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Anatomical and Physiological Plasticity of Dendritic Spines

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Key Words

spine remodeling, environmental enrichment, experience-induced structural plasticity, glutamate uncaging, 2-photon time-lapse imaging

Abstract

In excitatory neurons, most glutamatergic synapses are made on the heads of dendritic spines, each of which houses the postsynaptic terminal of a single glutamatergic synapse. We review recent studies demonstrating in vivo that spines are motile and plastic structures whose morphology and lifespan are influenced, even in adult animals, by changes in sensory input. However, most spines that appear in adult animals are transient, and the addition of stable spines and synapses is rare. In vitro studies have shown that patterns of neuronal activity known to induce synaptic plasticity can also trigger changes in spine morphology. Therefore, it is tempting to speculate that the plastic changes of spine morphology reflect the dynamic state of its associated synapse and are responsible to some extent for neuronal circuitry remodeling. Nevertheless, morphological changes are not required for all forms of synaptic plasticity, and whether changes in the spine shape and size significantly impact synaptic signals is unclear.

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INTRODUCTION

Since the early days of neuroscience, when Cajal was making camara lucida drawings of cells in the brain, scientists have been fascinated and perplexed by the diverse and complex morphology of neurons. Careful observation of neuronal morphology can reveal a great deal about the functional organization of a particular brain area and the neurons within it. For example, if we follow a neuron's axon, we will learn where its targets are, whereas by looking at the dendritic arbor we might predict the location of many of its inputs. A good example of the latter is the afferents to the locus coeruleus (LC). The somas of LC neurons are densely packed in this brainstem nucleus, and for a long time, investigators thought that their inputs projected to this region. It turns out that LC neurons extend their dendrites far out from the nucleus (similar to pyramidal neurons in the cortical layers), and thus, most of their inputs are located outside the nucleus (Shipley et al. 1996). Another clear example of a strong structure-function correlation is seen in pyramidal neurons of the hippocampus where there is approximately a one-to-one relationship between the numbers of dendritic spines and excitatory synapses (reviewed in Nimchinsky et al. 2004). Therefore, measurements of spine density provide an estimate of the synapse density in different regions of the cell.

However, the functional implications of other aspects of neuronal morphology are less clear. For example, in the past five years, a large body of work has emerged that thoroughly characterizes spine dynamics in vivo and has begun to examine the relationship between sensory experience and morphological plasticity. These studies revealed that dendritic spines can appear and disappear quickly in vivo and that the shape and size of spines also change over time. This structural plasticity is developmentally regulated and is affected by sensory stimulation. Nevertheless, the consequences of this structural plasticity are not well understood, and the idea that it might relate to functional plasticity of synapses is a provocative hypothesis that still needs to be tested further.

Here we review recent studies that examine spine plasticity in vivo and in vitro and consider what is known or can be predicted about the functional consequences of these structural changes.

DENDRITIC SPINE DYNAMICS IN VIVO

Spine Motility and Remodeling in Young and Adult Brain

Thanks to the generation of transgenic mouse lines that express genetically encoded

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fluorescent proteins in specific neuronal types and in a sparse pattern that creates a living "Golgi stain" (Feng et al. 2000), several research groups have published studies that examine the dynamics and motility of dendritic spines in vivo (Grutzendler et al. 2002, Trachtenberg et al. 2002, Majewska & Sur 2003, Oray et al. 2004, Holtmaat et al. 2005, Zuo et al. 2005, Zuo et al. 2005, De Paola et al. 2006, Holtmaat et al. 2006, Knott et al. 2006, Majewska et al. 2006, Oray et al. 2006). By creating a window viewing on the head of such mice and stabilizing the animal under a 2-photon laser scanning microscope (2PLSM) (Denk et al. 1990), researchers can repeatedly image the same portion of dendrites at the surface of the brain over periods of weeks and months. Using this approach the extent of morphological plasticity of dendrites and axons in the mature brain could finally be determined.

Dendritic spines in the apical tufts of cortical layer 5 pyramidal neurons are, for the most part, stable and persist for weeks to months. During a 1-month observation period, the percentage of stable spines in a young adult mice (1–2 months old) ranged from ~55% in the somatosensory cortex (S1) (Trachtenberg et al. 2002, Holtmaat et al. 2005) to ~75% in the visual cortex (V1) (Grutzendler et al. 2002, Majewska & Sur 2003). However, in mature adult mice (4–5 months old) spine stability is enhanced such that more than 70% of spines in S1 and more than 90% in V1 are persistent spines that remain throughout the period of the experiment (Trachtenberg et al. 2002, Holtmaat et al. 2005, Zuo et al. 2005). Thus, there is consensus that most spines from cortical layer 5 pyramidal neurons are stable and that spine stability is developmentally regulated.

Although these studies agree that there is an age-dependent decline in spine turnover, they report a wide range of values for the percentage of spines that are stable. True biological diversity among the different regions of the cortex seems to account for part if not all of the difference. Using the same experimental approach, and sometimes comparing between different cortical regions within the same animal, two independent labs reported that spines from layer 5 pyramidal neurons in the visual cortex are less dynamic than spines in the barrel cortex (Holtmaat et al. 2005, Majewska et al. 2006). In addition, dendritic branches from pyramidal neurons in the visual cortex have a smaller percentage of highly motile filopodia and thin spines than do those from somatosensory and auditory cortex (Majewska et al. 2006).

