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.

Synapse dynamics
Excitatory synapses at spines exhibit several forms of structural plasticity regulated by activity, including changes in the size of pre- and postsynaptic complexes, and spine disappearance and appearance events.

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[1]. 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[2] and the fact that their morphologies and stabilities change over time[3]. This phenomenon is regulated by activity, and the size of spine heads correlates with synaptic strength[4], presynaptic properties[5] and the long-term stability of the synapse[6]. 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

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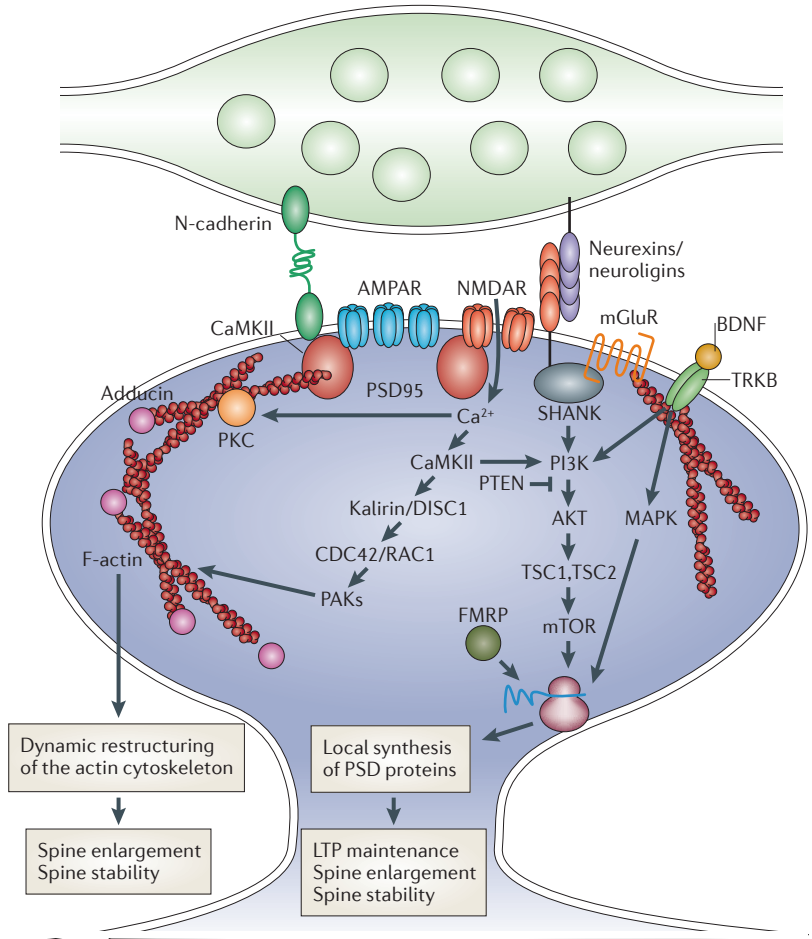

Figure 1 | **Molecular mechanisms regulating activity-mediated stabilization of dendritic spines.** Induction of synaptic plasticity at individual synapses is associated with a rapid enlargement of the spine head, an increase in synaptic efficacy and a switch in the stability of the synapse that could make them persistent. Recent findings implicate an important role of protein kinases (such as PKC (protein kinase C) and CaMKII (calcium/calmodulin 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[7]. Later, two-photon glutamate uncaging and imaging experiments demonstrated a close association between increased synaptic strength and an enlargement of the spine head[4]. 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[8]. It could also result from the mobilization of subcellular resources to potentiated synapses, such as ribosomes or additional cytoskeleton-associated proteins[9]. In addition, this restructuring could be part of a more global set of changes that promote the stabilization of the synapse[10]. 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*[6]. 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[11,12]. 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[13]. 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[14]. 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 (FIG. 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/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC) have been directly implicated in LTP maintenance and behavioural learning[15,16]. CaMKII activity is required for activity-mediated spine enlargement[17], and PKC contributes to *in vivo* spine stabilization[18]. 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)

Spine dynamics
The spines of excitatory synapses exhibit structural plasticity, including changes in shape and size and spine disappearance and appearance events.

