

Experience-dependent structural synaptic plasticity in the mammalian brain

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Abstract | Synaptic plasticity in adult neural circuits may involve the strengthening or weakening of existing synapses as well as structural plasticity, including synapse formation and elimination. Indeed, long-term *in vivo* imaging studies are beginning to reveal the structural dynamics of neocortical neurons in the normal and injured adult brain. Although the overall cell-specific morphology of axons and dendrites, as well as of a subpopulation of small synaptic structures, are remarkably stable, there is increasing evidence that experience-dependent plasticity of specific circuits in the somatosensory and visual cortex involves cell type-specific structural plasticity: some boutons and dendritic spines appear and disappear, accompanied by synapse formation and elimination, respectively. This Review focuses on recent evidence for such structural forms of synaptic plasticity in the mammalian cortex and outlines open questions.

Neural circuits are defined by the structure of axons and dendrites and the synapses that connect them. Axons route a neuron's output to diverse target regions, which can span most of the brain. Individual dendrites integrate inputs from several sources over hundreds of micrometers. In the adult brain, circuit changes mediated by structural plasticity, accompanied by synapse formation and elimination, are thought to underlie aspects of long-term memory formation¹. Given that in most areas of the brain, including the cerebral cortex, neurons are sparsely connected, structural plasticity could provide a substantial boost in the memory storage capacity, compared with plasticity due to changes in synaptic strength alone². Structural rearrangements over long distances allows more variability and therefore a larger number of potential circuits to be generated, implying a larger memory capacity per synapse. Structural plasticity might also be involved in recovery from brain injury^{3–5}.

Neuronal processes are studded with a high density of synapses. A synapse is typically defined by the presence of a presynaptic active zone with synaptic vesicles, a well-defined synaptic cleft and a postsynaptic density (PSD)^{6,7}. Most excitatory cortical synapses occur at contacts between axonal *en passant* boutons and dendritic spines. *En passant* boutons are small axonal varicosities that typically contain one active zone and one cluster of

synaptic vesicles⁸. A subpopulation of axons, for example those of cortical layer 6 pyramidal cells, harbours a high density of *terminaux* boutons that often form synapses with dendritic shafts⁹. Spines are tiny protrusions that emanate from the dendritic shaft¹⁰. Typically one bouton and one spine correspond to one synapse^{6,8}. A small number of boutons⁸ and spines^{11–14} lack synapses and a few boutons participate in more than one synapse (multiple synapse boutons, MSBs)^{8,14–17}. Electron microscopy (EM) reconstructions have revealed that spines are structurally extremely diverse: their volumes can range from 0.001 to 1 μm^3 ^{18,19}; their shapes include thin, filopodia-like protrusions ('thin spines'), short spines without a well-defined spine neck ('stubby spines') and spines with a large bulbous head ('mushroom spines')^{18,20}. Different cortical cells, and perhaps even individual cells within a class, can have dramatically different spine densities^{21–23}.

Changes in synaptic connectivity through the *de novo* growth and retraction of dendritic spines and axonal boutons might contribute to functional changes in the brain. Compared with synaptic strength changes alone, such structural spine plasticity would hugely increase the memory storage capacity of the brain, because a large number of synaptic connectivity patterns are attainable by spine or bouton growth, even without large-scale remodelling of dendritic and axonal

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arbors^{2,24–26}. Consistent with this notion, in cortical neuronal cultures that are still undergoing developmental circuitry changes, spines appear and disappear over tens of minutes^{27–29}, a process that can be triggered by synaptic activity^{29–34}. Furthermore, *de novo* spine growth has been linked to synapse formation in cultured preparations^{32,35–37}. Axonal boutons also grow, retract and remodel in an activity-dependent manner *in vitro*, but much less is known about this process^{38,39}.

Are these forms of structural plasticity ongoing in the adult brain? To what extent does structural plasticity contribute to experience-dependent rewiring *in vivo*? Answers to these questions will shed light on the storage capacity of neural circuits and also on the cellular and molecular mechanisms underlying learning and memory. Here we review recent work on structural plasticity in the adult mammalian brain, focusing on longitudinal imaging experiments in the mouse neocortex (see [Supplementary information S1](#) (table) for a summary of results) that used two-photon excitation laser scanning microscopy^{40–42} of neurons expressing fluorescent proteins^{39,43,44} (see [Supplementary information S2](#) (box))

The dynamics of axonal and dendritic arbors

Most studies of large-scale structural plasticity so far have relied on static measurements and comparisons between neurons in different animals. They suggest that neurons can undergo large-scale structural changes after relatively drastic long-term manipulations, leading to the conclusion that synapses are eliminated or formed with changes in connectivity. More recent studies have used long-term time-lapse imaging to probe structural dynamics in more detail and have yielded a different picture.

Axonal dynamics. Evidence for axonal growth in the adult brain has come mostly from experiments involving focal brain injury or lesions of the sensory periphery. For example, in squirrel monkeys ischaemic injury to the primary motor cortex induces axonal sprouting of premotor cortical projections near the infarct site⁴. Inducing epilepsy in rats leads to axonal reorganizations in the hippocampus⁴⁵. Limb amputation in macaques causes growth of intracortical axons across several millimeters in the somatosensory cortex⁴⁶, a phenomenon that may underlie functional reorganization of cortical maps⁴⁷ and phantom limb syndrome⁴⁸. Similar rewiring is observed in the cat visual cortex after focal retinal lesions⁴⁹ and in the rat barrel cortex after partial vibrissotomy⁵⁰. Although these experiments reveal that cortical axons maintain the capacity to grow and branch in the adult neocortex, their relevance to experience-dependent plasticity is unclear. However, in the hippocampus there are indications that axonal sprouting occurs during spatial learning^{51,52} or in response to environmental enrichment⁵³.

Long-term imaging experiments have shown that in naive adult mice a subset of axonal branches in the cortex can undergo structural rearrangements over lengths of tens of micrometers over several days⁵⁴. As

these retractions and elongations are associated with turnover of numerous boutons, they are likely to be associated with synapse formation and elimination⁵⁴. Similarly, in the cerebellum a subset of olivocerebellar climbing fibre branches are dynamic, whereas other branches of the same neurons are stable⁵⁵. Overall, these changes are modest, amounting to a few percent of axonal length within the terminal arbor ([Supplementary information S1](#) (table)). The large-scale organization of axonal arbors in the mouse^{54,56} and macaque⁵⁷ neocortex are therefore relatively stable. Similar stability has been observed in the terminal arborizations of the parasympathetic submandibular ganglion⁵⁸; this stability stands in dramatic contrast to the rapid and large-scale rearrangements that occur in the developing cortex^{59,60}. Long-term imaging studies of axons in response to behavioural training have not been reported so far.