Filopodia: thin, long protrusions that are highly motile and have lifespans of hours

Thin spines: protrusions with a small or ill-defined head and that are shorter and more stable than filopodia

We do not know if these differences reflect diversity of intrinsic properties of pyramidal neurons from different cortical areas or if they result from different activity patterns of each brain area. In experiments designed to answer this question, Sur and collaborators showed that in animals in which retinal inputs were routed into the auditory thalamus, spine motility was not affected and remained larger than in the visual cortex of control mice (Majewska et al. 2006). However, the extent of the rewiring is unknown and it is possible that it did not include all areas of the auditory cortex. Furthermore, because of the large feedback projection from cortex to thalamus, it is unclear if this manipulation will substantially alter the level of activity of the rewired inputs.

In addition, differences in experimental design may also impact on the degree of spine stability. For example, the chronic imaging can be accomplished either by imaging directly through a thinned skull or by removing the bone and establishing an agar-filled imaging window. On one hand, removal of the skull can trigger an injury response that rapidly activates microglia and may acutely affect spine dynamics (Davalos et al. 2005, Nimmerjahn et al. 2005). Whether the injury caused during the initial surgery affects spine dynamics weeks and months later is unknown. On the other hand, imaging through the skull may degrade the resolution of the microscope and reduce laser power delivery to the brain, which may hinder the detection of thin and highly motile structures such as filopodia. Last, another possible source of

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variability comes from the fact that in the visual cortex, most layer 6 pyramidal neurons are also labeled in the YFP-H mice line and, even though most of their apical tufts do not reach the surface of layer 1, a small percent of them do and might contaminate analysis of layer 5 neurons (Holtmaat et al. 2005). For this reason, researchers must confirm that the cell bodies of the imaged pyramidal cells are located in the expected cortical layer. Nevertheless, despite these discrepancies, many common conclusions can be extracted from this body of work, and with them a clearer picture of the extent and relevance of structural plasticity is emerging.

Mechanisms of Spine Stability in Adults

The spine stability observed in adult mice is reflected in stabilization of the number of spines and of their location, and it occurs through a progressive slow-down of the rate of spine elimination (Zuo et al. 2005). Although the rate of spine formation remains constant for most part of the postnatal life (from postnatal day 14 to 5-month old mice: 5%–15% depending on the region), the rate of spine elimination is developmentally regulated (Holtmaat et al. 2005, Zuo et al. 2005). In young mice, spine elimination occurs more frequently than does spine formation, leading to a net loss of ∼15%–20% of spines (Grutzendler et al. 2002, Holtmaat et al. 2005, Zuo et al. 2005). This spine loss was observed in both visual and somatosensory cortices and takes place between the first and the third month of age with slight differences in timing reported by different groups (Holtmaat et al. 2005, Zuo et al. 2005) (**Figure 1**).

In mature adult mice (>4-month old), the rates of spine elimination and formation become comparable, greatly reducing the net rate of spine loss (Holtmaat et al. 2005, Zuo et al. 2005, Zuo et al. 2005). Thus, these studies show that as the brain matures, the stability of neuronal circuits is achieved by a decline in spine elimination and an increase in the half-life of spines.

Functional Consequences of Morphological Plasticity In Vivo

What can we learn about a spine from its shape? Although appearances can be deceiving, nature exhibits many examples of how the shape, for example of an organ or a flower, reflects and facilitates its function. In addition to formation and elimination, dendritic protrusions undergo more subtle structural rearrangements in the form of changes in volume, length, and width that might reflect or be the cause of functional changes at the synapse. Structural plasticity might reflect

Figure 1

(*A*) Pyramidal neuron from the hippocampus expressing a genetically encoded fluorescent protein (EGFP), allowing for imaging of spine morphology in living tissue. This neuron is from a rat organotypic slice and was imaged by 2-PLSM. (*B*) Time-lapse imaging every 5 min of a dendritic branch from a neuron as in (*A*) permits the detection of persistent spines (always present, *asterisks*), transient spines (*arrow*), and transient filopodia (*arrowhead*). Note that persistent spines undergo changes in morphology over time. (*C*) The rate of spine elimination is developmentally regulated. The graphs shows that in young mice, the rate of spine elimination (*red*) exceeds the rate of formation (*blue*) and that between 1 and 3 months of age, spine elimination declines to reach rates similar to formation. Consequently, there is an increase in the number of stable spines (*green*) and a decrease in the total number of spines (*black*) during this period. (Model based on data from Grutzendler et al. 2002, Holtmaat et al. 2005, Zuo et al. 2005). (*D*) In the adult, newly formed spines tend to be transient and not form synapses. Reconstruction of dendritic segments by serial electron microscopy after time-lapse imaging every 4 days for 1 month shows that only one third of the spines that are less than 4-days old form synapses, whereas all the newly formed spines that survive for at least 4 days and all the stable spines (>28 days) have synapses (Data from Knott et al. 2006).

