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Experience-driven brain plasticity: beyond the synapse

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Abstract

The brain is remarkably responsive to its interactions with the environment, and its morphology is altered by experience in measurable ways. Histological examination of the brains of animals exposed to either a complex ('enriched') environment or learning paradigm, compared with appropriate controls, has illuminated the nature of experience-induced morphological plasticity in the brain. For example, this research reveals that changes in synapse number and morphology are associated with learning and are stable, in that they persist well beyond the period of exposure to the learning experience. In addition, other components of the nervous system also respond to experience: oligodendrocytes and axonal myelination might also be permanently altered, whereas changes in astrocytes and cerebrovasculature are more transient and appear to be activity- rather than learning-driven. Thus, experience induces multiple forms of plasticity in the brain that are apparently regulated, at least in part, by independent mechanisms.

Keywords

enrichment; complex environment; exercise

INTRODUCTION

The temptation to understand behavior in terms of either nature or nurture has become considerably less compelling in recent decades, in part because of the recognition that experience induces measurable, morphological changes in cells of the brain, both neurons and glia, and in their connections with one another. Alterations in gene expression, neurochemistry and the physiological properties of these cells often accompany environment-induced reorganization of brain connectivity, as do measurable adjustments in behavior that are adaptive in the context of the new environment. This dynamic interplay between the environment (nurture) and brain biology (nature) is being called upon to further the understanding of complex psychopathological disorders that are characterized by abnormal brain morphology and/or functional activation, even those that are known to have a strong genetic component. For example, the concordance rate for schizophrenia is 17% in dizygotic twins and 50% in monozygotic twins (reviewed in Tsuang, 2000), which indicates the contribution of both genetic and environmental factors. Similarly, polymorphism in the serotonin transporter gene modulates the onset of major depression in response to stressful life

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events (Caspi *et al.*, 2003). It is suggested that interaction between an individual's genetic makeup and his or her environment can account not only for individual differences in susceptibility to psychopathology, but for such things as personality characteristics and neural and behavioral responses to the normal aging process as well.

Elucidation of experience-induced alterations in brain morphology is facilitated by conducting studies in a laboratory setting where environmental conditions can be better controlled. The complex environment paradigm, pioneered by Donald Hebb and his students (e.g. Hebb, 1949; Forgays and Forgays, 1952; Hymovitch, 1952), involves housing a group of animals, typically either rats or mice, in a large cage that contains numerous toys, such as balls, tunnels and ladders, which are changed daily to provide a continuously stimulating environment. This complex environment is sometimes referred to as an 'enriched condition' (EC), but it is important to note that these animals are only enriched relative to rats housed in isolation or 'impoverished condition' (IC) and rats housed in a group but without toys ('social condition' or SC) rather than animals living in the wild. As Hebb's work first demonstrated, animals exposed to EC demonstrate superior performance in several tests measuring higher-order cognitive ability, such as the Hebb-Williams maze (Mohammed *et al.*, 1986; Galani *et al.*, 1997), the Morris water maze (Whishaw *et al.*, 1984; Mohammed *et al.*, 1990) and the radial-arm maze (Galani *et al.*, 1998).

The first use of the EC paradigm as a tool to study experience-induced morphological plasticity in the brain was by Bennett *et al*. (1964), who reported that rats raised in EC possessed heavier and thicker cerebral cortices than did IC rats. Because the differences were largest in the visual cortex, much of the subsequent work was aimed at determining the underlying morphology that contributes to the gross difference in size in this region (e.g. Diamond *et al.*, 1964; Diamond *et al.*, 1966; Volkmar and Greenough, 1972). Although not a ubiquitous phenomenon, morphological plasticity following exposure to EC has been demonstrated in several other brain regions that are involved in the processing and/or response to environmental stimuli, including the auditory cortex (Greenough *et al.*, 1973), primary somatosensory cortex (Coq and Xerri, 1998), hippocampus and entorhinal cortex (Fiala *et al.*, 1978; Moser *et al.*, 1997; Rampon *et al.*, 2000), amygdala (Nikolaev *et al.*, 2002), basal ganglia (Comery *et al.*, 1995; Comery *et al.*, 1996) and cerebellar cortex (Greenough *et al.*, 1986). Initially, alterations in neuronal structure were the focus of investigation, however, more recently it has become clear that other components of the nervous system, such as macroglial cells and cerebrovasculature elements, also exhibit robust plasticity in response to experience.

Morphological plasticity in the brain occurring in response to an increase in the complexity of the environment appears to reflect brain substrates of adaptation to the demands and opportunities provided by experience, including both relatively typical forms of learning and memory and adjustments associated with fundamental processes such as sensory, motor and cognitive processing. Some important points concerning how this conclusion has been reached deserve mention, but for a more comprehensive discussion see (Grossman *et al.*, 2002). Following the initial reports of increased cortical thickness in EC rats, it was suggested that the observed plasticity might not be caused by learning-dependent processes, but by changes in overall body growth, increase in social contact, differences in stress that might occur as a result of differential housing and to other hormonal or metabolic responses to behavioral manipulations. The evidence, however, does not support any of these mechanisms. In the original publication by Bennett and colleagues in 1964, it was reported that the increased cortical thickness of EC rats could not be attributed to differences in body size between the groups, because the EC rats weighed ~7% less than the IC rats, presumably because they were more active. The finding that young EC rats have slower somatic growth at the time that brain regions are expanding has been replicated (Black *et al.*, 1989b). Additionally, although somatic growth of adult rats is slow and not very responsive to housing condition, EC still induces

