specifically confined to projection neurons driving distal limb muscles and did not affect those driving proximal muscles. The specificity was particularly remarkable considering that the different projection neurons are locally intermingled within the primary motor cortex. Notably, the extent of the structural plasticity was correlated with the magnitude of the learned movement. Evidence for specificity was also provided in experiments in which sensory deprivation in the adult produced specific patterns of growth and retraction in cortical axons and dendrites.

In support of the notion that the local structural plasticity was specifically associated with learning, re-learning the same task or a second occurrence of the same kind of sensory deprivation did not elicit further plasticity in the same neurons. These findings suggest that learning-induced structural plasticity can initially affect a substantial fraction of the neurons involved in the learning, and that less abundant but more persistent alterations reflect ‘lasting structural traces’ of learning. The number of structural traces of learning that become long lasting may depend on intrinsic processes that regulate plasticity and on the amount of repeated training that triggers memory consolidation and reconsolidation processes. Elucidating the extent to which the new synapses may truly mediate the encoding of memories (that is, whether they represent ‘engrams’) will require more sophisticated methods to combine structural and functional imaging of synapses in vivo (see below). Nevertheless, two recent studies have provided some evidence that there may indeed be a direct correspondence between new synapses and engrams in learning. In one study, fear learning and its extinction affected the formation and disappearance of spines within two microns of distance on the same dendrites, suggesting that opposite changes in the numbers of spatially closely related synapses are associated with opposite behavioural outcomes. Evidence for specificity was provided by the observation that learning–extinction cycles for different tones, which produced separate regulation behaviourally, were associated with distinct stretches of dendrites.

In a second study, new spines assembled upon repeated motor learning had a high probability to appear in the close vicinity of spines that had appeared at previous days during the same motor learning process, suggesting a striking correspondence between the gradual encoding of specific new memories and the spatial position of new spines along particular dendrites.

In addition to alterations at subsets of neurons and synapses, behavioural learning can produce more global alterations in the numbers of specific types of synapses within systems involved in the particular learning. For example, different forms of behavioural learning can lead to up to a doubling in the numbers of excitatory synapses onto fast-spiking inhibitory interneurons in the hippocampus and/or cerebellar cortex (feedforward inhibitory (FFI) growth). Using targeted virus-mediated rescue experiments in a β-adducin mutant background deficient in learning-induced synaptogenesis, the same study provided causal evidence that this plasticity is critically important for the behavioural precision of the memory, but not for the memory of the learned association itself. Although the high level of local prevalence of the FFI growth might suggest a lower circuit level specificity for this form of structural plasticity, this may in fact not be the case. Thus, fast-spiking interneurons are thought to detect local levels of circuit excitation through the convergence of large numbers of weak excitatory synapses onto them and to broadcast that signal to most excitatory neurons within their local environment. Accordingly, the broad FFI growth plasticity may be specifically adjusted to the connectivity properties of fast-spiking feedforward excitation targeting cell bodies and proximal dendrites. Whether learning produces additional broadly distributed alterations in defined elements of neuronal circuits remains to be determined.

**Plasticity affecting multiple systems and neurons.** Several factors have been shown to influence future learning and behavioural outputs by inducing major modifications in the numbers, arrangements and dynamics of synaptic connections. For example, environmental enrichment and oestrogen both produce large increases in synapse turnover and synapse numbers at multiple neuronal systems. Conversely, stress can reduce synapse numbers in some systems (for example, in the hippocampus), while increasing them in other systems (for example, in the amygdala). Synapse dynamics and numbers are further influenced by seasonal changes and developmental age. For environmental enrichment, the increased synapse turnover has been causally related to improved learning. Common to these influences of external and internal contingencies on structural plasticity is the fact that they do not involve specific learning processes. The structural alterations related to experience, hormones and age are not confined to a few neuronal systems, but their distribution has not yet been investigated in sufficient detail to extract possible patterns. It is possible that these alterations may reflect the properties of the signals that induced them, such as the distribution of hormone receptors and the ways through which novel sensory experience influences circuit function.