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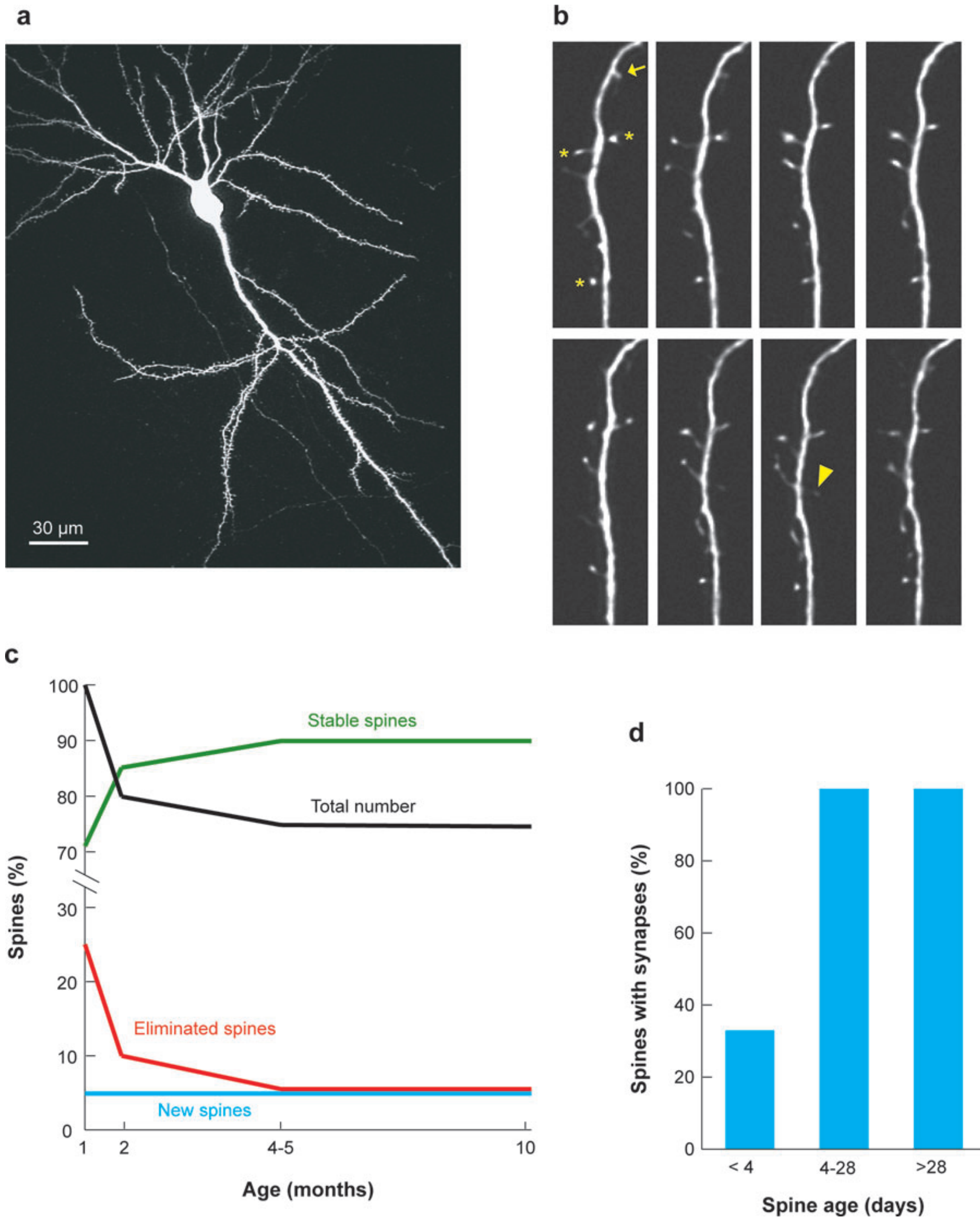

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PSD: postsynaptic density

Mushroom spines: dendritic protrusion with thin neck and bulbous head

changes in the size or protein constituents of the postsynaptic density (PSD), whether PSD is perforated, the number and composition of synaptic glutamate receptors, or even the release probability of the associated presynaptic terminal.

In the apical tufts of layer 5 pyramidal neurons of cortex, there is large diversity in the morphology of dendritic protrusions exemplified by the wide range of spine volumes (from 0.015 to 0.77 μm^3; Knott et al. 2006). In an elegant study that combined in vivo time-lapse imaging over one month with retrospective reconstruction of imaged dendrites by serial electron microscopy (EM), Knott and collaborators showed that new protrusions that have not formed synapses have smaller volumes and larger surface-to-volume ratios than do persistent stable spines (Knott et al. 2006). In addition, newly formed spines grow in volume as they become stable. Conversely, most spines show a reduction in volume before disappearing (Holtmaat et al. 2006). Given the correlation between spine head size and PSD area (Harris & Stevens 1989, Holtmaat et al. 2006), the observation that the volume of persistent stable spines is larger than those of transient spines suggests that persistent spines have larger PSDs with higher numbers of AMPA-type glutamate receptors and are associated with stronger synapses.

Further analysis of the data suggests that persistent spines are not all the same and that some are enlarging while others are getting smaller. Over time, there is no net change in the average volume or diameter of the spines from young or adult mice (Zuo et al. 2005, Holtmaat et al. 2006). However, in adult mice, the standard deviation of the mean diameter change increases over time (Zuo et al. 2005), possibly indicating that, in adult mice, spines that are shrinking or enlarging at one time point continue to shrink or enlarge over time, respectively.

Filopodia are the most motile type of protrusions (40–50 nm/min; Majewska et al. 2006) and turn over within hours (Zuo et al. 2005). Their density decreases with the age of the animal from 60% at P14 to less than 2% in 4–5-month-old mice (Grutzendler et al. 2002, Majewska & Sur 2003, Zuo et al. 2005). Does a filopodium represent an early stage of the spine? Will it become a mushroom spine over time? Conversely, can mushroom spines revert to filopodial morphology? These questions were examined by Sur and collaborators who reported that in one-month-old mice, with the exception of filopodia, all other protrusions were likely to maintain their classification over a period of three weeks (Majewska et al. 2006).

Filopodia can transform into mushroom or thin spines; however, only a small percentage actually does (0.2%) (Majewska et al. 2006). In agreement with this observation, another study showed that only a small percentage of filopodia turn into spines, whereas the vast majority of these newly formed protrusions (80%) disappear within the next 48 h (Zuo et al. 2005). Similarly, only 3% of newly formed spines survive for one month (Holtmaat et al. 2005, Holtmaat et al. 2006). However, if a newly formed spine persists for at least 4 days, it always formed a synapse, as defined by the presence of a postsynaptic density and apposed an active zone (Knott et al. 2006) (**Figure 1**). Thus, the consensus seems to be that most filopodia and newly formed spines have a half-life of just a few days and that the addition of a new stable spine and synapses is a rare event in adult mice.