plasticity in the adult brain that is similar to that observed in young animals (Black *et al.*, 1989b). The concern about increased social contact has been addressed by the inclusion of SC control animals (housed in a group but not in a complex environment); plasticity observed in EC animals cannot be explained by social contact if comparable changes are not demonstrated by SC animals, as is often the case (e.g. Rosenzweig *et al.*, 1978; Turner and Greenough, 1985; Sirevaag *et al.*, 1988; Sirevaag and Greenough, 1991; Jones and Greenough, 1996; Briones *et al.*, 2004) (see below for discussion of these studies). Neither is experience-dependent plasticity attributable to changes in levels of stress or stress hormones, although stress is capable of altering the morphology of neurons in some brain regions (Sapolsky, 2003). In one study, the adrenal weights of young male EC, SC and IC rats were compared with the density of astrocytic processes in the dentate gyrus of the hippocampus to examine the relationship between group experience and potential individual differences in stress reactivity (Sirevaag *et al.*, 1991). Because experience-induced astrocytic changes typically accompany alterations in dendritic structure, the dentate gyrus was selected for examination because EC had previously been shown to induce little or no effect on the dendritic architecture in this region of the brains of weanling male rats (Juraska *et al.*, 1985). Although there was a positive correlation between the density of astrocytic processes in this region and adrenal weight on an individual animal basis, there were no group differences in either adrenal weight or astrocytic process density (Sirevaag *et al.*, 1991). Thus, changes that correlate with adrenal hypertrophy can be distinguished from changes that correlate with experience. This is not to mitigate the substantial effects of significant exposure to stressors on the brain (reviewed in Sapolsky, 2003), which are evident here at the individual level, but rather to indicate that the effects of the complex environment are, by and large, not mediated in this manner.

One way the potential confounds of stress hormones and other, nonspecific hormonal and metabolic changes accompanied by EC exposure can be addressed is to employ paradigms in which the effects of learning are expected to be restricted to specific brain regions, and to compare plasticity in these regions to other ‘control’ regions. This approach has shown plastic anatomical effects of training to be concentrated on the side of the brain to which training is delivered compared to the opposite ‘untrained hemisphere’ (Chang and Greenough, 1982; Greenough *et al.*, 1985) and to distinguish between activity-induced and learning-induced plasticity (Black *et al.*, 1990; Anderson *et al.*, 1996; Kleim *et al.*, 1996; Kleim *et al.*, 1998) (discussed below). Furthermore, specificity of plasticity is seen even in subpopulations of neurons and synapses within the same brain region (Withers and Greenough, 1989; Kleim *et al.*, 1998); for example, EC causes dendritic hypertrophy of cerebellar Purkinje cells but not granule cells (Floeter and Greenough, 1979). Learning and memory remain the most, if not the only, plausible explanation of brain plasticity observed following complex experience because (1) both social and non-specific hormonal and metabolic factors can be ruled out, (2) direct interaction with the environment, not just visual exposure, is required to induce morphological plasticity in the brain (Ferchmin and Bennett, 1975), and (3) the brain undergoes similar remodeling in response to both training paradigms and EC exposure, specifically in regions that are associated with the processing of either environmental or task-related information.

Neuronal plasticity

Synapse number—Synaptogenesis in response to exposure to a complex environment has been demonstrated many times. Animals raised in EC have greater dendritic arborization, increased dendritic spine density and more synapses per neuron in a number of brain areas as compared with IC animals (reviewed by Greenough and Chang, 1988). For example, both dendritic branching and the number of synapses per neuron in the visual cortex are greater in rats raised in EC compared with either IC or SC rats, which are equivalent on this measure (Volkmar and Greenough, 1972; Turner and Greenough, 1985; Sirevaag and Greenough, 1987). Interestingly, the magnitude of these two effects is similar (20–25% increase in EC),

which might indicate that synaptogenesis in the visual cortex in response to visual experience is the result of elongation and/or elaboration of dendritic branches on which new synapses are formed, rather than an increase in the packing density of synapses along an existing length of dendrite. Dendritic elaboration as a result of EC also occurs in other neocortical areas (e.g. Greenough *et al.*, 1973; Kolb *et al.*, 2003) and in the dentate gyrus and area CA3 of the hippocampus, although, interestingly, the direction of the changes in dendritic arbor here varies by sex (Juraska *et al.*, 1985; Juraska *et al.*, 1989). This increase in dendritic length can be detected after as few as 4 days in EC in the visual cortex (Wallace *et al.*, 1992), and contributes to the greater thickness of the visual cortex in EC animals that was reported initially (Bennett *et al.*, 1964). However, increases in dendritic spine density in response to EC exposure are also reported (e.g. Globus *et al.*, 1973; Comery *et al.*, 1995; Rampon *et al.*, 2000; Kolb *et al.*, 2003). Also, repeated *in vivo* imaging of dendritic spines on pyramidal neurons in the adult mouse barrel cortex (using transgenic mice that express green fluorescent protein in a subset of cortical neurons) revealed that spine turnover is influenced heavily by sensory experience (Trachtenberg *et al.*, 2002). Sensory deprivation (whisker trimming) increased the proportion of spines that were transient (present for ≤1 day) and decreased the proportion of spines observed to be stable over several days (Trachtenberg *et al.*, 2002).

Experience-induced synaptogenesis persists well beyond the exposure to EC and can also be induced later in life. A recent study illustrates both findings: rats that were exposed to EC for either 30 or 60 days as adults had significantly (and equivalently) more synapses per neuron in layer IV of the visual cortex than did IC animals of the same age, as revealed by electron microscopy. Importantly, animals that had experienced EC for 30 days and then were placed in individual housing (IC conditions) for a subsequent 30 days also exhibited the full EC effect, reflecting the persistence of EC-induced synaptic plasticity (Fig. 1) (Briones *et al.*, 2004). Similarly, animals raised in EC for 30 days beginning at weaning followed by 30 days of IC housing conditions had comparably greater dendritic arborization of visual cortical neurons (both stellate neurons of layer IV and pyramidal neurons of layer III) compared with IC animals, as did animals that were examined immediately after 30 days of EC housing (Camel *et al.*, 1986). Finally, increases in synapse number and dendritic branching of neurons have been demonstrated in response to EC in aging rats (Green *et al.*, 1983;Greenough *et al.*, 1986).

Training animals results in similar increases in synapses, which indicates that the effects of EC on this measure are a result of the learning process and not merely increases in general activity levels. In a study designed to tease apart morphological changes associated with learning versus those associated with general activity, a group of adult female rats that had been trained on a motor skill-learning task (using a challenging 'acrobatic' course) were compared with animals allowed to exercise freely (on a treadmill) but with minimal opportunity for learning. The number of synapses per neuron in both motor and cerebellar cortices was greater in rats trained on the acrobat course when compared to both rats that exercised but did not engage in learning and to rats who were sedentary during the experiment (Black *et al.*, 1990; Kleim *et al.*, 1996). These learning-induced changes in synapse number persist for at least 4 weeks after training has completed (Fig. 2B) (Kleim *et al.*, 1997). Thus, it appears that learning, not merely neural activity, is required to induce synaptogenesis.