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 enlargement[19–23], whereas *in vivo* blockade of protein synthesis results in synapse destabilization[18]. 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 size[23–25]. Furthermore, phosphorylation of the cytoskeleton-stabilizing protein β-adducin through PKC is required for the stabilization of populations of synapses induced by environmental enrichment[18]. 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 enlarge[26,27]. 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 stability[28,29]. Several adhesion molecule systems have also been linked to spine stability, including neuroligin 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 stimulation[31]. 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 complexes[32]. 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 experience[1]. The magnitude of this turnover process varies strongly during development, decreasing significantly in adult brain[6,33,34], but a substantial capacity for circuit rewiring is maintained throughout life and can be reactivated by lesions[1]. 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 experience[33]. Whereas initial *in vitro* experiments mainly focused on spine growth and synapse formation in response to neuronal activation[34–36], more recent experiments have shown that activity also destabilizes existing synapses[10,35]. 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 skills[11,12]. Spine elimination and formation caused by fear conditioning and extinction, respectively, occur in a cue- and location-specific manner[14]. Similarly, a major correlate of environmental enrichment is a marked increase in synapse remodelling, including synapse formation and destabilization[18].

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 spines[10,37]. 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 spines[38]. 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 other[39]. 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 density[40]. 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 density[11,12]. By contrast, the enriched environment protocols greatly promoted spine growth, leading to an increase in the absolute numbers of spines[18]. 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 plasticity[41]. 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 development[42,43]. 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 vivo*[44] 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 gain[45].

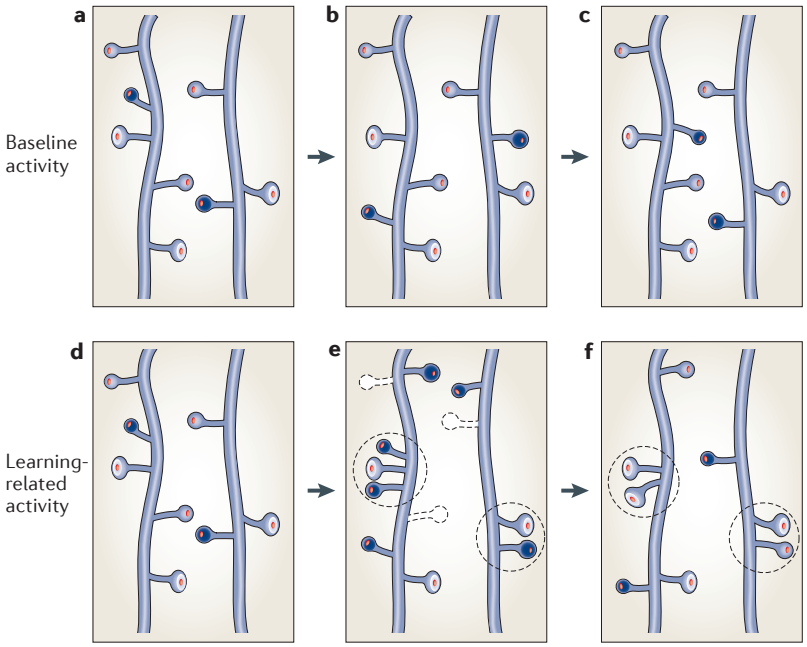

Figure 2 | **Learning-induced structural rewiring of synaptic networks. a–c** | Schematic showing a characteristic spine turnover sequence under baseline activity conditions, which includes both loss of existing spines and gain of new ones, and affects a small subpopulation of transient spines (small dark spines), leaving a larger population of more stable, persistent spines unaffected. **d–f** | Under conditions of behavioural learning, this turnover is markedly enhanced, leading to the formation of additional new spines (small dark spines), and the elimination of pre-existing spines (dashed spines). Although connectivity is modified, spine density can remain unchanged. The new spines formed following learning tend to occur in clusters (encircled areas) and exhibit a higher probability to become stabilized as persistent spines, introducing a lasting modification of the synaptic network.

Several additional molecular mechanisms have also been reported to modify spine numbers and dynamics. Oestrogens, for example, can rapidly shift the balance of spine turnover towards increased growth and stabilization, thus leading to an increase in spine density in the hippocampus[46,47]. The effect is reversible and probably accounts for the variations in spine density reported during the oestrous cycle. Brain-derived nerve growth factor (BDNF) also affects spine formation mechanisms by enhancing both destabilization of spines and spine formation in the cortex and hippocampus, and could thus contribute to some of the activity-dependent regulations of synapse dynamics[48,49]. The mechanisms through which BDNF influences spine growth are as yet unclear, but could be linked to a regulation of protein synthesis. Thus, PI3K, which interacts with AKT and has functional links with mTOR signalling, also regulates spinogenesis[50]. Furthermore, protein synthesis, mTOR signalling and spine turnover are affected in fragile X mental retardation protein (FMRP) knockout mice, a mouse model of fragile X syndrome[51]. A further group of molecular mechanisms affecting spine growth includes proteins implicated in the regulation of the cytoskeleton, such as Rho GTPases and their regulatory proteins. The extent to which some of these factors can diffuse locally could account for the mechanisms of clustered spinogenesis[52,53]. Notably, RAS, which is activated by LTP induction, has been shown to diffuse locally and promote plasticity in neighbouring spines[37]. Through its activation of the MAPK pathway and its effects on protein synthesis, it could also locally modulate spine growth. Although substantial progress has been made recently, more work will be needed in order to better understand how precisely these molecular mechanisms control spine turnover.