In Vivo Dynamics of Presynaptic Boutons and Dendritic Branches

In general, presynaptic boutons are less dynamic than dendritic spines (De Paola et al. 2006, Majewska et al. 2006). At first glance, this seems to contradict most of the evidence on spine motility and synapse formation. However, the finding that most (67%) of new spines that form synapses do so onto boutons that make multiple synapses may explain the discrepancy in pre- and post-synaptic turnover rates (Knott et al. 2006).

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The dendritic arbor of most neurons is remarkably stable in the mature brain. Branches of pyramidal neurons in cortical layers 5 and 2/3 (Trachtenberg et al. 2002, Lee et al. 2006) and mitral and tufted cells in the olfactory bulb (Mizrahi & Katz 2003) of adult rats show no branch addition or retraction over weeks and months. However, the degree of remodeling of the dendritic arbor seems to vary according to cell type because interneurons (possibly GABAergic) in layer 2/3 show remodeling on a weekly basis (Lee et al. 2006). This remodeling was excluded from primary, first-order branches and mainly involved extensions and retractions from higher-order branches and a small percentage of branch-tip additions. Thus, in the mature brain, remodeling of neuronal circuits among projection neurons appears to involve mainly morphological rearrangements at the dendritic spine level.

ACTIVITY-DEPENDENT STRUCTURAL PLASTICITY IN VIVO

Experience and learning affect connectivity in the brain. Nevertheless, several major questions remain unresolved. For example, how is it that sensory experience affects brain connectivity? Which key aspects of activity are required to do so, and by which mechanism does the rewiring occur? In principle, two basic scenarios should be distinguished: activity might regulate the formation of new synapses or it might control the elimination or destabilization of synapses once they are formed. In the first model, synaptogenesis is driven by experience-related neuronal activity in a way that is both temporally and spatially discrete and that mainly creates connections that encode relevant, meaningful information. In the second model, synapse formation occurs in a constitutive manner at a constant rate that generates an excess of synapses and that allows for activity-dependent elimination of synapses to occur. In this later model, experience-related activity prunes brain connectivity such that only meaningful connections are preserved.

Rather than a single strategy driving experience-dependent remodeling of circuits, it is possible that different types of neurons take advantage of one or the other strategy for which their intrinsic properties and network connectivity are better suited. Alternatively, one mechanism might predominate during early brain development and the other may dominate in the adult brain (e.g., activity-dependent spine elimination during early development of circuits followed by regulation of synapse formation in adulthood).

In the following sections, we discuss the effect of activity on spine motility, density, and morphological plasticity. In the first two sections, we focus on two experimental models of in vivo sensory deprivation and stimulation: whisker trimming and environmental enrichment. In the final section, we review the results of recent studies of structural plasticity that utilize in vitro models.

Sensory Deprivation

Rodent barrel cortex has been used extensively for the study of cortical critical periods and of circuit level plasticity. Trimming or removal of whiskers can trigger changes in connectivity at multiple cortical layers depending on the developmental timing of the deprivation (Diamond et al. 1994). In the studies described below, two different experimental models of whisker trimming are employed. In one case, all whiskers in the contralateral mystacial pad are trimmed (Zuo et al. 2005); in the others, whiskers are trimmed in a chessboard pattern such that every trimmed whisker is surrounded by intact whiskers (Trachtenberg et al. 2002, Holtmaat et al. 2006) and vice versa. Whether these models are significantly different in regard to their ability to trigger spine remodeling is unclear. Thus far, some general conclusions can be extracted from them, even when the analysis of the data and other experimental variables

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are so diverse that the results of each study cannot be directly compared.

Chessboard deprivation increases the number of transient spines and decreases the number of stable spines in layer 5 pyramidal neurons of the somatosensory cortex of young mice (6–10-week-old) after 3–4 days of sensory deprivation (Trachtenberg et al. 2002). The increase in spine turnover was significant by 48 h after trimming, the same time window during which changes in the receptive field were apparent (Trachtenberg et al. 2002). Similarly, binocular deprivation increases spine motility, although in this latter case the effect is seen only during the critical period (Majewska & Sur 2003). Thus, plasticity of spine structure and synaptic function are correlated in time, are triggered within days after sensory deprivation, and precede larger-scale rearrangements in connectivity.

In a different type of study that used acute brain slices from control and deprived mice, brief sensory deprivation (24 h of monocular deprivation) was reported to induce structural plasticity that was mimicked and occluded by enzymatic degradation of the extracellular matrix (Oray et al. 2004). However, this study differs in so many aspects from the in vivo studies described here that it is unclear whether a similar mechanism plays a role in vivo.

Sensory deprivation selectively affects spine elimination such that long-term sensory deprivation reduced the rate of spine elimination with no effect on the rate of formation (Zuo et al. 2005). This effect was limited to dendritic spines and was not observed for filopodia (Zuo et al. 2005). In addition, whisker trimming increased the stability of newly formed spines while decreasing the stability of persistent stable spines (Holtmaat et al. 2006). Thus, the emerging model is that sensory deprivation destabilizes old spines while making new spines more stable, with the net effect being an increase in spine motility that leads to short-term structural rearrangement of synapses and long-term changes in brain connectivity.