Dendritic plasticity. Classic studies of dendritic plasticity, mostly in rats, have relied on the Golgi method. Curiously, it is still not known which neurons are labelled by this method and how complete the labelling is. Such experiments have revealed that environmental enrichment⁶¹, extensive training⁶², stress levels⁶³ and drugs of abuse^{64,65} all might have profound influences on the complexity of dendritic arbors in some cortical areas, but not in others. Large-scale reorganization of dendritic arbors has also been observed in the rat sensorimotor cortex after damage to the contralateral homotopic cortex⁶⁶, and in the somatosensory cortex after vibrissal deafferentation⁶⁷ and forepaw denervation⁶⁸. Training-induced changes in dendrites seem to be more subtle⁶⁹. As these static measurements are only sensitive to robust changes in morphometric parameters, the studies might underestimate the actual changes in arbors in response to manipulations.

In contrast to the Golgi method, long-term imaging experiments in mice have shown that the large-scale organization of dendritic arbors is relatively stable ([Supplementary information S1](#) (table)). The dendritic arborization of cortical layer 5 (REF. 13) and layer 2/3 pyramidal neurons^{22,70,71}, and of mitral/tufted cells in the olfactory bulb⁷², remains unchanged over several months, even in response to learning⁷² and after enrichment²². Small length changes (a few micrometers) can be detected at the branch tips of pyramidal neurons (A. H. and K. S., unpublished observations). A subset of inhibitory dendritic branches show a larger degree of structural plasticity (~10 μm over 4 weeks), but the fractional change of the entire dendritic arbor is very small^{70,73}, in the order of 1–5 % of the overall dendritic length. Apart from the growth of dendritic trees of adult-born neurons^{74,75}, complete retractions or *de novo* growth of dendritic branches are extremely rare under any of the conditions studied in wild-type mice.

Thus, results from static and longitudinal experiments seem to be at odds. The discrepancies might be due to differences in the imaged cell types, brain region, animal species or the experimental manipulations. The capriciousness of the Golgi method is

Golgi method

A method that is used to label a sparse subset of neurons in fixed tissue using potassium dichromate and silver nitrate; neurons are stained by microcrystallization of silver chromate. The labelling seems stochastic, but the mechanisms underlying sparse labelling remain unknown.

another complication: neurons with different average dendritic complexities could be selectively labelled under particular experimental conditions, leading to the reporting of spurious structural plasticity. It is therefore of considerable interest to probe the effects of enrichment, training, stress, drugs and lesions on dendrite structure using genetic labelling methods⁷⁶ in combination with long-term imaging.

The dynamics of synaptic structures

Even in the absence of large-scale remodelling of dendritic and axonal arbors, changes in synaptic connectivity through the *de novo* growth and elimination of boutons and dendritic spines may contribute to functional rewiring. Numerous studies have examined cortical tissue by EM for synaptic changes after behavioural enrichment, learning or long-term potentiation (LTP) induction¹. These studies have provided evidence for synaptogenesis in the adult brain after

behavioural enrichment⁷⁷ or prolonged sensory stimulation *in vivo*¹⁷. More recently, several groups have directly observed the dynamics of synaptic structures in the intact mouse neocortex (FIG. 1, [Supplementary information S1](#) (table)).

Turnover of synaptic structures. Diverse types of axonal arbors, arising from cortical and subcortical sources, are intermingled in superficial layers of the neocortex. The dynamics of boutons on three types of axons have been compared in the mouse somatosensory cortex under baseline conditions (that is, in mice housed with same sex littermates in standard laboratory cages)⁵⁴. For all axon types, a subpopulation of boutons appear and disappear, while bouton densities remain stable ([Supplementary information S1](#) (table)). Boutons on thalamocortical axons are remarkably stable (FIG. 1a), with a large fraction persisting for 9 months or more. *En passant* boutons on intracortical layer 2/3 and layer 5