**Widespread dynamics followed by confined consolidation.** How can the presence of broadly distributed structural alterations upon experience and learning be reconciled with the specificity necessary for the structural modifications to selectively reflect learned relationships? It is possible that some of the broad changes in circuit structure affect function in ways that are unrelated to mechanisms of learning. However, many of the alterations as a result of experience, hormones and ageing are likely to affect learning and memory by acting on the same cellular and molecular processes. As...
discussed in previous sections, LTP and learning are accompanied by enhanced rates of synapse assembly and disassembly events. Several studies of learning-related synapse dynamics in vivo have provided strong evidence that enhanced dynamics is specifically correlated with new learning in intact birds, rodents and primates, and with recovery after stroke in the human adult. Similar studies have further shown that a subpopulation of new synapses is subsequently stabilized during a process depending on repeated training, which lasts for many days and even weeks. A study of how zebra finches learn to sing from a tutor provides a particularly compelling case for the relationship between behavioural learning and synapse turnover. Thus, at the appropriate developmental stage, enhanced spine turnover was detected on sensorimotor neurons involved in the learning, and the learning experience stabilized some of these spines. An age-related decline in spine dynamics was delayed if the birds were raised without a tutor. Furthermore, enhanced learning upon environmental enrichment was dependent on increased gains and losses of synapses. These were, in part, provided by the population of additional dynamic synapses that were induced upon enrichment. Similar principles seem to apply to the increase in labile synapses induced by oestrogen. It is likely that several types of signals, some acting locally and directly related to new learning, and others acting more globally and related to experience, hormones and age, may all produce alterations in synapse turnover and in the numbers of dynamic synapses that provide potential substrates for learning. The presence of larger numbers of dynamic synapses before learning may facilitate learning, whereas the selective stabilization of small subsets of dynamic synapses upon repeated learning may provide structural traces of learning (FIG. 3). As enhanced learning upon environmental enrichment also depends on synapse loss, it is likely that learning also involves the selective elimination of synapse subpopulations.

It is conceivable that learning and memory, under a regime of previously enhanced (for example, after environmental enrichment) or reduced widespread synapse dynamics, might be subject to regulation that differs, in part, from that involving synapse dynamics specifically induced during learning. That may, for example, involve distinct molecular compositions and stabilization mechanisms at synapses involved in learning. Such differences could have important implications for how experience (for example, stress) influences internal states and learning, but an adequate investigation of these phenomena will probably depend on the establishment of more sensitive experimental paradigms to study specific relationships between the structure and function of neuronal networks in living animals (see below).

**Figure 3** | Global and local synapse turnover regulation processes affecting learning and memory. The schematics represent dendrites of two excitatory neurons and their spine synapses. Increasing plasticity is represented as darker grey tones. Dynamic spines are green (gains) and red (losses); spine changes upon learning are indicated by green and red arrows; orange spines appear upon learning, but do not persist during consolidation; and structural traces of learning upon consolidation include spine gains (blue) and spine losses. Learning-induced structural plasticity enhances the turnover of subpopulations of new and pre-existing synapses specifically in excitatory neurons involved in the learning, and leads to the selective stabilization of some learning-induced spines. The enhanced structural plasticity baseline levels underlie improved behavioural learning upon enrichment, and improved song learning in the presence of a tutor during zebra finch development.
Plasticity regulation

What mechanisms regulate the potential for structural plasticity (metaplasticity) in the brain? Much of the current knowledge and concepts about plasticity regulation are derived from studies of juvenile animals in which time windows of enhanced plasticity facilitate adjustments that are important for adult function\textsuperscript{43,42,64–66}. Whereas most of the studies have investigated plasticity to adjust for malformations such as strabism or monococular deprivation, a recent study revealed that within the binocular visual cortex, critical period plasticity produces a matching of the orientation preferences of individual neurons in response to each eye\textsuperscript{67}. Critical period studies in the visual and auditory system have provided evidence for profound structural plasticity during learning, including the assembly and long-term retention of alternative extra circuits that can be recruited in the adult under appropriate conditions\textsuperscript{43,65,67–69}. Studies in barn owls have revealed that the additional learned circuits that had been assembled during a sensitive period in juvenile birds were turned on and off in the adult through mechanisms distinct from those that turn innate natural circuits on and off (disinhibition versus AMPA/NMDA ratios for the innate and learned circuits, respectively), suggesting that innate and acquired circuit arrangements can be distinguished functionally\textsuperscript{64,65}. At the mechanistic level, the studies of critical periods have uncovered a major role for the maturation of inhibitory circuits, and in particular those established by parvalbumin-positive (PV\textsuperscript{+}) fast-spiking interneurons, in opening and closing plasticity windows\textsuperscript{1,66,70}. Recent findings suggest that similar mechanisms may regulate plasticity in the adult, and that the regulatory mechanisms may in part involve structural plasticity at inhibitory interneurons.