Distribution of the structural plasticity

Circuit rearrangements can be confined to the neurons involved in the particular learning process, or to neuronal subpopulations within systems involved in the learning process. However, under different circumstances, structural rearrangements can also be induced in a broad range of systems in the brain (for example, upon environmental enrichment, see below). An issue that arises is whether the differences in plasticity distribution reflect different roles of structural plasticity or whether a common logic may underlie these distinct phenomena. In this context, it is useful to take into account that synapse gains and losses related to a particular learning process are mostly specified subsequent to the initial learning event. Accordingly, if memory consolidation upon learning involves the selective stabilization and strengthening of some synapses combined with the weakening and loss of other synapses, the different spatial scales of the structural plasticity may involve the distinction between the potential substrates of memory consolidation, which may be distributed locally or broadly, and the actual substrates of the consolidation, which may be specifically associated with the neurons involved in the particular learning process. Consequently, two crucial issues concern the specificity of the structural changes at the local level and whether more global structural alterations may serve as potential substrates for specific local modifications.

Plasticity within local microcircuits. A remarkable aspect of the recent studies relating learning to changes in dendritic spines and axon terminals is that the structural plasticity could be detected readily using sparse labelling approaches *in vivo*, provided that cortical areas relevant to the particular form of learning were analysed repeatedly during an appropriate time window. One might expect that changes in synapse numbers that correlate with new learning may only affect a very small fraction of the synapses within a relevant network, and for that reason methods that only sample 0.1–1% of the neurons of a given kind[1,6,35] may not be adequate to detect such changes. The dramatic detection sensitivity of these structural plasticity studies is probably owing to the fact that these experiments have involved longitudinal analysis of the same large ensembles of synaptic structures, an approach that is far superior to comparisons of synapse groups, which tend to underestimate the extent of the structural plasticity. In addition, the detection of structural changes was probably facilitated by the fact that behavioural learning initially increases the dynamics of a fraction of spine synapses that is larger than the fraction ultimately retained as a structural trace

Fragile X syndrome
X-linked syndrome caused by triplet repeat expansions (CGG) resulting in reduced expression of *FMR1* (fragile X mental retardation 1). The mutations are the most common single-gene cause of autism and intellectual disability.

Memory consolidation
The processes through which memory traces become long lasting. Synaptic consolidation mechanisms include protein synthesis-dependent long-term potentiation and structural plasticity.

of learning[11,12,40]. 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[54]. 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[54]. 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[55,56].

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[11,12,40]. 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[40]. 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*[57] (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[14]. 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[14]. 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[38].

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)[58]. 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[58]. 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[18,46,47]. 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)[59]. Synapse dynamics and numbers are further influenced by seasonal changes and developmental age[60]. For environmental enrichment, the increased synapse turnover has been causally related to improved learning[18]. 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