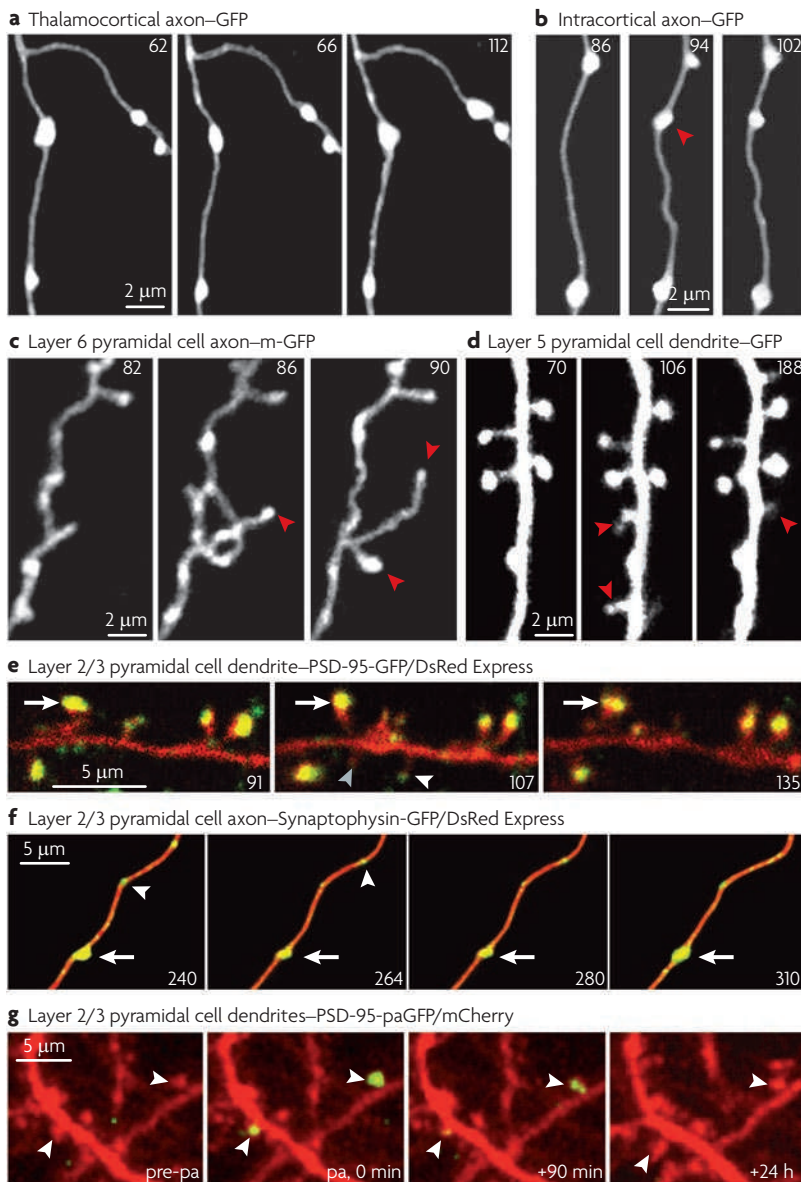


Figure 1 | *In vivo* time-lapse imaging of axonal boutons and dendritic spines. All panels show time-lapse images of axonal boutons (panels a–c, and f) or dendritic spines (panels d, e and g) in adult (panels a–f) or juvenile (postnatal day 17, panel g) mice. Images are from layer 1 or layer 2 in the barrel cortex under baseline conditions, except panel d (after chessboard whisker trimming). The numbers in the corners of the panels indicate the age (postnatal day) on which the image was taken. Neurons were labelled with green fluorescent protein (GFP) in Thy1 transgenic mice^{39,43} (a–d) or with multiple fluorescent proteins using *in utero* electroporation (e–g). **a** | Thalamocortical axon showing high levels of stability. **b** | Intracortical axon with stable and new (arrowhead) *en passant* boutons. **c** | Axon from a layer 6 neuron labelled with membrane-bound green fluorescent protein (m-GFP), with dynamic *terminaux* boutons (arrow heads). **d** | Dendritic spines on a layer 5B pyramidal cell dendrite. Some spines appear and disappear (arrowheads), whereas others are stable over long time periods. **e** | Dendrite of a layer 2/3 pyramidal neuron, transfected with postsynaptic density protein 95 (PSD-95) fused with GFP and dsRed Express. Most of big spines with PSD-95 are stable (see arrows). Some thin spines appear transiently, either with (white arrowhead) or without (grey arrowhead) PSD-95-GFP. **f** | Axon of a layer 2/3 pyramidal neuron transfected with synaptophysin-GFP and dsRed Express. Synaptophysin-GFP labels stable axonal boutons (arrows) and small transient varicosities (arrowhead). **g** | Measuring protein trafficking in individual dendritic spines *in vivo*. Layer 2/3 neurons were transfected with PSD-95 tagged with photo-activatable GFP (paGFP) and mCherry (red). paGFP is non-fluorescent under baseline conditions. paGFP was photo-activated in two spines (arrowheads) by brief two-photon excitation at $\lambda = 810\text{nm}$. The dissipation of fluorescence as a function of time can be used to measure protein turnover. Parts a, b and c are reproduced, with permission, from REF. 54 © (2006) Cell Press. Part d is reproduced, with permission, from REF. 182 © (2008) Elsevier. Images in part f are courtesy of V. De Paola and R. Weimer. Part g is reproduced, with permission, from REF. 44 © (2006) Public Library of Science.

Critical period

The developmental age when an animal displays a heightened sensitivity to certain environmental stimuli, such as sensory experiences, which impact (often irreversibly) the development of neural circuits.

pyramidal cell axons are more plastic, with a turnover of 20% over one month of imaging (FIG. 1b). These numbers are in quantitative agreement with studies of the same axon types in the macaque visual cortex⁵⁷ and with a study in the mouse visual cortex, although in the latter the identity of the imaged axons was not analysed⁵⁶. The turnover of *en passant* boutons is lower than dendritic spines, suggesting that spine growth and that of *en passant* boutons are not tightly coupled^{54,56}. Axons from layer 6 pyramidal cells are rich in small *terminaux* boutons. *Terminaux* boutons appear and disappear at higher rates, with more than 50% turnover in a month (FIG. 1c). Thus the extent of bouton plasticity depends on the cell type. All reported real-time measurements *in vivo* so far have come from mice that were studied under baseline conditions. Experiments using plasticity paradigms have not yet been reported.

Several groups have imaged dendritic spines in the developing^{22,78,79} and adult neocortex *in vivo*^{5,13,22,56,80–86} (Supplementary information S1 (table)). During the second week of life, spines appear and disappear at a rapid rate⁷⁸. Spine densities in the mouse neocortex were found to increase during the second and third week of life, followed by a period of net spine pruning^{22,79}. This corroborates former findings in the developing primate and rodent cortex that synapse densities increase rapidly during a relatively brief postnatal period, followed by a protracted period of net synapse elimination^{87,88} (but see REFS 89,90). As the brain matures, spine turnover decreases; this trend continues at least up to the sixth month of a rodent's life, long after the closure of all known critical periods^{13,22,56,79,80}.

In adult mice, a subpopulation of spines continues to turn over (FIG. 1d). Some spines appear and disappear over days, whereas others persist for months, perhaps even for the life of the animals^{13,22,56,79,80}. The

reported fractions of persistent, appearing and disappearing spines differ considerably between studies (see BOX 1, Supplementary information S1 (table) and Supplementary information S3 (box)) The consensus is that spines are less persistent in young adults (65–85% over 1 month) than in mature adults (75–95% over 1 month), and that the majority of spines persist over long periods, at least under baseline laboratory conditions.

Spine turnover has been measured in various cell types (layer 5B and layer 2/3 pyramidal cells) and cortical areas (somatosensory, motor, auditory, visual and frontal)^{13,22,56,79,80,83}. Like bouton turnover, the rate of spine turnover is cell type-dependent and might be brain region-specific. For example, apical dendrites of layer 2/3 pyramidal cells show a lower fractional spine turnover rate compared with layer 5 pyramidal cells²². Some studies have reported lower spine turnover²² or spine motility⁵⁶ (see below) in the visual cortex compared with the somatosensory cortex, but other studies have failed to detect a difference^{56,79}.