Finally, the effects of sensory deprivation on spine elimination are reversed by restoring normal sensory experience and, even though they are more pronounced in young mice (6-week-old), are also seen in adult mice after prolonged sensory deprivation (Zuo et al. 2005). This latter study challenges the notion of a fixed critical period of cortical plasticity that prevents sensory-evoked changes in the adult brain. Other studies have shown that sensory deprivation can also induce plasticity in the adult somatosensory cortex (Diamond et al. 1994, Glazewski et al. 1998) and visual cortex (Sawtell et al. 2003). In the mature brain, plasticity induced by sensory deprivation shares many features with plasticity in young animals, although it usually requires longer periods of deprivation. These studies suggest that, at least for some types of plasticity, the critical periods do not close abruptly and absolutely, but gradually and often incompletely.

Environmental Enrichment

Environmental enrichment is a well-characterized experimental model to study the role of sensory experience in neuronal plasticity. Since the initial observation made by Hebb that rats he had taken home and raised as pets performed better in problem-solving tasks than did rats raised in cages in the laboratory (Hebb 1949), enrichment has been studied in the laboratory by rearing rats in 2 ways: (*a*) a restricted, impoverished environment, in small cages (usually solid walls) with food and water ad libitum but no social interactions or (*b*) an enriched environment, reared in larger cages with toys, tunnels, and obstacles, and in groups to allow for ample opportunities for problem-solving and complex social interactions. It might be worth mentioning that the standards for animal rearing in the laboratory better resemble the physically impoverished environment than the enriched environment.

In addition to improving cognitive performance and learning (Hymovitch 1952, Brown

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1968, Gardner et al. 1975, Rampon et al. 2000, Duffy et al. 2001; reviewed by Lewis 2004, Leggio et al. 2005), enrichment enhances long-term potentiation (LTP) in the hippocampus (Duffy et al. 2001), affect gene expression (Rampon et al. 2000, Lazarov et al. 2005), cell proliferation and survival (Altman & Das 1964, Szeligo & Leblond 1977, van Praag et al. 1999), synaptogenesis (Greenough et al. 1985, Rampon et al. 2000, Briones et al. 2004), and neuronal morphology (Schapiro & Vukovich 1970, Volkmar & Greenough 1972, Globus et al. 1973, Greenough et al. 1978, Kozorovitskiy et al. 2005, Leggio et al. 2005). For the purpose of this review, we focus on its effect on structural remodeling in neurons with tangential references to the behavioral, electrophysiological, and biochemical changes.

In terms of neuronal morphology, environmental enrichment was shown to: (*a*) increase dendritic branching in pyramidal neurons of specific regions of the cortex and hippocampus (Schapiro & Vukovich 1970, Volkmar & Greenough 1972, Kozorovitskiy et al. 2005, Leggio et al. 2005); (*b*) increase spine density (Schapiro & Vukovich 1970, Globus et al. 1973, Rampon et al. 2000, Leggio et al. 2005); (*c*) increase the number of synapses (Altschuler 1979, Rampon et al. 2000, Briones et al. 2004); (*d*) increase the number of perforated postsynaptic densities and polyribosomes in spine heads (Greenough et al. 1978, Greenough et al. 1985); and (*e*) increase the average length of the postsynaptic density (Diamond et al. 1975). More recently, some of these findings have been confirmed in primates where environmental enrichment increases dendritic branching and spine density in pyramidal neurons of the CA1 region of the hippocampus and prefrontal cortex (Kozorovitskiy et al. 2005).

One of the most interesting aspects of this experimental model of experience-dependent plasticity is that enrichment exerts its effects at all ages. Cognitive improvement and structural plasticity are induced when animals are reared from birth in an enriched environment and when adult animals are exposed to it (Duffy et al. 2001, Briones et al. 2004, Kozorovitskiy et al. 2005). Even prenatal enrichment improves specific behaviors in rats (Koo et al. 2003). This suggests that whatever the mechanism is for enrichment-induced plasticity, it is able to operate at all stages of development.

Interactions between enrichment and disease and enrichment and other forms of plasticity have been demonstrated. For example, exposing animals to more complex environments attenuates or prevents the deficits caused by brain injury, ischemia, and seizures (reviewed in Lewis 2004). Environmental enrichment can prevent the loss of spines observed in aging rats (Saito et al. 1994) and can promote morphological and behavioral recovery in mouse models of fragile X syndrome (Restivo et al. 2005). In addition, enrichment reduced the deposition of Aβ in an animal model of Alzheimer's disease by increasing the activity of a Aβ degradating endopeptidase (Lazarov et al. 2005) and reversed the cognitive deficits of transgenic mice lacking NMDA-type glutamate receptors in the hippocampus (Rampon et al. 2000). Recently, Ebner and collaborators showed that placing animals in a rich environment after trimming the whiskers accelerated the development of functional plasticity in the barrel cortex (Rema et al. 2006).

Enriched environments usually involve group housing, so the question was raised whether social interaction alone could induce structure plasticity (Volkmar & Greenough 1972). Others have tried to dissociate the effect of enrichment from those induced by voluntary motor behavior and exercise. Gauge and colleagues studied the role of two components of enrichment, learning and motor activity, on neurogenesis and found that learning had no effect on neurogenesis and exercise induced neuronal proliferation (van Praag et al. 1999). Further enrichment mainly increased neuronal survival. However, it has not been addressed yet what the effect is of these components of enrichment on the structure

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Ca: calcium

NMDAR: (N-metyl-D-aspartate)-type glutamate receptor

plasticity. More important, the mechanisms by which sensory experience increases the density and total number of spines and synapses are still unknown. In vivo studies of spine dynamics such as those previously described are essential to start answering this question.