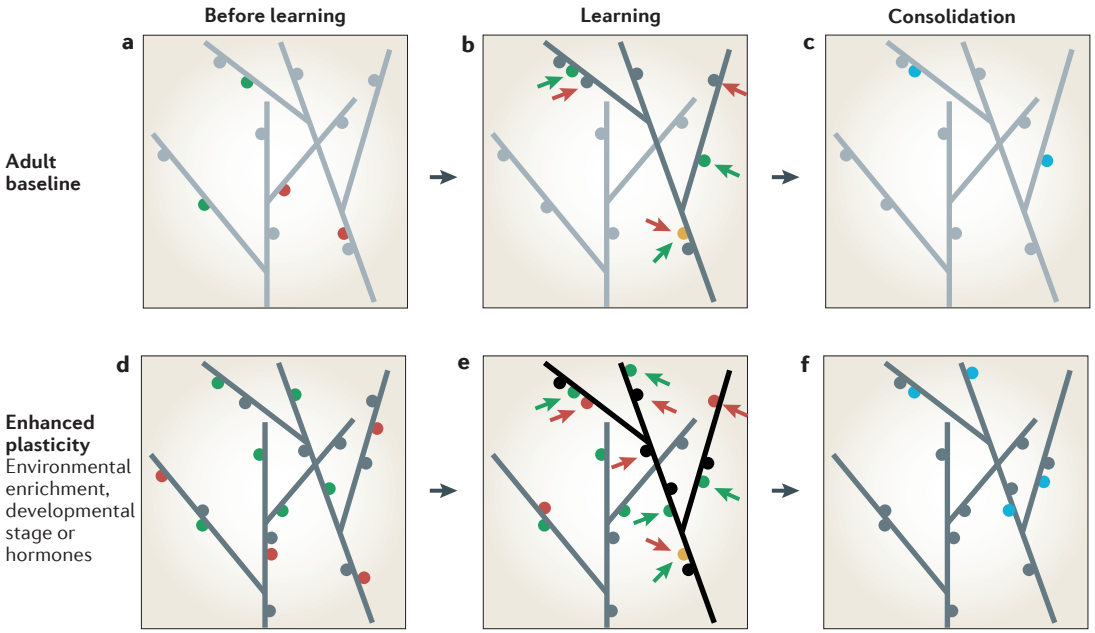

Figure 3 | **Global and local synapse turnover regulation processes affecting learning and memory.** The schematics represent dendrites of two excitatory neurons and their spine synapses. Increasing plasticity is represented as darker grey tones. Dynamic spines are green (gains) and red (losses); spine 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. **a**–**c** | Learning-induced structural plasticity enhances the turnover of subpopulations of new and pre-existing synapses specifically in excitatory neurons involved in the learning (**a** versus **b**), and leads to the selective stabilization of some learning-induced spines (**c**). **d**–**f** | Enhanced baseline levels of synapse turnover as a consequence of enrichment, developmental stage or hormones (**d** versus **a**) may augment the magnitude of learning-induced spine gains and losses (**e** versus **b**), and may lead to more robust structural traces of learning (**f** versus **c**). The enhanced structural plasticity baseline levels underlie improved behavioural learning upon enrichment[18], and improved song learning in the presence of a tutor during zebra finch development[13].

discussed in previous sections, LTP and learning are accompanied by enhanced rates of synapse assembly and disassembly events[10,61]. 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[13,44,59,62,63]. 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[11–13,63]. 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[13]. 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[13]. Furthermore, enhanced learning upon environmental enrichment was dependent on increased gains and losses of synapses[18]. These were, in part, provided by the population of additional dynamic synapses that were induced upon enrichment[18]. Similar principles seem to apply to the increase in labile synapses induced by oestrogen[46,47]. 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[18], 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).

Critical period
A developmental period of enhanced plasticity during early postnatal life whose opening and closing is regulated by experience. Learning during critical periods can leave long-lasting structural traces that influence adult learning.

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[41,42,64–66]. Whereas most of the studies have investigated plasticity to adjust for malformations such as strabism or monocular 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[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[64,65,67–69]. 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[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^+) fast-spiking interneurons, in opening and closing plasticity windows[41,66,70]. 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.