Factors promoting and inhibiting plasticity. Some of the molecular pathways known to regulate plasticity are illustrated in FIG. 4. In most cases, plasticity regulation involves signalling pathways relating neuronal activity to the expression of key activity-regulated genes\textsuperscript{71–73}. Consistent with its central roles in mediating signalling downstream of synaptic activity, calcium has prominent roles in activity-regulated gene expression. One of the genes regulated by calcium is the transcription factor MEF2 (myocyte enhancer factor 2), which reduces excitatory synapse numbers. Genes regulated through MEF2 include the synaptic components ARC and HomoER1, and the neurotrophin BDNF, which augments inhibitory synapse numbers\textsuperscript{71}. Although many growth factors can enhance plasticity when applied to cultured neurons or in vivo, only a few of them, particularly BDNF, have been related conclusively to endogenous plasticity regulation under physiological conditions\textsuperscript{74}. Strong evidence supports the notion that BDNF signalling has a key role in promoting plasticity, and that this signalling pathway is recruited upon enhanced excitation\textsuperscript{48,49}. Intracellular signalling molecules and pathways relating excitation and BDNF signalling to plasticity include: ARC, MAPK, CaMK, CREB (cAMP response element-binding), UBE3A (ubiquitin protein ligase E3A) and histone acetylation or reduce the formation of such synapses (such as MEF2 (myocyte enhancer factor 2) and miR-134). Expression of the transcription factor NPAS4 (neuronal PAS domain-containing protein 4) promotes the formation of inhibitory synapses (indicated by a curved arrow). Structural plasticity can result from neuromodulatory modifications of the excitatory–inhibitory balance (including the cholinergic system, LYNX1 (Ly-6/neurotoxin-like protein 1), serotonin and dopamine). LYNX1 inhibits nicotinic acetylcholine (nACh) receptors, which can be found presynaptically, on dendrites and around somas. Furthermore, structural plasticity can be achieved through diffusible factors (including brain-derived neurotrophic factor (BDNF) and WNT, indicated by the green shading) that can affect synaptic signalling pathways (such as CaMKII, MAPK (mitogen-activated protein kinase), ARC and UBE3A) or through alterations of the extracellular matrix (matrix metalloprotease 9 (MMP9) and perineuronal nets (PNNs)).
Innate natural circuits
Connectivity that may support innate processing such as tuning to positions or orientations in space or matching visual and auditory inputs. Adaptive alternative circuits can be assembled during critical periods and retained in the adult.

Fluoxetine
A selective serotonin reuptake inhibitor used to treat major depression (trade names include Prozac, Eli Lilly) that can enhance plasticity in the adult.

Perineuronal nets
Specialized extracellular matrix surrounding soma and proximal dendrites of parvalbumin-positive interneurons. The assembly of perineuronal nets correlates with local closure of critical periods, and their removal reactivates plasticity in the adult.