Synapse morphology—In addition to inducing the formation of new synapses, experience can also either modify the morphology of existing synapses or induce the formation and/or loss of synapses exhibiting particular characteristics (reviewed in Greenough and Chang, 1988). Animals exposed to EC have larger synaptic components, both pre- and post-synaptic. For example, the average size of the postsynaptic density (PSD), which is proportional to dendritic-spine volume (Sorra and Harris, 2000), is increased by ~5–8% in the visual cortex following exposure of rats to EC for 1 month beginning around weaning (West and Greenough, 1972; Diamond *et al.*, 1975; Sirevaag and Greenough, 1985; Turner and Greenough, 1985). In

synaptic boutons, the cross-sectional area of vesicle aggregate profiles in layer IV of the visual cortex is ~one-third greater in EC compared with IC rats (SC rats were intermediate) (Sirevaag and Greenough, 1987). The size of the synapse is thought to be related to its strength, and in support of this notion, it has been found that in monocularly-deprived cats, synapses innervated by fibers carrying information from the non-deprived eye are larger, both pre- and post-synaptically, than synapses that are innervated by fibers carrying information from the deprived eye (Tieman, 1984; Tieman, 1985).

In addition to size, the shape of dendritic spines, which are the primary location of excitatory synaptic input onto principal neurons in the neocortex and hippocampus, is important for their function, because shape influences a spine's conductive properties (Sorra and Harris, 2000). Reflective of the spine's relative maturational state, spine shape changes in similar ways over development (from the initial sessile shape, to exhibiting a clearly discernible head or neck and, finally, to the large mushroom shape with a mature spine apparatus) (Harris *et al.*, 1992) and in response to EC (Sirevaag and Greenough, 1985) and to LTP (Chang and Greenough, 1984) (reviewed by Greenough and Chang, 1988). For example, Comery *et al*. (1996) reported 60% greater density of multiple-headed dendritic spines on spiny neurons in the striatum of EC compared with IC rats. On the pre-synaptic side, boutons in animals exposed to EC are more concave than those in IC rats, with SC rats intermediate (Wesa *et al.*, 1982). Additionally, Tieman (1985) showed that in monocularly-deprived cats, synapses associated with the deprived eye are more presynaptically convex than those associated with the open eye. Finally, Dyson and Jones (1980) reported that synaptic contacts in the rat visual cortex become increasingly less convex with age. Therefore it appears that the shapes of both pre- and post-synaptic components indicate the maturational state of a synapse.

Recently, it has become clear that other aspects of synaptic morphology are also sensitive to experience. For instance, perforated synapses (those whose PSD has enlarged, resulting in discontinuities) are associated with synaptic plasticity, in part, because they increase in the visual cortex both across development and in response to EC (Greenough *et al.*, 1978; Jones and Calverley, 1991), in the motor cortex following training on a motor skill learning task (Jones, 1999), and in the hippocampus in response to either kindling or LTP induction (Geinisman *et al.*, 1990; Geinisman *et al.*, 1991). Additionally, postsynaptic expression of AMPA receptors, the number of which is considered to be the major determinant of synaptic efficacy, was found to be a ubiquitous characteristic of perforated synapses in the hippocampus using immunogold electron microscopy (Ganeshina *et al.*, 2004). By contrast, only a fraction (64%) of the non-perforated synapses examined expressed these receptors (Ganeshina *et al.*, 2004). Experience also induces the formation of multiple synaptic boutons (MSBs; two post-synaptic contacts innervated by the same pre-synaptic varicosity). Specifically, animals trained on a motor skill task had more MSBs in the cerebellum than animals who either exercised without the opportunity for learning or were sedentary during the course of the experiment (Federmeier *et al.*, 2002). Similarly, the number of MSBs per neuron that contacted both a dendritic spine and a dendritic shaft were increased greatly in layer IV of the visual cortex of rats exposed to EC for 60 days compared with either SC or IC controls (Jones *et al.*, 1997). From these examples it is clear that the formation of novel dendritic contacts onto existing axonal boutons and varicosities is a common form of experience-driven synaptic plasticity, one that would seem to have the effect of altering the efficacy of a pre-existing pathway rather than creating novel connections. Although the steps leading to the formation of perforated synapses and MSBs, and their ultimate function, is less clear, some interesting hypotheses have been proposed. Carlin and Siekevitz (1983) advanced the model of the dividing synapse to account for the induction of perforated synapses. Initially, the synaptic junction was thought to enlarge, then develop a perforation, then split into two separate synaptic junctions within a single synaptic terminal and, finally, the spine itself would divide into two spines, each containing one synaptic junction. However, when Kristin Harris' group examined synapses

carefully in CA1 of hippocampus using unbiased stereological techniques as applied to electron microscopy, they failed to observe a single branched ('multiple-headed' spine) with the different 'heads' in synaptic contact with the same presynaptic bouton (Sorra *et al.*, 1998), an intermediate stage that is predicted by the splitting hypothesis. Subsequently, they examined the issue of spine splitting directly by serially reconstructing synapses on hippocampal dendrites and the surrounding neuropil across development and in response to hippocampal LTP (Fiala *et al.*, 2002). Their results indicate that the postsynaptic components of MSBs actually frequently arise from separate dendritic processes. When two postsynaptic components from the same dendrite contact the same presynaptic bouton, they do not appear to have derived originally from the same dendritic spine, because other stable structures, such as mature axons, pass between them. This topic is still hotly debated and is an exciting avenue for future research in the neuroanatomical correlates of plasticity.

Neurogenesis—Most neurons in the brain proliferate during gestation and, until recently, the notion that neurogenesis does not occur in the adult mammalian brain (outside of the olfactory bulb) was part of neuroscience dogma. Although there were earlier indications to the contrary (Altman, 1962; Altman, 1963; Kaplan, 1981), these were largely ignored until several key studies were published within the last decade. These studies confirm the phenomenon of adult neurogenesis in the dentate gyrus of rodents and primates; the question of whether neurogenesis occurs in the adult primate neocortex remains controversial (Eriksson *et al.*, 1998; Gould *et al.*, 1999b; Kornack and Rakic, 1999; Gould *et al.*, 2001).