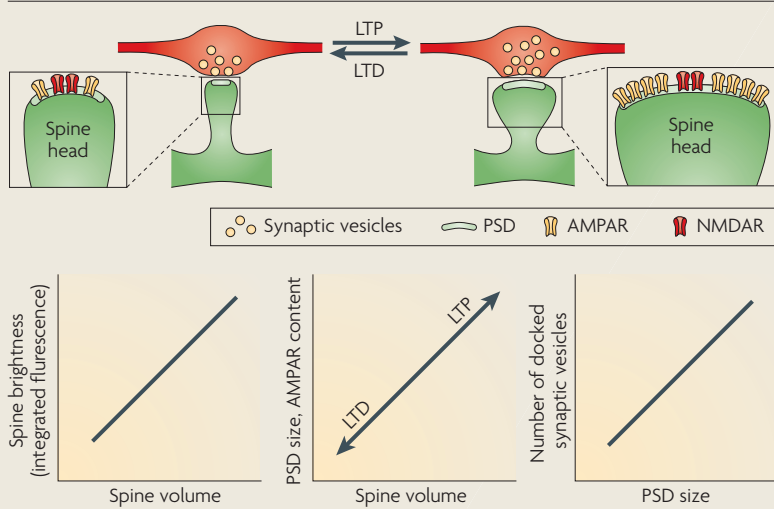
Time-lapse studies have revealed that spines have widely differing lifetimes. Under baseline conditions, spines that appear are likely to disappear again within a few days^{13,22,83}; we have called these structures 'transient' spines. Conversely, spines that persist for over a week are likely to persist for months^{22,79} (FIG. 1d); we have called these structures 'persistent' spines. Similarly, in other studies thin, filopodia-like protrusions were dynamic and had short lifetimes, whereas the majority of the big mushroom-type spines were stable^{56,79,80}. Thus, on average, there is a relationship between spine stability and spine size; transient spines are typically small, whereas persistent spines are large²². However, the correlation between structure and stability is not absolute. Small spines can persist, and large spines can disappear. In some studies spines were separated for analysis into structural categories: spines (protrusions with a well-defined head) and filopodia (thin protrusions), with the implication that only spines correspond to synapses^{56,79,80}. However, expression of cytoplasmic green fluorescent protein (GFP) does not allow segregation of spines into structural classes by imaging, let alone to assess for the presence of synapses. In addition, detailed imaging and EM studies indicate that all dendritic protrusions do not fall neatly into a limited number of recognizable structural subtypes, but rather form a structural continuum^{14,22,91,92}. Furthermore, even the thinnest protrusions often make synapses^{13,14,19,36,93} or express synaptic proteins (FIG. 1e). It might therefore be preferable to class all dendritic protrusions as spines and to analyse their dynamics as a function of objective structural parameters^{22,91}.

Spine motility. In addition to spine growth and retraction, several studies have measured rapid (seconds to minutes) spine head motility (also called twitching) as an indicator of developmental and experience-dependent synaptic plasticity^{56,81,94–96}. This work has been inspired by the finding that the actin cytoskeleton of dendritic spines is constantly rearranging⁹⁷, and the supposition that spine twitching modulates synaptic

Box 1 | Quantitative differences between imaging studies

Even after taking differences in animal age, cell type and cortical area into account, it seems that the extent of spine structural plasticity found in several recent imaging studies is still quantitatively inconsistent^{13,22,56,79,80,83,86,164,165}. At least three factors could contribute to the discrepancies¹⁶⁶. First, differences in surgical preparations could matter¹⁶⁷, but the contributions are likely to be small (Supplementary information S3 (box))¹⁶⁵. Second, spine quantification is performed manually. Although individual researchers and research groups have established consistent and rational analysis criteria, these criteria differ between groups. Some of these differences in criteria are necessitated by variations in image quality in different brain regions and in different types of transgenic mice (Supplementary information S2 (box)). Nevertheless, these differences can cause up to a twofold variation in reported spine turnover¹⁶⁶. The scoring of changes in structural plasticity, for example after sensory deprivation, is likely to be more robust as identical criteria are used before and after the manipulation, provided the analyser is blind to the experimental manipulation. Third, differences in sampling are likely to matter as well. Cell type-dependent differences in spine turnover are difficult to detect in densely labelled brains as only short dendritic segments of individual neurons can be traced (Supplementary information S2 (box)); spines must be pooled over multiple neurons and therefore turnover measurements might be biased to the lower rates that are characteristic of cells with the highest spine densities²². Furthermore, turnover rates based on the average of a modest number of single cells in sparsely labelled mice might not adequately represent spine turnover of the entire neuronal population^{22,23}. A combination of these three factors probably explains the discrepancies in reported spine turnover rates.

Box 2 | Correlates of synaptic strength



High-resolution *in vivo* imaging can be used to track the appearance and disappearance of synaptic structures. Can imaging also be used to measure the strengthening or weakening of existing synapses? There are indications that measurements of spine volume could provide an excellent indication of synaptic strength.

Spine volume is proportional to the area of the postsynaptic density (PSD)⁶, which in turn is proportional to the synaptic AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor (AMPA) content^{168–170} and to the postsynaptic sensitivity to glutamate¹¹⁰. These quantities also co-vary with presynaptic parameters that indicate the efficiency of glutamate release, including the size of the active zone and the number of docked vesicles¹⁷¹. Spine volumes increase after long-term potentiation (LTP)^{172–175}, as does the postsynaptic sensitivity to glutamate uncaging^{172,174}. Similarly, long-term depression (LTD) causes spine head shrinkage¹⁷⁶. Spine volumes can be estimated from *in vivo* images by measuring spine brightness, the fluorescent signal integrated over a dendritic spine^{22,177,178}. Changes in spine volume tracked *in vivo*^{23,179} can thus be interpreted as changes in synaptic strength (see figure).

Synaptic strength might also be tracked more directly with recombinant fluorescent synaptic proteins. For example, the AMPAR subunit GluR1 tagged with pH-sensitive green fluorescent protein (GFP) has been used to report the synaptic GluR1 content in the membrane of single spines^{173,180}. Postsynaptic density protein 95 (PSD-95) tagged with GFP can be used to measure the size of the PSD^{44,104}. Furthermore, synaptic transmission is associated with Ca²⁺ influx through glutamate receptors and voltage-gated Ca²⁺ channels. Thus, genetically encoded Ca²⁺ indicators targeted to synaptic compartments^{163,181} can directly report synaptic transmission in single synapses^{108,109}.

In summary, imaging experiments could be used to directly read out the weights of single synapses *in vivo*.

NMDAR, N-methyl-D-aspartate receptor.

function^{98,99}. Spine motility decreases with developmental age *in vitro*^{94,100} and *in vivo*, and is regulated by sensory experience^{56,78,81}. Changes in spine motility might be triggered by synaptic activity and precede more prominent morphological changes, such as spine retraction or stabilization^{56,78}.