Receptive fields
In the visual system, these are the regions to which a neuron responds effectively to the presence of a stimulus. More generally, neurons in sensory systems are selectively tuned to particular stimuli from the environment.

critically important for skill learning and for functional recovery after brain injury. In addition to enhanced excitation, reduced inhibition augments plasticity under a number of different conditions, including environmental enrichment, the effects of fluoxetine treatments and the reduction of perineuronal nets around the cell body and proximal dendrites of PV+ interneurons. Several lines of evidence have directly related reduced inhibition to enhanced plasticity during critical periods and in the adult in rodents. Finally, important recent studies have introduced the notion that the potential for plasticity in the adult may be as robust as that detected in juvenile animals, but that adult plasticity is effectively prevented through 'brake' mechanisms. The reduced plasticity in the adult may prevent aberrant plasticity after the formation of lesions and may ensure the transmission of adaptive behaviours learned from conspecifics across generations. In addition to perineuronal nets and myelin-associated inhibitors, which may in part have structural roles, LYNX1 (Ly-6/neurotoxin-like protein 1) has been identified as a specific inhibitor of nicotinic cholinergic signalling that suppresses plasticity in the presence of widespread cholinergic innervation in the adult. An important transcripational pathway involving Npas4 (neuronal PAS domain-containing protein 4) also specifically links excitation to the establishment of a higher number of inhibitory synapses onto activated neurons. Furthermore, miR-134 has been identified as a major negative post-transcriptional regulator of plasticity downstream of SIRT1 (NAD-dependent protein deacetylase sirtuin 1) and upstream of CREB.

Inhibitory circuit rearrangements. Whereas most studies of structural plasticity initially focused on excitatory neurons, several recent studies have revealed that structural plasticity by inhibitory neurons precedes that by excitatory neurons and may have a critical role in regulating plasticity during learning. An initial series of studies documented structural plasticity of dendritic tips by GABAergic neurons in adult mouse cortex, with most of the plasticity contained within a superficial strip of layer 2/3 (REFS 94–96). A subsequent study documented pronounced structural plasticity of inhibitory axons upon sensory deprivation, which preceded sprouting by excitatory axons, and several-fold enhanced spine and axonal bouton turnover. Changes in structural plasticity were detected within hours following peripheral lesions, suggesting that they might account for rapid changes in functional plasticity of receptive fields. Furthermore, dramatic changes in structural plasticity by fast-spiking striatal inhibitory neuron axons that specifically target the indirect striatal pathway were detected following lesions that result in dopamine deprivation. Finally, two recent studies in sensory-deprived visual cortex provided evidence that regulation of structural plasticity by inhibitory interneurons may provide permissive conditions for subsequent plasticity by excitatory neurons. One study reported an early loss of spines, thus reducing excitatory inputs onto a subpopulation of inhibitory interneurons (mainly neuropeptide Y-positive), and a subsequent loss of axonal boutons, thus reducing inhibitory output by the same interneurons upon sensory deprivations. The second study reported a loss of excitatory inputs onto inhibitory neurons in layer 2/3 upon visual deprivation. Together, the studies suggest that early structural plasticity in sensory-deprived cortex may lead to a diminished excitatory drive onto inhibitory interneurons, suggesting a possible structural basis for disinhibition and enhanced excitation.

Is it inhibition or excitation? The recent discovery of early structural plasticity at inhibitory interneuron subpopulations preceding plasticity at excitatory neurons suggests a possible conceptual framework to account for how excitation–inhibition balances may regulate short- and long-term structural plasticity in the adult. The mechanisms involved appear to resemble those regulating plasticity during circuit maturation, consistent with the notion that plasticity is controlled in similar ways in young animals and in adults. Instead of focusing on excitatory or inhibitory neurotransmitter levels, or on global levels of excitation and inhibition, this emerging framework addresses plasticity regulation at the circuit level, thus offering possible mechanistic solutions to account for fine-tuned regulation and specificity in learning-related plasticity. Findings discussed in previous sections that may be particularly relevant are: at the level of individual neurons, structural plasticity is augmented by enhanced excitation; reducing inhibition is sufficient to enhance plasticity in the adult; and salient activity (for example, exposure to light after dark rearing) can produce disinhibition of excitatory neurons by activating ‘second layer’ (dissinhibiting) inhibitory interneurons, partly through structural plasticity. Accordingly, signals that trigger plasticity may initially reduce the activation of GABAergic neurons, such as PV+ interneurons that target excitatory neurons; depending on the extent of the plasticity, this may involve recruitment of disinhibitory interneurons and/or structural plasticity to reduce the connectivity of PV+ interneurons, in turn leading to enhanced excitation and structural plasticity of excitatory neurons (FIG. 5). Targeting inhibitory neuron networks first might have a plasticity-facilitating effect at the network level. The enhanced potential for plasticity could then serve as a basis for more specific synapse remodelling processes at the level of individual excitatory neurons. The validity of the model, the identity of the particular interneuron subpopulations and the circuit mechanisms involved in short- and long-term plasticity regulation processes remain to be determined.