Although the number of neurons added to the adult brain is small in comparison to both total neuron number and glial cell genesis, several environmental factors influence this process. In general, stress (during both development and adulthood), and stress hormones, alcohol exposure and the aging process decrease the number of new neurons added to the adult brain, whereas antidepressants, estrogen, exercise and EC increase it (Gould *et al.*, 1997; Cameron *et al.*, 1998; Kempermann *et al.*, 1998; Tanapat *et al.*, 1999; van Praag *et al.*, 1999; Malberg *et al.*, 2000; Brown *et al.*, 2003; Mirescu *et al.*, 2004). The mechanisms by which voluntary exercise and environmental complexity result in greater numbers of new cells added to the dentate gyrus of the adult rodent appear to be different: exercise increases the rate of neurogenesis, whereas EC exposure promotes the survival of new neurons (Kempermann *et al.*, 1998; van Praag *et al.*, 1999).

Recently, our laboratory has investigated the interacting influences of voluntary exercise and aging on adult neurogenesis for the first time in a primate model. In collaboration with Judy Cameron's group at the University of Pittsburgh, young adult (10–12 years) and mature adult (15–17 years) female *M. fascicularis* monkeys were assigned to one of three treatment groups: Runners, who ran on a treadmill for one hour a day, five days a week, for 24 weeks; sedentary Controls, who sat on an immobile treadmill for an equivalent amount of time for 24 weeks; and Run-Stops, who exercised for 24 weeks exactly as the runners did, but then subsequently sat on an immobile treadmill for the allotted time for an additional 12 weeks. Preliminary results confirm that significant neurogenesis occurs in the dentate gyrus of adult monkeys. Additionally, our findings to date indicate that neurogenesis is increased in this area in young adult monkeys in response to exercise, but that the ability of exercise to increase neurogenesis in this region might be reduced with age (Kohler, Cameron, Williams and Greenough, unpublished).

Aided by the discovery that brain-derived neurotrophic factor (BDNF), which is known to be crucial for use-dependent synaptic plasticity, is common to many of the factors known to impact adult neurogenesis, the potential functions of adult-generated hippocampal neurons are beginning to be explored. Stress is a risk-factor for depression, and both exercise and antidepressants can relieve behavioral correlates of depression (Cotman and Berchtold,

2002). BDNF can alleviate behavioral symptoms in animal models of depression (e.g. Siuciak *et al.*, 1997; Shirayama *et al.*, 2002) and is increased by factors that increase neurogenesis in the adult dentate gyrus (i.e. exercise, estrogen and antidepressants) and decreased by those factors known to reduce neurogenesis (i.e. stress, aging) (reviewed by Cotman and Berchtold, 2002). Furthermore, antidepressant administration either blocks or attenuates the stress-induced decrease of neurogenesis in the dentate gyrus and BDNF levels (Nibuya *et al.*, 1995; Xu *et al.*, 2004). Interestingly, the time-course necessary for antidepressant administration to influence hippocampal neurogenesis is similar to that required for therapeutic benefit (i.e. chronic rather than acute) (Nibuya *et al.*, 1995; Russo-Neustadt *et al.*, 2000). Finally, inhibiting hippocampal neurogenesis blocks the behavioral effects of antidepressant drug administration (Santarelli *et al.*, 2003). Thus, hippocampal neurogenesis might play a role in the behavioral effects of mood-stabilizing factors, such as antidepressants and exercise, in the adult brain.

Increases in BDNF levels have also been found in the dentate gyrus and cerebral cortex of rats housed in EC (e.g. Ickes *et al.*, 2000). Because EC rats exhibit superior learning and memory ability compared to IC rats, and because exercise and EC-exposure during adulthood increase neurogenesis in the dentate gyrus but not in the olfactory bulb (Brown *et al.*, 2003), a learning-specific role for neurons added to the adult brain has also been proposed. It has been shown that training on associative learning tasks that require the hippocampal formation, but not training on hippocampal-independent tasks, increases the number of new neurons in the dentate gyrus (Gould *et al.*, 1999a). Because exposure to EC is known to improve animals' performance on tests of learning and memory, it seems likely that EC-generated hippocampal neurons participate in the improved memory performance. Earlier this year, Rampon's group confirmed the benefit conferred on both memory performance and hippocampal neurogenesis by EC exposure, and furthermore reported that blocking adult neurogenesis with the antimitotic agent methylazoxy-methanol acetate abolished the EC-induced improvement in hippocampal-dependent memory (Bruel-Jungerman *et al.*, 2005). Interestingly, it might be the neurons born prior to the learning experience, and not those generated by the learning experience itself, that are crucial for memory performance. Mild irradiation, which inhibits adult neurogenesis, disrupts performance on the spatial (hippocampal-dependent) version of the Morris water maze (but is without effect on performance of the hippocampal-independent, visible platform version of the maze) when administered 4–28 days before maze training, but not when administered just before or immediately following maze training (Snyder *et al.*, 2005). This finding is perhaps unsurprising in light of the fact that the brain must rely on past experiences to predict future ones. Thus, cells may be added to the adult hippocampus in anticipation of their need to mediate the acquisition, storage, and/or consolidation of future memories.

Astrocytes

Although the focus on environmentally-driven plasticity in the brain has traditionally been on altered morphology of neurons and in particular the synapse, astrocytic glia also demonstrate robust changes in response to experience. Exposure to EC, which was originally designed to investigate the relationship between neuronal and behavioral changes, was also shown in some early studies to induce changes in astroyctic morphology (Diamond *et al.*, 1964; Szeligo and Leblond, 1977). However, limitations in quantification techniques available at the time resulted in inconsistent findings that made subsequent interpretation difficult. With the advent of improved quantification methods, in general referred to as unbiased stereological methodology, EC-induced increases astrocytic-cell size (hypertrophy) and number (hyperplasia) have been confirmed (Sirevaag and Greenough, 1987; Sirevaag and Greenough, 1991).