STIMULUS-DEPENDENT ALTERATIONS OF SPINE MORPHOLOGY IN VITRO

The hypothesis that changes in synaptic function are intimately linked to changes in dendritic spine structure has been tested many times. EM studies of the neuropil were used to make population comparisons of the structure of dendritic spines across developmental or plasticity states. During development, this type of study revealed clear changes in the density, shape, and size of dendritic spines of hippocampal pyramidal neurons (Harris et al. 1992). However, in terms of detecting changes in spine structure and density that accompany stimulus-induced synaptic plasticity, these studies may have been hindered by the inability to examine the same set of spines before and after the induction protocol. Thus, these beautiful studies achieved the statistical power necessary to detect increases in the density of relatively rare structures such as perforated PSDs (Harris et al. 1992, Toni et al. 2001, Stewart et al. 2005), bifurcating spines (Toni et al. 2001), and multiple-synapse boutons (Fiala et al. 2002) but generally did not uncover changes in the size of the spine head now thought to accompany expression of long-term depression (LTD) and potentiation (LTP). These studies have been reviewed extensively elsewhere (Majewska et al. 2000, Sorra & Harris 2000, Nikonenko et al. 2002) and are not considered further here.

Three advances have allowed the detection of more subtle stimulus-induced changes in spine shape and number that may have been missed in earlier population comparisons. First, time-lapse microscopy allows for comparison of the morphology of dendrites and spines before and after a manipulation, greatly increasing statistical power. Second, the use of fluorescent reporters of intracellular calcium (Ca) levels allows identification of spines that contain the activated synapses and that may express LTP. Last, 2-photon glutamate uncaging (Matsuzaki et al. 2001, Carter & Sabatini 2004, Sobczyk et al. 2005, Losonczy & Magee 2006) allows the experimenter to select nearly any visualized spine and deliver an arbitrary pattern of glutamate stimulation to the postsynaptic terminal of the selected spine.

Growth and Shrinkage of Dendritic Spines

Using 2-photon glutamate uncaging, Kasai and collaborators delivered LTP-inducing stimuli to selected spines of GFP-expressing hippocampal pyramidal neurons in rat organotypic slices (14–20-day-old animals, room temperature, perforated patch) (Matsuzaki et al. 2004). Spines that received LTP induction increased in volume approximately twofold (stimulation of a single spine by glutamate uncaging at 1–2 Hz for 60s while holding at 0 mV or holding at –60 mV in 0 Mg extracellular solution). This increase persisted for more than 1 h after stimulation and required signaling through NMDA-type glutamate receptors (NMDAR), calmodulin, and calcium/calmodulin protein kinase II (CAMKII), as well as reorganization of the actin cytoskeleton (Matsuzaki et al. 2004). When CAMKII activity or actin-based cytoskeletal rearrangements were blocked, spines receiving the LTP-inducing stimuli exhibited only transient increases in spine head volume. Furthermore, the persistent increase in glutamate-evoked currents and spine head volume could be achieved only by uncaging onto small spines, suggesting that the synapses made onto large spine heads are refractory to this form of LTP induction. The same group had previously shown that glutamate uncaging evokes larger AMPA-type glutamate

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receptor (AMPAR) currents in spines with bigger heads (Matsuzaki et al. 2001), which is consistent with EM studies showing a positive correlation between spine head size, PSD area, and AMPAR immunolabeling (Harris & Stevens 1989, Schikorski & Stevens 1997, Takumi et al. 1999).

Similar increases in spine volume were seen using a chemical LTP protocol (chemLTP) in rat hippocampal pyramidal neurons in organotypic slice cultures (20–23 days in vitro, 30°C, whole cell recordings) (Kopec et al. 2006). The chemLTP protocol, consisting of strong activation of NMDARs and PKA for 15 min, results in translocation of GFP-tagged AMPARs into the spine heads. This finding is in agreement with the previously reported changes in AMPAR trafficking triggered by LTP (Lissin et al. 1999, Shi et al. 1999, Malinow & Malenka 2002). A persistent increase in spine volume (~50%) was observed; however, in contrast with the studies of Matsuzaki et al. (2004), chemLTP-induced spine enlargement was independent of the initial size of the spine head.

Studies using time-lapse imaging of fluorescently labeled neurons previously revealed that the induction of LTP is associated with the growth of new spines or filopodia (Engert & Bonhoeffer 1999, Maletic-Savatic et al. 1999). Three properties of the synapses on hippocampal pyramidal neurons help make the prediction of changes in spine number and spine head size: (*a*) the postsynaptic nature of LTP expression in the hippocampal CA1 pyramidal neurons (Nicoll & Malenka 1999) (although see Zakharenko et al. 2001); (*b*) the nearly one-to-one relationship between synapse and spine number in hippocampus (Harris et al. 1992); and (*c*) the positive correlation between spine head size, PSD area, and AMPAR number (Harris & Stevens 1989, Knott et al. 2006).

Are changes in spine morphology necessary for the induction or expression of synaptic plasticity? At least two studies suggest that this is not the case. Lang et al. (2004) imaged GFP-expressing CA1 pyramidal neurons in acute slices of adult transgenic mice during delivery of LTP-inducing stimuli. They imaged hundreds of spines and found only transient increases in spine head size. Although simultaneous field recordings confirmed induction of LTP, the approach did not allow the authors to identify specifically which spines had been potentiated. In a separate study of hippocampal pyramidal neurons in acute rat slices, LTD induction was accompanied by decreases in the spine head diameter (14–18-day-old, 32°C, whole-cell recording). LTD and spine shrinkage both required activation of NMDAR and calcineurin (Zhou et al. 2004) but whereas the electrophysiological changes relied on signaling through PP1/2A, spine remodeling was independent of the phosphatase activity and was mediated by activation of the actin depolimerization factor cofilin. Thus, changes in spine morphology could be induced without concomitant LTD induction indicating that at least the pathways involved in these two processes are divergent.