Synapse stability. An emerging consensus is that a subpopulation of dendritic spines and axonal boutons is remarkably stable, with lifetimes on the order of the lifespan of the mouse. Even more remarkable is that the relative sizes of individual dendritic spines and boutons can be maintained for months (FIG. 1), suggesting that synaptic weights could also be stable for months

(BOX 2). By contrast, *in vitro* studies indicate that synaptic protein complexes are highly labile, with protein lifetimes of a day or two, orders of magnitude shorter than synapse lifetimes¹⁰¹. In addition, synaptic molecules (such as Ras, PSD-95, Shank3, bassoon and synaptophysin) continuously redistribute between synapses on the same neurite over minutes to hours^{44,102,103,104}. How can stable synapses exist in the context of high protein turnover either through redistribution or through unstable protein components? Answers to this question might come from experiments that track the fates of synaptic proteins *in vivo*. For example, PSD-95 is an abundant multi-domain postsynaptic scaffolding protein that clusters glutamate receptors and organizes the associated signalling complexes¹⁰⁵. PSD-95 is thought to determine the size and strength of synapses. Using two-photon photo-activation of PSD-95 tagged with photo-activatable GFP (paGFP), the trafficking of PSD-95 molecules in and out of single PSDs was measured *in vivo*⁴⁴. Synaptic PSD-95 in single PSDs *in vivo* turned over remarkably quickly (with a half-life of approximately one hour) and exchanged with PSD-95 in neighbouring spines by diffusion (FIG. 1g). Large PSDs in large spines captured more diffusing PSD-95 and also retained PSD-95 longer than small PSDs. Changes in the sizes of individual PSDs over days were associated with concomitant changes in PSD-95 retention times. In other words, the kinetic interactions between PSD-95 molecules and individual PSDs are tuned to regulate and maintain synapse size.

Spine growth and synapse formation

High-resolution optical microscopy alone typically cannot detect synapse formation and elimination. Contact of dendrite and axon is a poor predictor of synapses, as several non-synaptic contacts occur per actual synapse^{24,26}. Furthermore, as a volume corresponding to the optical point spread function often contains multiple synapses, optical overlap of fluorescent presynaptic and postsynaptic molecules does not provide proof of a synapse. Instead, detection of synapses requires retrospective analysis using EM^{13,106}, array tomography¹⁰⁷, direct imaging of synaptic proteins *in vivo* (FIG. 1e,f), or perhaps optophysiological recordings with single synapse sensitivity^{28,108–110}.

To investigate the relationship between spine growth and synapse formation, *in vivo* time-lapse imaging experiments have been followed up by serial section EM analysis of previously imaged structures^{13,14,23,54}. Both, new dendritic spines and new axonal *terminaux* boutons were found to bear synapses (FIG. 2). An analysis of the time-course of spine maturation in the adult somatosensory cortex revealed that synaptogenesis could be remarkably slow: spines that were older than four days always had a synapse, however only 30% of spines that were two days old formed a synapse (FIG. 2 b,c and FIG. 3). The other ~70% of the newly formed spines probably corresponds to the small steady-state population of spines lacking synapses^{11,12}. Consistent with the *in vivo* studies, an EM study of cultured hippocampal brain slices showed ultrastructural hallmarks of synapses only 15–19 hours after the stimulus³⁶; another study in

Optical point spread function

The point spread function (PSF) describes the response of an imaging system to a point object. In microscopy the PSF is a measure of the resolution.

Optophysiological recording

Optical microscopy-based imaging of cellular function, such as calcium imaging.

brain slices (usually derived from early postnatal brain) showed a much shorter delay (< 1 hour) between spontaneous spine growth and synapse formation¹¹¹. It is not clear if all transient spines participate in synapses at some point during their life cycle. It could even be that a subpopulation of transient spines serves completely distinct, non-synaptic functions, such as chemosensation. These studies highlight the importance of combining time-lapse imaging with ultrastructural analysis to elucidate the role of structural plasticity.

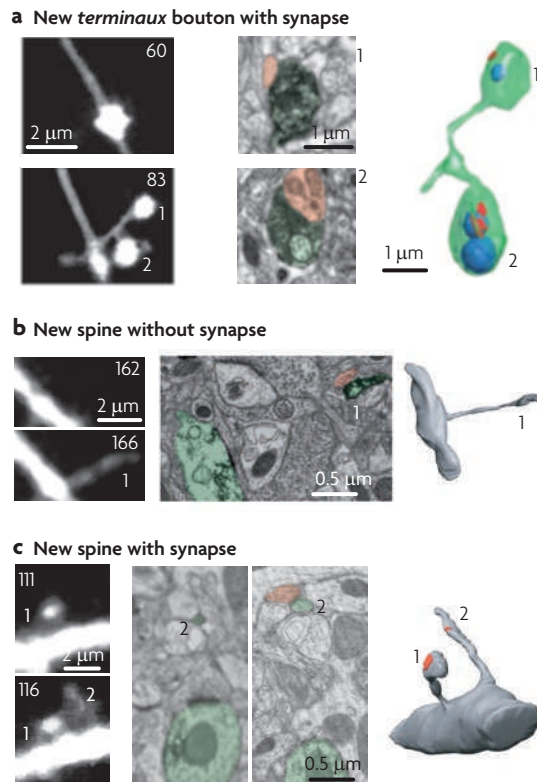


Figure 2 | Retrospective electron microscopy analysis of previously imaged neurons in vivo. **a** | New axonal terminaux boutons (numbered 1 and 2) that were imaged *in vivo* (left) were reconstructed using serial section electron microscopy (EM) (centre, right). Both new boutons (green) bear synapses, one with a dendritic spine (bouton 1) and one with a dendritic shaft (bouton 2). In the reconstruction, the axonal boutons are green, synapses are red and mitochondria are blue. **b** | A new (age < 4 days) thin spine (numbered 1, bottom left panel; green, centre panel) that was reconstructed using serial section EM (centre, right). The tip of the protrusion makes contact with an axonal bouton (pink), but there are no clear signs of a synapse. **c** | Thin new (age 1–5 days) spine (numbered 2, bottom left panel) that was reconstructed together with an older persistent spine (numbered 1, bottom left panel) using serial section EM (centre, right). The new spine (green) weaved its way through a dense neuropil to form a clear synapse with an axonal bouton (red), 2 μ m away from the parent dendrite. In the reconstruction synapses are colored red. Age (postnatal day) of mice is shown in top corner of the panels. Part **a** is reproduced, with permission, from REF. 54 © (2006) Cell Press. Parts **b** and **c** are reproduced, with permission, from REF. 14 © (2006) Macmillan Publishers Ltd. All rights reserved.