The hypertrophy of astrocytic processes in response to EC appears to depend on both duration of EC exposure and the cortical layer (reviewed in Jones, 2002). In general, morphological

plasticity of astrocytes in response to EC occurs on a time scale that is comparable to observed neuronal changes in this paradigm. For example, 4 days of EC housing during adolescence increases the surface density of glial fibrillary acidic protein-immunoreactive astrocytic processes in layer II/III of the rat visual cortex (Jones *et al.*, 1996), an exposure duration that induced detectable dendritic growth in this same layer (Wallace *et al.*, 1992). Additionally, after 30 days of differential housing, the astrocyctic volume per neuron in the visual cortex increased in EC rats by an amount comparable to the previously established increase in synapse number (Sirevaag and Greenough, 1985; Jones and Greenough, 1996). This might indicate that the astrocytic hypertrophy in the visual cortex of the EC rat is driven by synapse formation, as is the case in the cerebellar cortex following motor-skill learning (Anderson *et al.*, 1994). Because exercise without skill learning does not induce either synaptogenesis or astrocytic hypertrophy, and astrocytic and synaptic changes in the cerebellar cortex correlate on an animal-by-animal basis, increased astrocytic volume can be inferred to arise in association with learning-specific synaptogenesis, and not merely constitute a response to a general increase in neural activity (Anderson *et al.*, 1994).

It is not just the morphology of astrocytes that is altered by experience; the relationship between astrocytes and neurons is also refined. In the neocortex, astrocytes cover pre- and post-synaptic elements of axodendritic synapses but, typically, only partial covering is observed. The degree of synaptic ensheathement by fine astrocytic processes, as observed by electron microscopy, increases in the visual cortex of EC rats (Fig. 3) (Jones and Greenough, 1996), indicating that the function of demonstrated alterations in astrocytic processes in response to EC is related to enhancing synaptic function. Clearly there is an experience-dependent enhancement of astrocytic–synaptic communication, an important finding in light of the fact that perisynaptic astrocytes modulate synaptic transmission in response to synaptically released neurotransmitters, and themselves release neurotransmitter (Oliet *et al.*, 2001;Zhang and Haydon, 2005). Additionally, astrocytes are involved in GABA and glutamate re-uptake and metabolism (Schousboe *et al.*, 1992;Bezzi *et al.*, 1999) and can conduct excitation via propagated Ca^{2+} waves which can directly influence neuronal activity (reviewed by Zhang and Haydon, 2005). In CA1 of the hippocampus, where nearly 60% of the synapses are contacted directly by astrocytic processes (Ventura and Harris, 1999), postsynaptic glutamate receptors are activated by glutamate spillover from neighboring terminals (in addition to glutamate released from the presynaptic terminal) (see Kullmann *et al.*, 1999). Astrocytic coverage of synapses may thus also serve to enhance input specificity. Although to our knowledge it has not been examined whether activity influences the degree of astrocytic ensheathement of synapses in the hippocampus, it has been demonstrated conclusively in the supraoptic nucleus of the hypothalamus that the degree of synaptic coverage by astrocytes is associated with the degree of glutamate clearance, which, in turn, influences glutamate concentration and diffusion in the synapse (Oliet *et al.*, 2001). Thus the presence of astrocytes at the synapse has a profound influence on synaptic efficacy.

Uniquely anatomically and functionally positioned to manage these diverse roles at the synapse, astrocytes exhibit plasticity that, in addition to neuronal plasticity, might be crucial to the processes of learning and memory. However, their role in enhancing learning-dependent synaptic plasticity may be transient; astrocytic changes appear to fade rapidly in the absence of continued behavioral and environmental stimulation. For example, when animals were first trained on a motor skill learning task for 10 days and then left idle for the following 28 days, training-induced effects on astrocytes were reduced and no longer statistically significant, although synaptogenesis that had occurred during learning remained evident in these animals (Fig. 2A,B) as it paralleled that observed in animals that were trained continuously for the entire 38 days of the experiment (Kleim *et al.*, 1997; Kleim, in revision). It is tempting to speculate that astrocytic changes might be necessary to induce, but not to maintain, adaptive changes in the brain's 'wiring diagram' in response to experience.

Oligodendrocytes and myelination

There is evidence that both oligodendrocytes and the myelination process itself are sensitive to developmental experience. Early work in the optic nerve showed that visual deprivation resulted in reduced myelination (Gyllensten and Malmfors, 1963) and, conversely, that premature eyelid opening accelerated the onset of myelination (Tauber *et al.*, 1980). However, it should be noted that not all studies find a relationship between early visual experience and myelination in the optic nerve (Moore *et al.*, 1976; Fukui *et al.*, 1991). Szeligo and Leblond (1977), who were the first to examine the influence of rearing environment on brain fiber tracts, found increases in oligodendrocytes in the visual cortex of EC rats. Subsequently, Sirevaag and Greenough (1987) also found the volume fraction of oligodendrocyte nuclei in the visual cortex to be greater among EC-raised rats, compared with their IC littermates. The influence of developmental experience on oligodendrocytes is not limited to the visual cortex; raising rats in EC increases the number of myelinated axons (measured using electron microscopy) in the splenial corpus callosum, which contains axons of visual cortical neurons (the responses of these cells to EC are reviewed above) (Juraska and Kopcik, 1988). The positive effect of a complex rearing environment on the size of the corpus callosum has also been demonstrated in rhesus monkeys (Sanchez *et al.*, 1998).

Despite studies indicating that myelination continues well into adulthood in rodents and humans (Yakovlev, 1967; Benes *et al.*, 1994; Nunez *et al.*, 2000), this process is still often treated as if it were a strictly developmental phenomenon. The question of whether myelination remains sensitive to experience during adulthood is interesting and largely unexplored. Recently, our laboratory has begun to investigate this. Preliminary data indicate that the corpus callosum of rats exposed to EC during adulthood contains increased numbers of myelinated axons in the splenial portion and that these changes persist for at least one month after the animal has been removed from the complex environment (Briones, 1999). Thus, the ability of oligodendrocytes and the myelination process to respond to the demands of an animal's environment apparently extends beyond the developmental timeframe, and the experience-induced changes in the adult brain appear to be stable. At this time it is unknown whether the increased myelination in the EC rat is due to an increase in the number of axons that a typical oligodendrocyte helps to myelinate or by an increase in the proliferation and/or survival of new oligodendrocytes. Although many questions remain to be answered, the discovery that adult myelination is regulated by experience provides a functional correlate to continued myelination in the brain across the lifespan.