AMPAR: (α-amino-3-hydorxy-5-metyl-4-isoxazole-propionic acid)-type glutamate receptor

Functional Consequences of Spine Remodeling

What is the impact on synaptic function of the changes in spine morphology seen with LTP- and LTD-inducing stimuli? The spine neck poses a barrier to current flow and to diffusion of molecules between the spine head and the dendrite (see Parameters of Spine Morphology that May Affect Electrical and Biochemical Signaling). However, for the vast majority of spines, the effect on synaptically evoked electrical signals is expected to be inconsequential (Segev & Rall 1988, Harris & Stevens 1989, Koch & Zador 1993, Svoboda et al. 1996). Conversely, for the special case of Calcium (Ca) diffusion at physiological temperatures and in the absence of added exogenous Ca buffers, this barrier is sufficient to fully isolate spines with an identifiable neck and will thereby allow them to act as independent Ca signaling compartments (Sabatini et al. 2002).

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PARAMETERS OF SPINE MORPHOLOGY THAT MAY AFFECT ELECTRICAL AND BIOCHEMICAL SIGNALING

The spine neck is a thin, approximately cylindrical structure filled with proteins and salts. Therefore, the resistance (R) it poses to the flow of current is

$$R = \frac{\rho L}{A},$$

where L and A are the length and open cross-sectional area of the spine neck, respectively, and ρ is the resistivity of the cytoplasm that fills the neck. Anatomical and functional studies have estimated $R < 150\ \mathrm{M\Omega}$ for the majority of spines in hippocampal pyramidal neurons (Harris & Stevens 1989, Svoboda et al. 1996). Thus for typical unitary synaptic currents (10–100 pA) and spine neck resistances, the voltage drop across the spine neck during synaptic stimulation is not expected to exceed 10 mV or significantly impact electrical signaling (Koch & Zador 1993).

The spine neck also retards the diffusional exchange of molecules between the spine and dendritic shaft. The time constant of diffusional equilibration across the spine neck (τ) for a particular molecule is

$$\tau = \frac{VL}{DA},$$

where L and A are as above, V is the spine head volume, and D is the diffusion constant of the molecule in the neck. For GFP and PAGFP, ~28 kD proteins, τ ranges from ~100 ms to many seconds (Majewska et al. 2000, Bloodgood & Sabatini 2005), allowing for significant compartmentalization of activated proteins.

If spine neck resistance is generally too low to impact electrical signaling greatly and too large to impact Ca signaling within the spine head, is the spine neck of any functional relevance to the synapse? The induction of physiologically tractable forms of synaptic plasticity typically requires the repetitive activation of a synapse. For example, spike timing–dependent plasticity (STDP) is induced by prolonged and relatively low-frequency stimulation of the synapse (~0.1 Hz) in conjunction with postsynaptic APs. Which signal is retained and accumulated within the spine and is responsible for storing the history of activation of the synapse and eventually triggering the expression of plasticity? As described below, small free-diffusing molecules cannot accumulate in the spine head during these low-frequency stimulus protocols because they are expected to be fully cleared from the spine head during the interstimulus interval. Similarly, for most spines, it is unlikely that an activated but freely diffusing protein, such as a kinase, can accumulate near active synapses. However, because paired stimuli such as those used for STDP induction reduce diffusional exchange across the neck (Bloodgood & Sabatini 2005), an early step in plasticity induction may be to increase the diffusional isolation of the active synapse and push the morphology of the spine into a region (large head, thin neck) in which activated proteins can accumulate. Alternatively, perhaps the accumulating signal is not freely diffusing and is insensitive to changes in the spine neck. For example, each paired stimulus might promote phosphorylation of an integral component of the PSD. However, even in this case, regulation of the neck may influence plasticity induction. If the phosphatase that dephosphorylates the PSD protein is generally active in the dendrite but not normally found in the active state in the spine head, constriction of the spine neck may reduce the probability of the phosphatase gaining access to the active synapse and thereby prevent it from "erasing" the plasticity-promoting signal. Thus, even without affecting electrical or Ca signaling, changes in the neck may adjust the threshold for plasticity induction or the stability of plasticity expression at each synapse.

Parameters that Regulate Diffusional Equilibration Across the Spine Neck

For any signaling molecule, a concentration gradient across the spine neck will dissipate according to a characteristic time constant

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τ_{equ} that reflects the properties of the molecule and the morphological parameters of the spine (see Parameters of Spine Morphology that May Affect Electrical and Biochemical Signaling). For the morphology of the spine neck to influence the amplitude and kinetics of evoked biochemical signals significantly within the spine head, τ_{equ} must be on the same order or smaller than the lifespan of the signaling molecule within the head ($\tau_{lifetime}$). Consider a freely diffusing protein of similar size to GFP (i.e., $\tau_{equ} \sim 200$ ms) that is activated by phosphorylation in the spine head but can be dephosphorylated only in the dendrite (i.e., $\tau_{lifetime}$ is infinite). In this condition, activated protein is lost only by diffusion into the dendrite, and the time over which it signals in the spine is directly proportional to τ_{equ}. In contrast, if the phosphatase for the same protein is also active within the spine head, then the protein may be dephosphorylated quickly (i.e., short $\tau_{lifetime}$ compared with τ_{equ}). In this case, the exact parameters of the neck are relatively irrelevant, and the specific activity and concentration of the phosphatase in the spine head will determine the duration of signaling. Last, the protein may tightly interact with an integral component of the postsynaptic density (PSD). If this affinity is high, then the dissociation rate of the protein from the PSD (K_{off}) will determine the lifespan of the protein in the spine. For example, with affinity $K_D = 1$ nM, K_{off} is at most 0.5 s^{-1}, and the time constant of the protein coming off the PSD is ∼2 s, which dwarfs τ_{equ} for most proteins in most spines. Thus, the amplitude and kinetics of evoked signals will be limited by τ_{equ} only for signaling molecules that are not rapidly degraded, inactivated, or sequestered in the spine head.