From structural plasticity to memories
It is generally assumed that structural plasticity provides a mechanism for long-term storage of memory traces upon learning. However, the temporal sequences of events and the regulatory mechanisms relating learning and structural plasticity to long-term memory are still poorly understood. An important aspect involves
the temporal delay between the early potentiation of pre-existing synapses, spine growth and synaptogenesis upon learning. Such delays may differ among learning protocols and systems involved. Thus, some studies have suggested that synapses involving new spines or filopodia are assembled within the first 1–3 hours after potentiation, whereas other studies have provided evidence for delays of 12–18 hours. The longer delays provide a potential mechanism to relate learning to the consolidation of memories, for example, during sleep. Such scenarios may enhance the specificity of synapse remodelling processes upon learning by uncoupling contingencies present during learning from the consolidation of new synapses and their integration into memory networks. Further structural plasticity may occur during longer lasting system-level consolidation processes, but experimental evidence for such plasticity is not available yet. Likewise, whether and how memory retrieval and reconsolidation processes involve structural plasticity remains to be determined. Addressing these fundamental issues in learning and memory at the structural level will require the development of more specific and sensitive approaches to investigate circuit and network remodelling processes in vivo, at the level of identified synapse ensembles.

**Synapse remodelling and mental health**

The important contribution of structural plasticity to various behavioural learning situations highlights the importance of connectivity remodelling and synapse stabilization as substrates for learning processes and memory retention. Accordingly, any defect in synapse dynamics can be expected to have a significant impact on the development, organization or specificity of synaptic networks. Indeed, the mechanisms regulating synapse dynamics have been implicated in several developmental psychiatric disorders as discussed below.

**Synapse rearrangements in disease and upon lesions.**

Analyses of the synaptic defects associated with a number of synaptic proteins implicated in intellectual disability, autism spectrum disorders or schizophrenia show alterations of synapse structure or numbers (TABLE 1). Consistent with a key role for structural plasticity and the excitation–inhibition balance in controlling circuit maturation, many of the psychiatric conditions manifest during early life. SHANK3 (SH3 and multiple ankyrin repeat domains protein 3), PSD95, synapse-associated protein 97 and ubiquitin protein ligase E3A are involved in excitatory synapse stabilization. FMRF, PTEN (phosphatase and tensin homologue), TSC1 (tuberous sclerosis 1; also known as hamartin) and TCS2 (also known as tuberin) regulate local protein synthesis, possibly affecting mechanisms of synapse stabilization. Several molecules (such as DISC1, kalinir, EPAC2 (also known as RAPGEF4), PAK3 and ARHGEF6 (Rho guanine nucleotide exchange factor 6)) are implicated in signalling through Rho GTPases, and could perturb cytoskeletal functions that regulate spine and synapse dynamics. Finally, MECP2 (methyl-CpG-binding protein 2) and molecules of the neuroglin–neurexin complex appear to be important for regulating the balance between excitation and inhibition and could therefore interfere with spine formation and dynamics. All these observations point to the possibility that alterations of structural plasticity mechanisms may have an important role in these diseases. Consistent with this notion, defects in connectivity between layer 5 cortical neurons have been reported in a mouse model of Rett syndrome, and this is associated with important alterations of spine dynamics. Other recent evidence from in vivo imaging in a mouse model of fragile X syndrome suggests that synapse dynamics could be exaggerated, leading to an increased proportion of unstable synapses and an excessive remodelling of synaptic circuits. Similarly, mutation of the intellectual disability gene PAK3, which is an effector of the Rho GTPases RAC1 (Ras-related C3 botulinum toxin substrate 1) and CDC42 (cell division control protein 42 homologue), results in excessive