Several potential, activity-dependent axonal signals have been proposed to initiate myelination, including ATP and the neurotransmitters glutamate and aspartate (receptors for which are expressed by oligodendrocytes) (Butt and Tutton, 1992; Brady *et al.*, 1999; Stevens and Fields, 2000; Stevens *et al.*, 2002). The communication between axons and myelinating glial cells is also bidirectional and myelination leads to local changes in axonal cytoarchictecture (de Waegh *et al.*, 1992). Although the mechanism by which an increase in environmental complexity triggers myelination is uncertain, it is clear that some form of communication between neurons and oligodendrocytes is affected by experience.

One might speculatively view the addition of new axons to the myelinated fiber pool as a form of plasticity with a potential comparable to either the addition or strengthening of synapses, with one additional feature: speed rather than efficacy of communication is enhanced. Typical myelinated fibers, which are generally larger in axonal diameter than non-myelinated axons, conduct at velocities 50–100 times faster than their non-myelinated counterparts (see Brinley, 1980). Assuming a velocity of 60 msec for a large myelinated fiber, conduction of an action potential from one hemisphere to the other might require 1–2 msec, whereas a typical unmyelinated fiber conducting at 1 msec might take 50–100 msec to travel the same distance. During the difference in time of arrival between the myelinated and unmyelinated axonal input,

a typical large cortical pyramidal neuron could have fired a burst of several action potentials in response to the earlier input. Hence, it is hard to imagine that these two fiber types are working in synchrony on the same behavioral and thought processes. Moreover, if an unmyelinated fiber were to be recruited to the myelinated pool, as seems to occur during exposure to a complex environment, its input would subsequently arrive some 50–100 msec earlier than before –seemingly a qualitative difference in an asynchronously operating system in which the response to inputs does not typically wait until all afferents have had their say. In short, where relatively long-distance communication is involved (and for these purposes, communication between adjacent gyri in the human brain would be 'long distance', with disparities of perhaps 30–40 msec between myelinated and unmyelinated fiber inputs), myelinated fibers should largely determine the early response, if there is one. Hence, addition to the myelinated pool connecting brain regions involved in a particular task might qualitatively alter performance. One might wonder about the purpose of the vastly more numerically prevalent unmyelinated fibers. One possibility is that they allow utilization of a richer supply of information in cases where a rapid response is not essential.

Cerebrovasculature

In contrast to earlier studies (e.g. Diamond *et al.*, 1964; Rowan and Maxwell, 1981), data from our laboratory indicate that the brain's vasculature is responsive to experience. Animals raised in EC have larger, more elaborately branched capillaries in the visual cortex compared with IC- and SC-raised animals (Black *et al.*, 1987; Sirevaag *et al.*, 1988; Black *et al.*, 1991). The relative contribution of motor skill learning and increased motor activity to plasticity of cerebrovasculature cannot be distinguished using the EC paradigm. Physical exercise has since been shown to induce angiogenesis in motor brain regions; however, because motor skill learning is required to induce synaptogenesis, it can be inferred that angiogenesis is driven more by the repeated performance of unskilled movements rather than by the learning process *per se* (Black *et al.*, 1990). Also, the plasticity of cerebrovasculature in response to behavioral demands appears to be far greater than that of synapses because the volume fraction of capillaries (which combines diameter and density effects) nearly doubles following EC exposure (Black *et al.*, 1987; Sirevaag *et al.*, 1988). Additionally, the capacity of the vasculature in the primary motor cortex to supply blood in response to increased demand is greater in exercised animals, using functional magnetic resonance imaging to specifically address blood flow (versus the more common BOLD signal arising from deoxyhemoglobin) and reserve capacity under load in anesthetized rats (Swain *et al.*, 2003). Recently, our laboratory has investigated the interacting influences of exercise and aging on cerebrovasculature. Capillary volume in the precentral gyrus was found to be increased in mature (15–17 years) but not young (10–12 years) female *M. fascicularis* monkeys who had exercised for 1 hour a day, 5 days a week for 24 weeks before tissue collection, compared with sedentary controls (Fig. 4) (Rhyu, 2003). Interestingly, capillary volume fraction in monkeys that had a 12 week period of inactivity following the 24 weeks of exercise returned to inactive control levels, indicating that exercise-induced changes in cerebrovasculature are short-lived (Rhyu, 2003). Aside from this study, the persistence of experience-induced angiogenesis has not been well investigated; however the magnitude of the effect and its activity-dependent nature indicate that the impact of experience on cerebrovasculature is likely to be considerably more short-lived than the quite stable changes in synaptic reorganization and myelination discussed above. Because, like exercise, experimentally induced hypoxia also induces rapid angiogenesis (Harik *et al.*, 1995), information concerning blood oxygen levels or a related metabolic demand may be the physiological signal that triggers vascular proliferation. Although the precise signal is unknown, it is known that similar to synaptogenesis, angiogenesis in response to experience is greatest during development, is maintained during adulthood, and remains present, although diminished, during aging (Black *et al.*, 1989a).

CONCLUSIONS

Although much exciting work remains to be done in the field of experience-induced morphological plasticity in the brain, especially in terms of non-neuronal components of the nervous system such as astrocytes, oligodendrocytes and cerebrovasculature, some important observations may be drawn from the information available to date. The most general conclusion that can be made confidently is that the brain is an extremely plastic organ, the structure of which is exquisitely sensitive to experience. A major function of the brain is thus to continuously re-organize itself, and it does so in a way that is specifically tailored to result in behavior that is adaptive in the context of the individual's own unique environment. The nature of experience-driven plasticity is such that, although it has been demonstrated in many brain regions, in any given instance it is specific to those regions involved in processing the behaviorally-relevant features of the environment. For example, dramatic morphological changes occur in the visual cortex in response to a visually complex environment (EC). By contrast, learning a complex motor skill induces plasticity in motor areas of the brain such as the motor and cerebellar cortices. Such region-specific reorganization is not limited to experimental rodent models because it has also been observed in monkeys (e.g. Recanzone *et al.*, 1992) and humans, for example string musicians (see Pantev *et al.*, 2003) and those who have learned to read Braille (Pascual-Leone and Torres, 1993).