For this reason, the buildup of synaptically evoked Ca transients within the spine head under physiological conditions is unlikely to be affected by the size of the spine neck. At ∼ 34°C, Ca is rapidly extruded from the spine head ($\tau_{lifetime} \sim 15$ ms) (Sabatini & Svoboda 2000, Scheuss et al. 2006). Because τ_{equ} for Ca that interacts with endogenous Ca binding proteins is 10–100 times greater than this value, synaptically evoked Ca signals dissipate principally by extrusion of Ca across the head membrane, and the duration of these signals are insensitive to changes in τ_{equ} over a broad range (Sobczyk et al. 2005). This analysis holds only under physiological conditions in which the mobility of Ca in the spine neck is greatly diminished owing to interactions with Ca binding proteins (reviewed in Sabatini Maravall et al. 2001). In the presence of large concentrations of Ca indicators, and especially at room temperature where the extrusion of Ca due to active pumping is slowed, the properties of the spine neck may influence the time course of synaptically evoked transients in the spine head (Korkotian et al. 2004, Noguchi et al. 2005).

τ_{equ} = time constant of diffusional equilibration across the spine neck

$\tau_{lifetime}$ = lifespan of a signaling molecule within the spine head

The properties of the spine neck vary tremendously from spine to spine and are dynamically adjusted. Bloodgood & Sabatini (2005) measured the diffusion of photoactivatable GFP (Patterson & Lippincott-Schwartz 2002) in hundreds of spine in apical dendrites of hippocampal pyramidal neurons in rat organotypic slices and found that τ spanned nearly 3 orders of magnitude. The mean value for τ_{equ} for this 28 KD protein was ∼250 ms, but in the extreme cases (∼5% of spines), τ_{equ} exceeded 5 s, indicating a severe barrier to diffusion. These spines may represent a special class in which the spine neck resistance approaches 1 GΩ, permitting the spatially restricted activation of regenerating sodium and calcium currents in the spine neck (Segev & Rall 1988). In addition, τ_{equ} was regulated chronically by changes in activity (24 h) and acutely (minutes) by pairing back-propagating action potentials (bAPs) with synaptic stimulation. In particular, stimulation of a single spine with 2-photon glutamate uncaging followed by a trio of bAPs resulted in a rapid restriction of diffusional coupling, whereas bAPs or uncaging alone had no effect. Thus the activity of a single synapse can rapidly regulate the property of the spine neck with which it is associated.

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CONCLUSIONS

Dendritic spines undergo changes in shape and size in vivo and in vitro. Spines also appear and disappear through life, but their turnover rate declines with age. As the brain matures, spine stability is achieved by a slowdown in the rate of spine elimination. At all ages, neuronal activity also regulates spine turnover and these changes are likely to alter neuronal circuits functionally. For example, in young animals, sensory deprivation by trimming whiskers leads to a short-term increase in spine motility in barrel cortex and triggers both elimination of old spines and formation of new protrusions. New protrusions that persist for several days form synapses, and thus, the long-term effect of these structural rearrangements is a change in neuronal connectivity in the region of the cortex.

Morphological rearrangements of spines can be induced in vitro by plasticity-inducing stimuli. Induction of LTP is accompanied by an enlargement of the spine head and of LTD by decreases in spine size. Thus, spine size is dynamically regulated by the activity patterns of the associated synapses. The studies reviewed here represent important advances to the field and, although unresolved issues still remain, they bring us closer to understanding the role of structural plasticity in regulating the function of synapses, neurons, and circuits.

SUMMARY POINTS

1. Imaging of cortical neurons in transgenic mice that sparsely express genetically encoded fluorophores has permitted the long-term observation of neuronal morphology in vivo.
2. The lifetime of dendritic spines increases with age, but transient and motile dendritic spines are found in adult animals.
3. In adults, most new spines are transient and only a small percentage of them will ultimately form synapses and persistent for more than just a few days.
4. A balance between spine elimination and spine growth determines the net change in spine number in vivo. In young adult animals, the rate of spine elimination exceeds that of spine formation, and spine numbers do not stabilize until approximately the third month of age.
5. Spine stability is altered by in vivo sensory stimulation and sensory deprivation.
6. In vitro, plasticity-inducing stimuli can trigger alterations in the morphology and number of dendritic spines. However, synaptic plasticity can be induced without concomitant changes in spine morphology.

UNRESOLVED ISSUES

1. Which signals trigger the growth or retraction of a spine in vivo? Which intracellular signaling pathways mediate these morphological changes?
2. Which factors stabilize spines? Does a stable spine persist in vivo because it is associated with a continually active synapse? Or, alternatively, is a spine persistent because it has entered a new state in which it is, by default, stable and is insensitive to changes in activity patterns?

3. Do the differences in spine stability across cortical areas arise from differences in the inherent properties of the neurons or rather because of different local patterns and levels of activity?
4. Do changes in spine morphology have physiologically relevant effects on the biochemical and electrical consequences of synaptic stimulation?
5. Can an in vitro system be developed that accurately reflects the properties of spines in the adult animal?

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