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Figure 5 | Circuit mechanisms of plasticity regulation. Left: schematic representing a local circuit arrangement involving two inhibitory neurons (ovals 1 and 2, perisomatic and disinhibiting, respectively) impinging onto one excitatory cell (triangle 3). Circles: excitatory inputs; bars: inhibitory inputs. Right: circuit mechanisms leading to enhanced plasticity. Decreased connectivity (decreased synapse numbers (a,b) or decreased synapse function (c)) is represented by red colours; increased connectivity is represented by green colours. Structural plasticity in the excitatory cell is enhanced (shown in purple) under conditions of decreased excitatory connectivity (a), increased inhibitory connectivity (b) or perineuronal net reduction (c) on the perisomatic interneuron that directly inhibits the excitatory cell. Whereas the three scenarios involving structural plasticity at inhibitory interneurons lead to broad disinhibition of excitatory cells, a direct increase of the excitatory drive onto the excitatory neuron can also enhance plasticity.

**Rett syndrome**

Neurodevelopmental disorder caused by mutations of MECP2 (methyl-CpG-binding protein 2), a methylated DNA binding protein that maps onto the X chromosome. Some of the manifestations of Rett syndrome are characteristic of autism spectrum disorders.
Table 1 | Synaptic proteins with genetic defects that have been associated with developmental psychiatric disorders

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
<th>Synaptic contribution</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARHGEF6</td>
<td>RAC GEF, regulation of actin cytoskeleton</td>
<td>Synapse formation and maturation</td>
<td>Intellectual disability</td>
</tr>
<tr>
<td>CYFIP1</td>
<td>Protein synthesis</td>
<td>Unknown</td>
<td>Fragile X syndrome</td>
</tr>
<tr>
<td>DISC1</td>
<td>Scaffold protein</td>
<td>Synapse formation and maturation</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>EPAC2</td>
<td>RAP GEF</td>
<td>Spine maturation</td>
<td>ASD</td>
</tr>
<tr>
<td>ERBB4</td>
<td>Receptor tyrosine kinase</td>
<td>Regulation of excitatory transmission</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>FMRP</td>
<td>Protein synthesis</td>
<td>Synapse stabilization</td>
<td>Fragile X syndrome</td>
</tr>
<tr>
<td>GABRB3, GABRA5, GABRG3</td>
<td>GABA receptor subunits</td>
<td>Excitation–inhibition balance</td>
<td>ASD</td>
</tr>
<tr>
<td>IL1RAPL1</td>
<td>Scaffold protein</td>
<td>Synapse formation</td>
<td>Intellectual disability</td>
</tr>
<tr>
<td>Kalirin</td>
<td>RAC GEF, regulation of actin cytoskeleton</td>
<td>Synapse formation and maturation</td>
<td>Schizophrenia, ASD</td>
</tr>
<tr>
<td>LIMK1</td>
<td>Protein kinase, actin skeleton</td>
<td>Spine maturation</td>
<td>Williams syndrome, intellectual disability</td>
</tr>
<tr>
<td>MINT2</td>
<td>Presynaptic adaptor protein</td>
<td>Neurosecretion</td>
<td>ASD, schizophrenia</td>
</tr>
<tr>
<td>Neurexin 1</td>
<td>Trans-synaptic modulator of ERBB4</td>
<td>Regulation of excitatory transmission</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>Neurexin 1</td>
<td>Presynaptic adhesion molecule</td>
<td>Synapse stabilization</td>
<td>ASD</td>
</tr>
<tr>
<td>Neurexin 3, neurexin 4</td>
<td>Adhesion molecules</td>
<td>Synapse stabilization</td>
<td>ASD</td>
</tr>
<tr>
<td>Oligophrin 1</td>
<td>RhoA GAP, regulation of receptor trafficking</td>
<td>Spine maturation</td>
<td>Intellectual disability</td>
</tr>
<tr>
<td>PAK3</td>
<td>Protein kinase, actin cytoskeleton</td>
<td>Synapse formation and stabilization</td>
<td>Intellectual disability</td>
</tr>
<tr>
<td>Protocadherins</td>
<td>Adhesion molecules</td>
<td>Unknown</td>
<td>ASD</td>
</tr>
<tr>
<td>PSD95</td>
<td>Scaffold protein</td>
<td>Synapse plasticity and stabilization</td>
<td>ASD, schizophrenia</td>
</tr>
<tr>
<td>PTEN</td>
<td>Tyrosine phosphatase, protein synthesis</td>
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<td>ASD, macrocephaly</td>
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<td>RSK2</td>
<td>Protein kinase</td>
<td>Neurosecretion</td>
<td>Intellectual disability</td>
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<td>SAP97</td>
<td>Scaffold protein</td>
<td>PSD protein trafficking</td>
<td>ASD, schizophrenia</td>
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<tr>
<td>SHANK2, SHANK3</td>
<td>Scaffold protein</td>
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<td>ASD</td>
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<td>srGAP3</td>
<td>RAC1 GAP</td>
<td>Unknown</td>
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</tr>
<tr>
<td>SSCAM (also known as MAGI2)</td>
<td>Scaffold protein</td>
<td>Receptor trafficking</td>
<td>Intellectual disability</td>
</tr>
<tr>
<td>SynGAP</td>
<td>RAS/RAP/RAC-GAP</td>
<td>Receptor trafficking and actin cytoskeleton</td>
<td>ASD, intellectual disability</td>
</tr>
<tr>
<td>TSC1, TSC2</td>
<td>Protein synthesis</td>
<td>Synapse stabilization</td>
<td>Intellectual disability</td>
</tr>
<tr>
<td>UBE3A</td>
<td>Protein degradation</td>
<td>Synapse formation</td>
<td>Angelman syndrome, intellectual disability</td>
</tr>
</tbody>
</table>