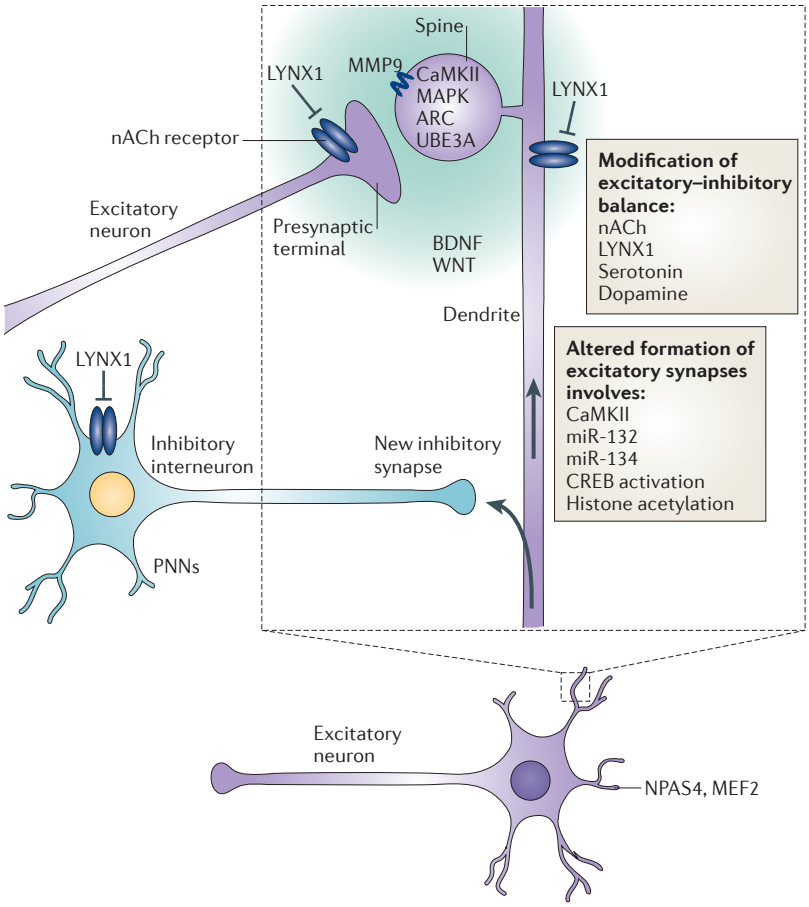

Figure 4 | **Mechanisms of structural metaplasticity regulation.** The capacity for structural plasticity can be regulated at various levels. Alterations in expression of certain genes in target neurons (shown in purple) and their transport into dendrites and to synapses (straight arrow) results in structural plasticity by mechanisms that may enhance the formation of excitatory synapses (such as calcium/calmodulin kinases (CaMKs), miR-132, 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)).

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[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 *HOMER1*, and the neurotrophin *BDNF*, which augments inhibitory synapse numbers[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[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[48,49]. Intracellular signalling molecules and pathways relating excitation and BDNF signalling to plasticity include: ARC, MAPK, CaMK, CREB (cAMP response element-binding) activation, histone acetylation and the microRNA miR-132 (REFS 19, 75–82). Mechanisms through which age influences plasticity regulation can involve chromatin remodelling pathways[81]. Extracellular factors that facilitate plasticity include the proteases matrix metalloprotease 9 and urokinase-type plasminogen activator[83]. In addition, WNT signalling can enhance synapse numbers[84]. Further important signalling molecules with a major role in regulating plasticity include the neuromodulators acetylcholine, noradrenaline, serotonin and dopamine. Among them, a particularly strong case has been made for a link between nicotinic cholinergic transmission and enhanced plasticity. Thus, cholinergic transmission is

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[85–87].

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[41,88–90]. Several lines of evidence have directly related reduced inhibition to enhanced plasticity during critical periods and in the adult in rodents[41,62,90].

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[62]. 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[43]. An important transcriptional 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[91]. 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[92].

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[93] 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[55,56]. 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[97]. 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 deprivation[98]. The second study reported a loss of excitatory inputs onto inhibitory neurons in layer 2/3 upon visual deprivation[99]. 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' (disinhibiting) 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[100]. 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

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.

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[101], whereas other studies have provided evidence for delays of 12–18 hours[102]. 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.