New spines preferentially form synapses with large axonal boutons that already bear a synapse, resulting in so-called MSBs^{14,36,112} (FIG. 3). In the somatosensory cortex most new spines (~65%) make synapses on MSBs, whereas ~35% make synapses with single synapse boutons, which are presumably new¹⁴. These numbers are consistent with the finding that in the cortex the turnover of *en passant* boutons is twofold lower compared with dendritic spines^{22,54,56}. Remarkably, quantitative analysis of cultured hippocampal slices³⁶ and the dentate gyrus *in vivo*¹¹² reveal similar results. These observations suggest that new spines on MSBs initiate a competition that leads to the pruning of one of the synapses at a later stage¹¹² (FIG. 3). Increases in the fraction of MSBs have also been observed after learning¹¹³ and hormonal stimulation¹¹⁴. This suggests that the formation of new synapses on MSBs is a common mechanism for adaptive plasticity in the adult brain and might help to ensure local homeostasis in neural networks.

Is spine growth deterministic or random? Does the presynaptic target site release factors that promote directed spine growth or do spines make synapses with targets they happen to encounter? These questions remain largely unanswered. It has been shown that presynaptic activity^{29–32} and glutamate^{33,115} can trigger spine growth. New protrusions seem to preferentially grow towards axonal boutons with active synapses, often ignoring other potential presynaptic elements in the direct vicinity of the dendrite^{14,36,112}. These observations suggest that glutamate may act as a trophic factor to guide new spines to active boutons. However, it is likely that numerous other cell-autonomous and secreted molecules also contribute to guide new spine growth¹¹⁶.

Experience-dependent spine growth

Functional circuits in the adult neocortex adjust to novel experience. It has long been hypothesized that spine growth and retraction could be a substrate or mechanism for experience-dependent plasticity in the developing and adult brain. In the developing cortex, spine pruning⁷⁹ or stabilization^{117,118} are driven by normal sensory experience. In the adult brain, spine and synapse densities can change upon manipulation of sensory experience¹¹⁹. Increased spine and synapse densities have been reported after rearing or training in enriched environments^{119–122,62}, and also after long-term sensory stimulation (for example, REF. 17) and deprivation (for example, REF. 79).

Long-term imaging experiments have provided further support for a role of spine growth and retraction in experience-dependent plasticity (FIG. 4). The mouse barrel cortex is a powerful model system to study experience-dependent plasticity¹²³. Trimming a subset of mystacial whiskers causes experience-dependent changes in receptive fields (FIG. 4b). For example, responses to deflection of the spared whiskers potentiate. This so-called response potentiation has several key properties¹²⁴: First, it can be detected after a day or so, but continues to progress over several weeks.

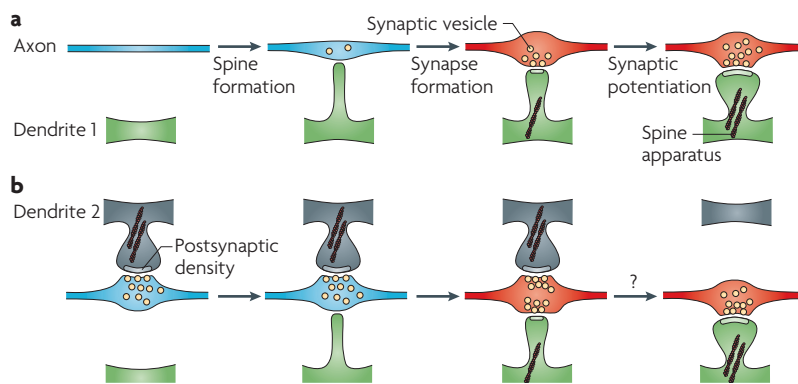


Figure 3 | Two modes of synapse formation by spine growth. a | A new spine (green) can grow towards an axon (blue) to make a synapse with a *de novo*-generated bouton (red), resulting in a single-synapse bouton. **b** | New spines often grow towards existing boutons (blue), contacting another spine (grey), resulting in multiple synapse boutons. The multi-synaptic state of the bouton could be transient. Spine maturation involves increases in spine volume and synapse formation, and might lead to the elimination of the previously existing synapse. Figure modified, with permission, from REF. 14 © (2006) Macmillan Publishers Ltd. All rights reserved.

Second, it is largest for neurons close to the interface between deprived and spared cortical barrel columns. And third, it is impaired in homozygous mice bearing a point mutation (T286A) in the calcium/calmodulin-dependent protein kinase II alpha (*CaMK2a*) gene that abolishes CaMKII autophosphorylation^{125,126}. To test the hypothesis that experience-dependent spine growth might contribute to response potentiation, a subset of whiskers were trimmed in a chessboard fashion and structural plasticity was analysed (FIG. 4a–e). Induction of plasticity was found to stabilize new spines (13–15%, compared with 5% under baseline conditions) on pyramidal neurons over 2–3 weeks after whisker trimming²³ (FIG. 4e). Growth of new persistent spines was especially pronounced close to the interface between deprived and spared barrel columns¹²⁷. Furthermore, in T286A homozygote mice, whisker trimming failed to increase the growth of new persistent spines¹²⁷. The properties of structural and functional plasticity are very similar. These findings provide strong support for a role of spine growth in response potentiation.

Does experience-dependent spine plasticity cause changes in the barrel cortex wiring diagram? In other words, do previously unconnected units become connected and vice versa? Paired recordings in brain slices indicate that connection probabilities can change dramatically in response to whisker trimming¹²⁸. Unlike changes in the strength of existing synapses, wiring changes are associated with structural plasticity and are expected to enhance the storage capacity of the brain because it provides flexibility to choose which presynaptic cells provide input to each postsynaptic cell².

Evidence for a role of spine growth in experience-dependent plasticity has also come from experiments in the adult mouse visual cortex. Monocular deprivation causes ocular dominance shifts that are accompanied by an increase in spine addition, leading to an increase (8%) in new persistent spines in the binocular

zone⁸⁴. These changes were specific to layer 5 cells, and more pronounced in structurally more complex dendritic arbors. Remarkably, the cell-type specificity of spine stabilization in the visual cortex is similar to that observed in the somatosensory cortex after whisker trimming²³. As the structural complexity of dendritic arbors is a defining feature of cell type and projection pattern¹²⁹, these observations imply circuit-specific structural plasticity.