Synaptic proteins for which genetic defects (single point mutations, deletions, translocations or copy number variations (CNVs)) have been associated with autism spectrum disorders (ASDs), intellectual disability or schizophrenia. Supporting references can be found in recent reviews. ARHGEF6, Rho guanine nucleotide exchange factor 6; CYFIP1, cytoplasmic FMR1-interacting protein 1; DISC1, disrupted in schizophrenia 1; EPAC2, Rap guanine nucleotide exchange factor 4; FMRP, fragile X mental retardation protein; IL1RAPL1, interleukin-1 receptor accessory protein-like 1; LIMK1, LIM domain kinase 1; MINT2, MUNC18-interacting protein 2; RSK2, p21-activated kinase 3; PSD, postsynaptic density; PTEN, phosphatase and tensin homologue; RSK2, ribosomal S6 kinase 2; SAP97, synapse-associated protein 97; SHANK, SH3 and multiple ankyrin repeat domains protein; srGAP3, SLIT-ROBO Rho GTPase-activating protein 3; SSCAM, membrane associated guanylate kinase, WW and PDZ domain containing 2; SynGAP, Ras GTPase-activating protein; TSC, tuberous sclerosis; UBE3A, ubiquitin protein ligase E3A.

Outlook: network structure–function
Studies of structural plasticity related to learning and memory have led to major advances during the past couple of years. First, specific synapse assembly and synapse loss processes have been related conclusively to animal learning, and to structural traces of the learning. How the new synapses contribute to memory is not yet clear, but the current evidence favours the notion that the new synapse arrangements do have specific roles in memory encoding. Second, causality relationships could be established between the new assembly of identified synapses upon learning and the behavioural expression of the learned memories. Third, important mechanisms and principles underlying the regulation of synapse

spine growth and defects in activity-mediated spine stabilization. Alterations in synapse dynamics, either through excessive or insufficient rewiring or defects in synapse stabilization, could perturb the specificity of the mechanisms through which learning shapes the formation of synaptic networks.