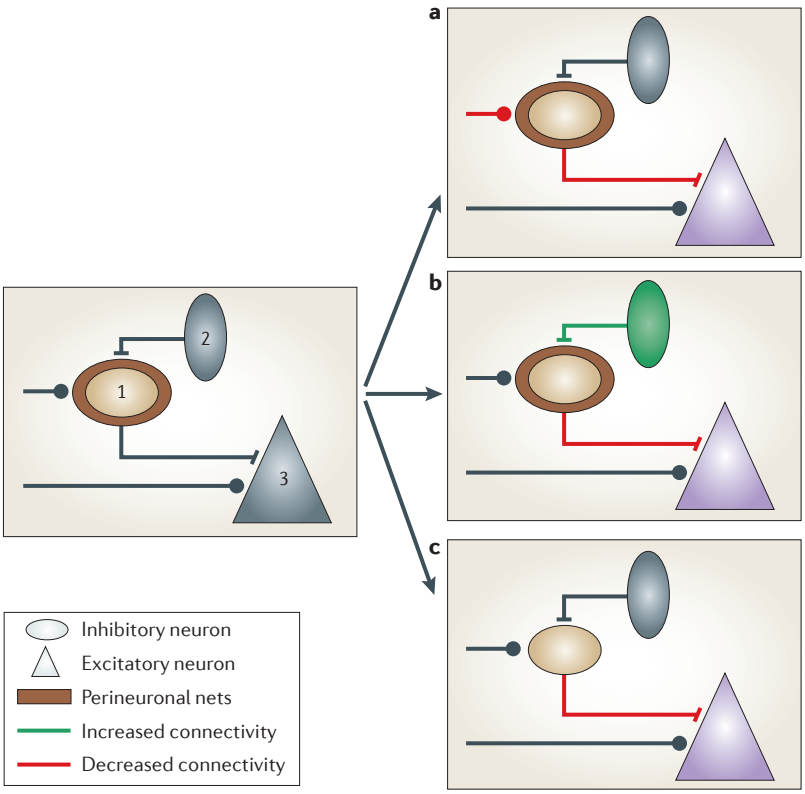

Figure 5 | **Circuit mechanisms of plasticity regulation.** Left: schematic representing a local circuit arrangement involving two inhibitory neurons (ovals 1 and 2, perisomatic and disinhibiting, respectively) impinging onto one excitatory cell (triangle 3). Circles: excitatory inputs; bars: inhibitory 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.

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. FMRP, 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, kalirin, 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 neuroligin–neurexin complex appear to be important for regulating the balance between excitation and inhibition and could therefore interfere with spine formation and dynamics[103]. 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[104], and this is associated with important alterations of spine dynamics[105]. 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[106,107]. 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

Table 1 | **Synaptic proteins with genetic defects that have been associated with developmental psychiatric disorders**

Protein	Function	Synaptic contribution	Disease
ARHGEF6	RAC GEF, regulation of actin cytoskeleton	Synapse formation and maturation	Intellectual disability
CYFIP1	Protein synthesis	Unknown	Fragile X syndrome
DISC1	Scaffold protein	Synapse formation and maturation	Schizophrenia
EPAC2	RAP GEF	Spine maturation	ASD
ERBB4	Receptor tyrosine kinase	Regulation of excitatory transmission	Schizophrenia
FMRP	Protein synthesis	Synapse stabilization	Fragile X syndrome
GABRB3, GABRA5, GABRG3	GABA receptor subunits	Excitation–inhibition balance	ASD
IL1RAPL1	Scaffold protein	Synapse formation	Intellectual disability
Kalirin	RAC GEF, regulation of actin cytoskeleton	Synapse formation and maturation	Schizophrenia, ASD
LIMK1	Protein kinase, actin skeleton	Spine maturation	Williams syndrome, intellectual disability
MINT2	Presynaptic adaptor protein	Neurosecretion	ASD, schizophrenia
Neuregulin 1	Trans-synaptic modulator of ERBB4	Regulation of excitatory transmission	Schizophrenia
Neurexin 1	Presynaptic adhesion molecule	Synapse stabilization	ASD
Neuroligin 3, neuroligin 4	Adhesion molecules	Synapse stabilization	ASD
Oligophrenin 1	RhoA GAP, regulation of receptor trafficking	Spine maturation	Intellectual disability
PAK3	Protein kinase, actin cytoskeleton	Synapse formation and stabilization	Intellectual disability
Protocadherins	Adhesion molecules	Unknown	ASD
PSD95	Scaffold protein	Synapse plasticity and stabilization	ASD, schizophrenia
PTEN	Tyrosine phosphatase, protein synthesis	Synapse stabilization	ASD, macrocephaly
RSK2	Protein kinase	Neurosecretion	Intellectual disability
SAP97	Scaffold protein	PSD protein trafficking	ASD, schizophrenia
SHANK2, SHANK3	Scaffold protein	Synapse stabilization	ASD
srGAP3	RAC1 GAP	Unknown	Intellectual disability
SSCAM (also known as MAGi2)	Scaffold protein	Receptor trafficking	Intellectual disability
SynGAP	RAS/RAP/RAC-GAP	Receptor trafficking and actin cytoskeleton	ASD, intellectual disability
TSC1, TSC2	Protein synthesis	Synapse stabilization	Intellectual disability
UBE3A	Protein degradation	Synapse formation	Angelman syndrome, intellectual disability

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[72,103,114,115]. 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; PAK3, 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.

spine growth and defects in activity-mediated spine stabilization[53]. 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[55,98,108]. Synapse-restructuring-associated growth and pruning correlates with functional changes recapitulating the structural plasticity seen in early development.

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[57], 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

Microcircuit
The minimal number of interacting defined neurons that can collectively produce a particular functional output. The term implies local computations, and usually distinguishes locally interconnected neurons (for example, within the hippocampus or within its dentate gyrus) from the long-range projections that interconnect brain regions.

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*[109–111]. 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[112,113]. 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.

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The authors declare no competing financial interests.