Experience-dependent plasticity is not limited to synapses or, even, to neurons. In fact, most if not every component of the nervous system exhibits robust, reproducible responses to experience. Thus, in addition to synaptogenesis, dendritic reorganization, and neurogenesis, other non-neuronal components are sensitive to experience, resulting for example in angiogenesis, increased myelination, astrocytic hypertrophy and increased astrocytic ensheathement of synapses. There are differences in both the type of experience that drive these changes and in their relative stability in the absence of the driving experience. For example, learning results in synaptogenesis, astrocytic hypertrophy and survival of newly generated dentate gyrus neurons, whereas physical exercise without learning induces angiogenesis and dentate gyrus neurogenesis. Changes in synapses and myelination appear to be more stable and, perhaps, permanent, possibly because they reflect re-organization of the brain's functional 'wiring diagram', in comparison to the more generally-acting transient effects on astrocytes and the brain's cerebrovasculature. The stability in morphological changes in synapses and myelination might be due to the fact that the brain must rely on past experiences to predict future ones. Therefore, organizational changes in these components of the nervous system at one point in time are very likely to be useful in the future. Another example in this regard is the addition of new cells in the dentate gyrus. As discussed above, whereas physical exercise increases the rate of neurogenesis, EC and, more specifically, learning, appears to enhance the survival of neurons generated earlier. Thus, cells may be added to the adult hippocampus in anticipation of their need to mediate the acquisition, storage and/or consolidation of future memories. From this brief review of the literature it can be inferred that experience induces multiple forms of plasticity in the brain that must be regulated, at least partially, by independent mechanisms. Finally, it should be emphasized that environmentally-induced plasticity in the brain does not simply consist of changes in different classes of cells independently; rather, interactions between neurons and glia are also altered to more optimally meet behavioral demands. The greater ensheathement of synapses by astrocytes in response to a complex environment is one example of this, and we believe the future of the investigation of environmentally-driven plasticity lies in understanding the integrative response of different brain elements to experience and in discovering the nature of their adaptive significance.

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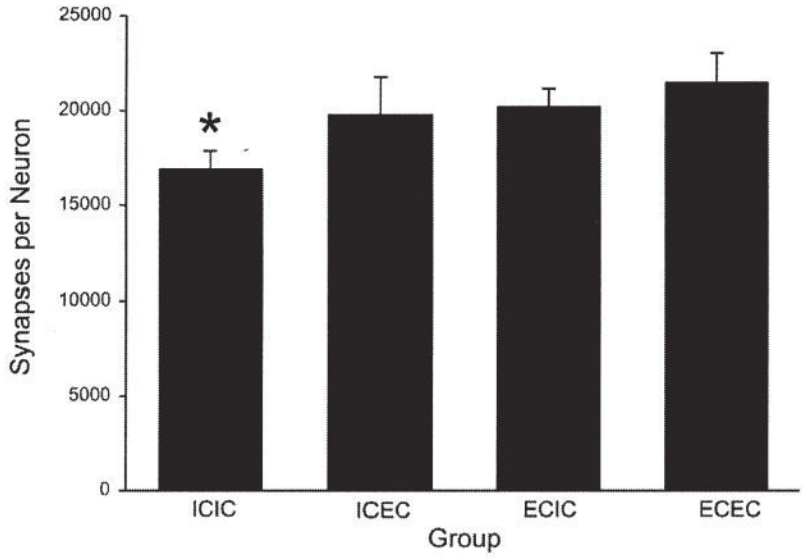

Fig. 1. Persistence of the EC-induced increase in the number of synapses per neuron in the adult rat visual cortex

The EC-induced increase in the number of synapses per neuron in the adult rat visual cortex persists for at least 30 days after animals are removed from EC. ICIC animals, which were individually caged (IC) for 60 days, were significantly different (*, $P<0.05$) from each of the three other groups: ICEC animals (housed in IC for 30 days followed by EC housing for 30 days); ECIC animals (housed in EC for 30 days followed by IC housing for 30 days); and ECEC animals (housed in EC for 60 days). Modified, with permission, from Briones *et al*. (2004).