Structural plasticity is also important to restore function following lesions. Several recent studies have highlighted the extensive remodelling of both dendritic spines and axons in cortical tissue recovering from stroke or in the visual cortex following lesions. Synapse-restructuring-associated growth and pruning correlates with functional changes recapitulating the structural plasticity seen in early development.
remodelling upon enhanced synaptic activity and learning are being defined at the molecular and cellular level. Among them, an important new insight involves the assembly of new synapses in spatial clusters, suggesting mechanisms of local co-regulation for synapses that may involve the same or connected learning-related memories. Finally, recent results suggest first conceptual frameworks to account for plasticity regulation mechanisms at the circuit level.

The emergence of structural plasticity as a growing research area in learning and memory raises new immediate and long-term challenges. Major unresolved mechanistic issues include: defining the relationships between gains and losses of identified individual synapses upon learning and the memory of what was learned at the microcircuit and systems level; identifying causal sequences of events that relate experience and learning to alterations in structural plasticity and the balance between excitation and inhibition, which includes elucidating how structural remodelling of identified inhibitory and excitatory neuron microcircuits impinge on long-term plasticity regulation during development, in the adult and in disease; and relating genes involved in psychiatric conditions to synapse and microcircuit maturation and remodelling and to the functional consequences of these remodelling processes for system function and animal behaviour.

What will be the probable impact of these new findings for research in neuroscience? The recent advances suggest that structural plasticity processes may be integral components of most aspects of learning and memory. Accordingly, this field of research is likely to have an increasing impact on cognitive neuroscience. The main limitations going forward are of a technical nature. Although functional imaging techniques in intact animals are extremely valuable for uncovering volume alterations in grey matter or axonal projections upon learning or in disease models, they still lack the resolution required to detect structural plasticity at the microcircuit level. Nevertheless, future research will have to tackle network functions at the level of ensembles of individual identified synapses and neurons in vivo. Further progress will probably depend on the development of methods to image synapses and their molecular components with high sensitivity and spatiotemporal resolution in situ.

Exciting recent developments mainly, but not exclusively, based on calcium imaging have achieved sufficient resolution to monitor function at the level of ensembles of spines in the neocortex. Combining such methods in vivo and in slice preparations should allow neuroscientists to bridge important gaps between the anatomy of microcircuits, their plasticity and their function. In parallel, modelling efforts will probably be important for the development of testable conceptual frameworks that take into account specific structural rearrangements within realistic neuronal networks. The addition of structural plasticity rules to current functional plasticity models may reveal new behaviours or properties that are important for learning capacity. Finally, targeted manipulations in situ — for example, through cellular, but possibly even subcellular, compartment-specific optogenetic methods — will be key in order to establish causal relationships between defined structural alterations in network architecture and network function in behaving animals. Combining cell- and synapse-specific imaging, modelling and optogenetic methods should allow neuroscientists to tackle learning, memory and cognition at the level of defined neuronal circuits.
In hippocampal slice cultures, oestradiol reversibly affects pre-established networks.

Bonhoeffer, T. & Lohmann, C. Activity-dependent clustering of functional synaptic inputs on developing dendrites with spines.


Hubener, M. Prior experience enhances plasticity in adult visual cortex.

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See comments to reference 68.


Transplanted and mature interneurons produce a second critical period window in the visual cortex of young mice, arguing for cell-intrinsic mechanisms of critical period regulation driven by interneuron maturation.


The antidepressant fluoxetine restores plasticity in the adult visual cortex.


Anesthetics rapidly promote dendritic spine plasticity in the adult visual cortex.


McCurry, C. L. et al. Unique contributions of distinct cholinergic projections to motor cortical plasticity and learning in barn owls and mice.


This study identifies the transcription factor Npas4 as a key activity-dependent regulator of inhibitory synaptogenesis onto excitatory neurons.

This study of inhibitory dendritic arbor structural plasticity in the adult mouse visual cortex.

The first live imaging study of inhibitory dendritic arbor structural plasticity in the adult mouse visual cortex.

The first live imaging study of inhibitory dendritic arbor structural plasticity in the adult mouse visual cortex.

This study of inhibitory dendritic arbor structural plasticity in the adult mouse visual cortex.

This live calcium imaging study in a mouse cortex provides a first view of spine ensemble function in situ.


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Competing interests statement
The authors declare no competing financial interests.