Recent imaging experiments tracked dendritic spines in the motor cortex during motor learning. Training in a forelimb reaching task rapidly induced the growth of new dendritic spines, which were preferentially stabilized by subsequent training sessions¹³⁰. Together the experiments in the somatosensory, visual and motor cortex demonstrate that spine addition and subtraction, with synapse formation and elimination, are likely to contribute to experience-dependent rewiring of cortical circuits (FIG. 5).

The experience-dependent effects described above are small (approximately 10% change in the total complement of persistent spines)^{23,79,84,127}. However, important inputs often constitute only a minor fraction of the synapses impinging on an individual neuron. For example, thalamic synapses make up only 10–20% of the total input within layer 4 stellate-cell dendrites¹³¹, but they still dominate the activity in layer 4. Most imaging studies have been performed in the layer 1 tufts of pyramidal cells. Layer 1 synapses are made by afferents from several sources. For example, in the barrel cortex these inputs include local layer 5 and layer 2/3 axons, thalamic axons as well as feedback from the motor cortex and other cortical areas¹³². It is possible that a 10% change in synapses corresponds to a much larger fractional change in connectivity between specific cell types.

What is the potential of spine plasticity in the adult brain? Answers to this question are beginning to come from the response of the visual cortex to small retinal lesions (FIG. 4f–j)⁸³. Immediately after the lesion, the corresponding projection zone in the cortex temporarily loses function. Over one to two months this region is invaded by neighbouring areas of the visual field (Fig. 4g). In parallel with the functional recovery, neurons in the centre of the lesion projection zone replace almost their entire complement of dendritic spines (FIG. 4h–j). These data provide compelling evidence for a role of spine growth and retraction in functional cortical rewiring in response to small peripheral lesions. They also show that the potential for plasticity by spine growth and retraction is very large. Support for a large potential for structural plasticity in the adult brain also comes from data showing that deletion of the phosphatase and tensin homolog (*Pten*) tumour suppressor gene triggers massive growth of dendrites and spines in pyramidal neurons in adult mice⁷¹. These results suggest that growth-related signal transduction pathways control neuronal morphogenesis in the adult brain. However, it remains to be determined if these pathways can be modulated under physiological conditions to allow large scale dendritic growth.

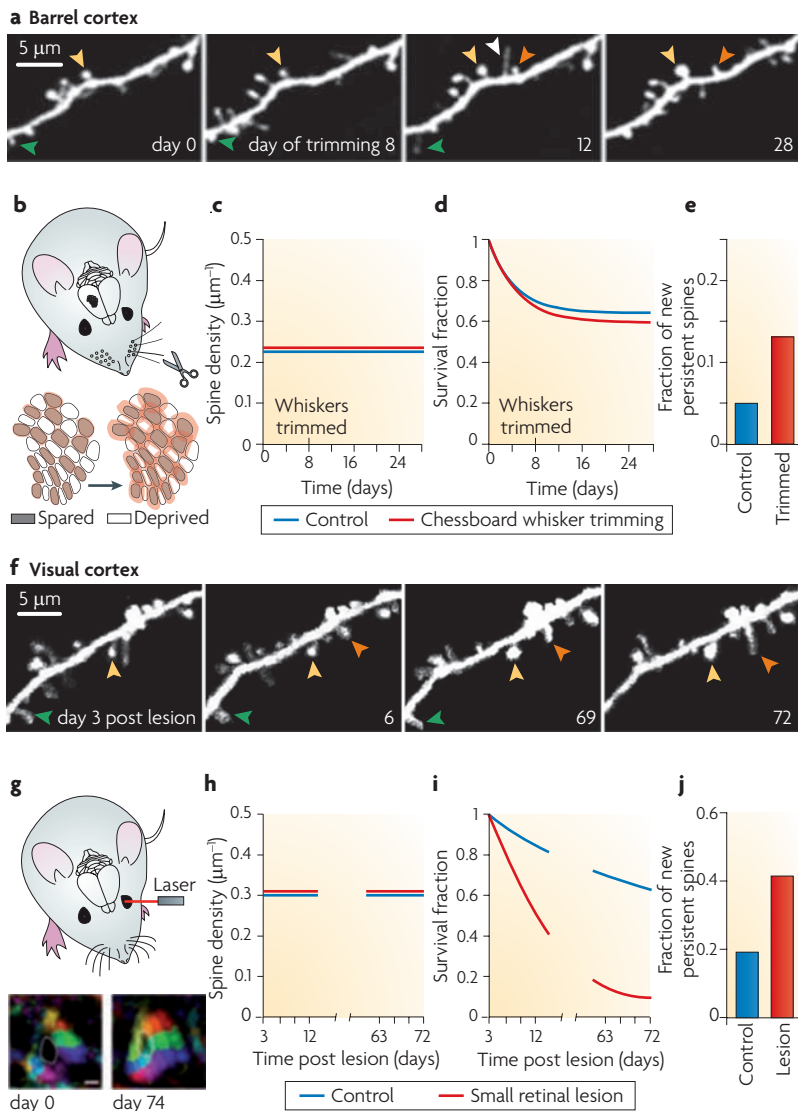


Figure 4 | Experience-dependent spine plasticity in the adult neocortex.
a | Time-lapse image of dendritic spines in the barrel cortex before and after chessboard whisker trimming (initiated at day 8). Many spines are stable (for example, yellow arrowhead), but some appear and disappear (for example, white arrowhead). New persistent spines (orange arrowhead) are more likely to grow after whisker trimming and previously persistent spines (green arrowhead) are more likely to disappear. **b** | The experimental paradigm; chessboard whisker trimming causes changes in the whisker representational map in the barrel cortex. **c** | Spine density in the barrel cortex remains unchanged after whisker trimming. **d** | The fraction of surviving spines is slightly decreased after whisker trimming due to an increased loss of persistent spines. **e** | The fraction of new persistent spines increases ~2.5 fold after whisker trimming. **f** | Time-lapse of dendritic spines in the visual cortex after a unilateral focal lesion in the retina. Although some spines are present over more than two months of imaging (for example, yellow arrowhead), most spines are lost (for example, green arrowhead) and replaced by new persistent spines (orange arrowhead). **g** | The experimental paradigm; immediately after a focal lesion in the retina the lesion projection zone (LPZ) in the neocortex is unresponsive to visual stimulation, as measured by intrinsic signal optical imaging (bottom, day 0). The size of the unresponsive region decreases with time on a timescale that matches structural plasticity. Scale bar, 700 µm. **h** | Spine density in the visual cortex remains unchanged after a small retinal lesion. **i** | Dendritic spines near or in the LPZ display high turnover rates; in the centre of the LPZ almost the entire spine population turns over. **j** | In the LPZs of small lesions the fraction of new persistent spines is increased several-fold. Parts **a–e** are modified, with permission, from REF. 23 © (2006) Macmillan Publishers Ltd. All rights reserved. Parts **f–j** are modified, with permission, from REF. 83 © (2008) Macmillan Publishers Ltd. All rights reserved.