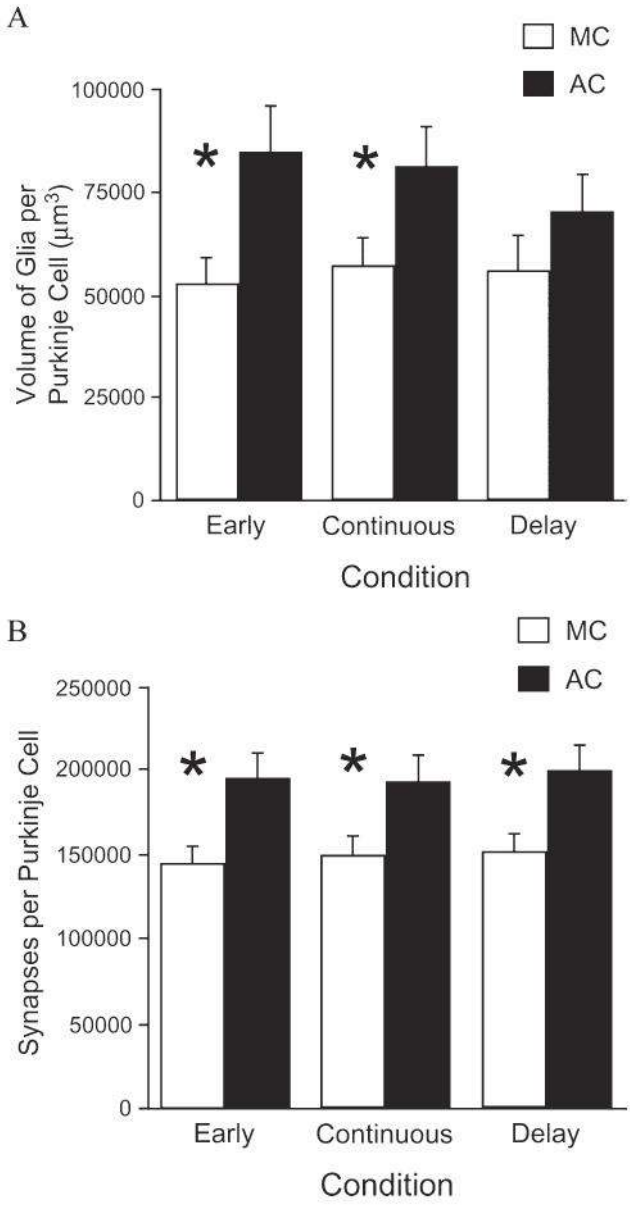

Fig. 2. The glial response to motor-skill learning

The glial response to motor-skill learning (A) is transient and requires persistent motor skill training for maintenance, whereas the increase in synapses per neuron (B) is stable in the absence of continued training. AC (acrobat) rats were trained on a motor skill learning task, whereas MC (motor control) animals ran on a treadmill but were not given an opportunity for learning. Animals in the Early group either participated in training (AC) or exercised (MC) for 10 days, animals in the Continuous group participated for 38 days, and animals in the Delay group participated for 10 days and then training (or exercise) was discontinued for the following 28 days before histological examination. * indicates $P<0.05$ for the comparison between the MC and AC animals of a particular group (Early, Continuous and Delay). Modified, with permission, from Kleim *et al*. (1997) and from Kleim *et al*. (in preparation).

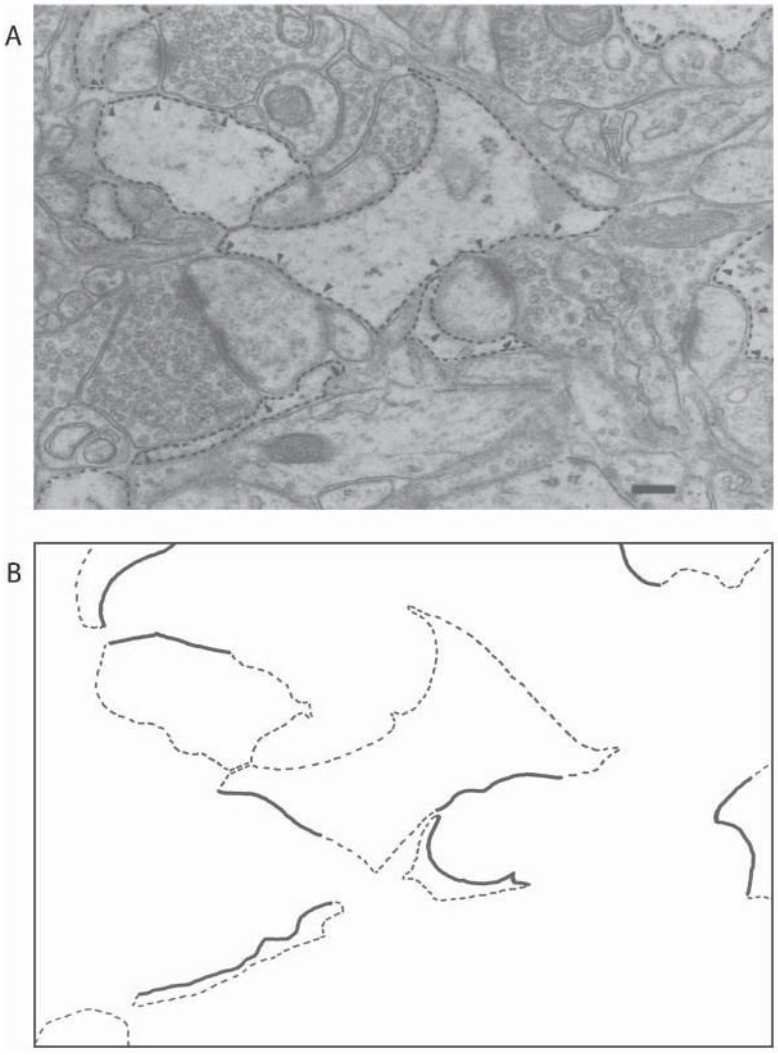

Fig. 3. Astrocytic processes in layer IV of the rat visual cortex

Astrocytic processes (dashed outline) in layer IV of the rat visual cortex revealed by electron microscopy (A), and tracing of these processes (B). Processes in direct apposition to synaptic elements are indicated (arrows, A; solid lines, B). Scale bar, 0.2 μm. Reprinted, with permission, from Jones and Greenough (1996).

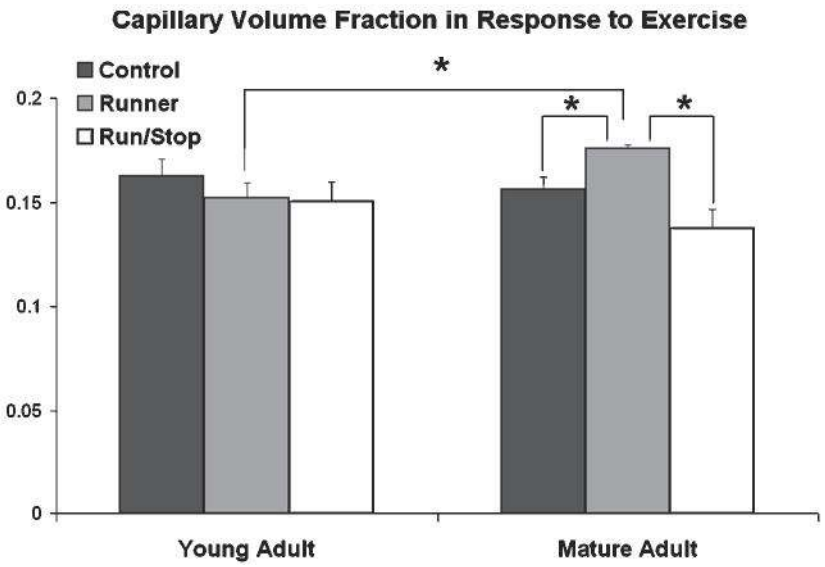

Fig. 4. Exercise increases changes in the vascular volume fraction in the cortex of mature, but not young, monkeys

Exercise increases the vascular volume fraction in the cortex of mature (15–17 years) but not young (10–12 years) monkeys. In mature animals, Runners (exercised for 24 weeks) had greater capillary volume fraction compared with both Run/Stop animals (that had a 12 week period of inactivity following 24 weeks of exercise) and sedentary Control animals. Reprinted, with permission, from Rhyu *et al*. (2003).