Spines in disease and injury

Synaptic dysfunction is one of the first hallmarks of neurodegenerative disease¹³³. Spine pathology has been observed in association with many brain disorders¹⁰ such as Alzheimer’s disease^{85,134–136}, Parkinson’s disease^{137,138}, Prion diseases^{86,139}, schizophrenia¹⁴⁰, mental retardation^{141,142} and epilepsy¹⁴³. It is currently unclear how these phenotypes causally relate to disease progression. Time-lapse imaging *in vivo* is beginning to reveal the mechanisms underlying spine abnormalities related to these brain disorders. For example, in the vicinity of a cerebral infarct in mice, dendrites become exceptionally plastic, characterized by a long-lasting increase in the rate of spine turnover^{5,144}. These structural changes might provide a substrate for the long-term functional changes in the representational cortical maps that are observed after stroke models^{144,145}. Similarly, in mouse models for Alzheimer’s disease, the vicinity of amyloid plaques is characterized by highly dysmorphic neurites and spine turnover^{146–148} causing a net loss of spines. This phenotype could be caused by β-amyloid oligomers, which have been shown to block LTP and directly induce long-term depression (LTD), spine loss and memory loss^{149–152}. Long-term imaging experiments have the potential to reveal the relationship between changes in spine dynamics and neuronal network dysfunction in neurodegenerative diseases and memory disorders¹⁵³.

Conclusions and outlook

Long-term *in vivo* imaging studies are providing a complex picture of structural plasticity in the adult brain. The large-scale organization of axons and dendrites is remarkably stable and some synaptic structures also persist, perhaps for most of the lifespan of the animal. By contrast, it is now clear that a subset of synaptic structures displays cell type-specific, experience-dependent structural plasticity: axonal boutons turn over in a cell type-specific manner. Spines grow and retract, and the dynamics of this turnover is regulated by sensory experience (FIG. 5).

But many questions remain. Deciphering the role of structural plasticity will require linking changes at the level of synapses, single cells and neural circuits. Putative presynaptic and postsynaptic elements could be labelled by using methods for stochastic¹⁵⁴ or cell type-specific expression⁷⁶, perhaps using different coloured fluorescent proteins or fluorescent indicators of synaptic contact¹⁵⁵. The structural plasticity of individual cells is extremely heterogeneous, probably reflecting the behaviours of distinct cell types²³ and perhaps, interdigitated subcircuits^{156–158} with distinct functions.

In the context of experience-dependent plasticity it will be of great interest to link receptive field changes of individual neurons to the structural plasticity of these neurons. This could be achieved by using long-term imaging of neuronal structures in combination with indicators of neuronal function¹⁵⁹. A clear link between structural plasticity in specific neural circuits and learning remains to be demonstrated. Future studies

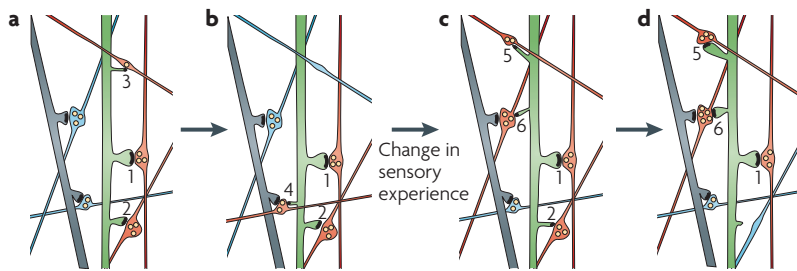


Figure 5 | A model for the relationship between transient spines, persistent spines and circuit plasticity. A spiny dendrite (green) is shown with its neighbouring connected (red) and unconnected (blue) axons (a). Postsynaptic densities are shown in black and neurotransmitter vesicles are yellow. Under baseline conditions, many spines are stable (spines numbered 1 and 2), whereas some new spines sample available presynaptic partners (spines 3 and 4). However, the majority of these spines retract. The transient spines may serve to generate large numbers of diverse, short-lived contacts (blue axons turning red) (b). A change in sensory experience causes the selection of a subset of these connections for stabilization (spines 5 and 6) (c), and loss of some previously persistent spines (spine 2) (d). Stabilization could be driven by long-term potentiation-like Hebbian plasticity, which is associated by an increase in the size and strength of synapses (BOX 2).

will have to combine long-term *in vivo* imaging with behavioural training.

Two-photon microscopy⁴⁰ has revolutionized high-resolution imaging *in vivo*⁴². However, current methods

are still limited to the most superficial ~500 µm of the brain. Deeper in the brain spherical aberration and scattering degrade the resolution and contrast of current methods so severely that synaptic structures cannot be resolved⁴¹. This means that less than 10% of the mouse brain can currently be imaged *in vivo*. High-resolution imaging in deep brain regions, like the hippocampus, requires invasive surgical methods^{160,161}. It remains to be seen if invasive methods can provide long-term high-resolution imaging at the level of single synapses.

Furthermore, it is now possible to image the molecular anatomy of single synapses *in vivo*: by imaging the dynamics of tagged synaptic molecules, the sequential assembly of new synapses can be analysed one molecule at a time¹⁶² in the intact brain⁴⁴. Other fluorescent probes, such as genetically encoded calcium indicators^{159,163}, will provide additional insight into the function of synapses as they form and stabilize. In summary, dynamic images of the function and plasticity of individual synapses in the intact brain is likely to provide a key to understanding experience-dependent synaptic plasticity *in vivo* and will also deepen our understanding of events leading to synaptic dysfunction in cases of neuronal injury or degeneration.

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DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
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FURTHER INFORMATION

Karel Svoboda's homepage: <http://research.janelia.org/Svoboda>

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ERRATUM

Experience-dependent structural synaptic plasticity in the mammalian brain

Anthony Holtmaat and Karel Svoboda

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On page 654 of the above article, the scale bar in parts a and f of figure 4 should represent 5 μm rather than 5 mm. This has been corrected in the online version.