I. INTRODUCTION

In the last half of the nineteenth century, scientists in fields ranging from histology to psychiatry were proposing that memory and development were intimately linked to subtle changes in neural processes. Because synapses were not visible with the microscopic techniques available at the time, these connections between neurons became the focus of much speculation. Ramon y Cajal (1893) suggested that learning might involve the formation of new synaptic connections between neurons. Tanzi (1893), noting that the resistance to transmission between neurons might vary with the size of the connection, proposed that frequent use of a synapse might produce growth similar to that produced by exercising a muscle, thereby strengthening preexisting connections. Both theories assumed that the structural plasticity seen in development extended into adulthood, a concept that was not demonstrated until nearly a century later.

Little theoretical or empirical progress was made along this line until Hebb (1949) suggested how experience could be represented in new or modified neural organizations. Rejecting the “one memory — one neuron” concept,
Hebb proposed that memory involved large structures of interconnected neurons, termed cell assemblies. Memory thus became a process more than a place, since encoding and retrieval depended on the cooperation of many neurons rather than a small subset. Hebb, who also felt that developmental plasticity and adult memory might share mechanisms, proposed what is now termed the Hebb synapse, a model synapse with a rule that concurrent pre- and postsynaptic activity increases synaptic efficacy. This basic concept of a cooperative set of modifiable connections as the basis of learning and memory continues to have substantial influence on neural network theory (McClelland and Rumelhart, 1988; Kesner and Rolls, 2001). Initially, alterations in neuronal structure were the focus of investigation; more recently, however, it has become clear that other nervous system components, such as macroglial cells and cerebrovasculature, also exhibit robust plasticity in response to experience. Regardless of the particular brain substrate under investigation, the goal of much scientific effort is to understand the degree to which adult memory processes are related to developmental plasticity (Yuste and Bonhoeffer, 2001).

II. EXPERIENCE-EXPECTANT AND EXPERIENCE-DEPENDENT NEURAL PLASTICITY

The organization of functional brain circuits is governed by the interplay of an individual's genetic makeup and his or her experiences. In Piaget's view (1980), an organism's interaction with its environment results in two types of experiences: those that are commonly shared by all members of a species (general information, or expected environmental features), and individual-specific experiences (unexpected environmental features), which allow the organism to adapt its behavior to its own unique surroundings. It has been proposed that these represent two types of brain information storage, termed experience-expectant and experience-dependent processes, respectively, and that they are supported by different brain mechanisms (Black and Greenough, 1986).

On the one hand, experience-expectant processes mean that the amount of information carried by the genome can be greatly simplified — if an individual member of a given species will almost certainly encounter particular features of the environment during its development, then the neural circuit can be genetically programmed to respond to those features, allowing experience of those features thenceforth to drive development of the system. In this case, the genome is programmed to anticipate particular experiences, which themselves must occur in order for proper development to take place. Thus experience-expectant information storage occurs during a brief window of time during development (termed a critical or sensitive period), which is both species and system specific, when the organism is optimally primed to respond to a par-
ticular type of information. Experience-dependent information, on the other hand, is idiosyncratic for each individual and thus is not able to be anticipated by evolution, so storage of this type of information is not programmed by the genome. Experience-dependent information storage, which includes what is commonly regarded as learning, can happen at any time across the life span and so does not involve a critical period. The brain substrates of experience-expectant and experience-dependent information storage may differ, although it seems likely that some mechanisms would be shared between them. We discuss what is known about these mechanisms following a brief description of the methods used in this research.

III. QUANTITATIVE METHODS IN DEVELOPMENTAL NEUROBIOLOGY

Nerve cell bodies and processes, glial cells, and the brain's vasculature are tightly packed and intertwined in all brain regions. Figure 2-1A is a drawing of a thin section of rat visual cortex in which all tissue components were stained. While larger dendrites, somata, nuclei, and some other features can be observed, it is impossible to identify other parts of the tissue that are associated with any particular cell. Stains such as that used in Figure 2-1A are useful

![Figure 2-1 A. Drawing of a cortical section stained with toluidine blue. Note the tangled packing of glial somata (g), neuronal somata (n), dendrites (d), and capillaries (c). The density and size of the neuronal somata allow the cortex to be divided into six layers. B. Drawing at lower magnification of a Golgi-stained cortical section. Fewer pyramidal (p) and stellate (s) neurons are stained, with complete dendrites reaching across cortical layers. Unstained tissue is relatively transparent.](image)
FIGURE 2-2 The amount of dendritic material for cortical neurons can be analyzed in two ways: (1) The intersections between dendrites and a superimposed series of concentric rings are counted. Here there are six basilar intersections at the fourth ring on the right. (2) The number and length of dendrites at each order are measured. Here there are six basilar segments of third order on the left. The apical dendrite is that emanating from the top, or apex, of the pyramid-like cell body of a pyramidal neuron. Basilar dendrites radiate from its base. A (axon).

for quantifying numbers of neurons, glial cells, blood vessels, etc.; however, since all of them are stained, they are all visible. Figure 2-1B is a drawing from a much thicker section stained via the Golgi method, named after its inventor, the turn-of-the-century anatomist Camillo Golgi, which stains only a few neurons in a region and thus allows their dendrites (and their axons to varying degrees) to be viewed. To obtain accurate light microscopic measurement of neuron morphology, the dendritic field must be traced completely without becoming lost in the tangle of other neural processes. A camera lucida can be used to collect data by superimposing the slide's image onto a two-dimensional drawing, or a computer-aided microscope can record the three-dimensional coordinates of points on the dendritic branches, storing a mathematical representation of the neuron.

Dendritic branches are commonly described in terms of order of bifurcation, as indicated in Figure 2-2. A first-order segment is defined as originating from the soma (cell body), and a second-order segment has its root in the forked end of a first-order segment. Another method of measuring dendritic trees uses a two-dimensional transparent overlay of concentric rings (Sholl, 1956) or concentric spheres for three-dimensional computer-microscope data. The fre-
FIGURE 2-3. Drawings of a synapse before sectioning and of the electron microscope image of the tissue at the plane of section. One can count and classify synapses from micrographs as well as measure the synaptic cleft, postsynaptic thickening, and pre- or postsynaptic areas. Note how difficult it would be to identify which axon and dendrite a synapse belongs to by examining a single electron micrograph.

The frequency of ring intersections indicates dendritic volume distribution. Neurons stained using the Golgi method also allow analysis of dendritic spine numbers, their sizes, and their shapes. Spines are small postsynaptic extensions on the dendrites of many kinds of neurons (see Figure 2-1B and Figure 2-3). The size and shape of spines may affect their conductive properties with regard to the effectiveness of transmission from one neuron to another, and hence the possible modification of spine structure by learning has been investigated in several studies (Chen et al., 2000).

Electron microscopic studies are useful for measurement of the number of synapses and their size and other characteristics. Figure 2-3 illustrates the appearance of synapses in an electron micrograph. Since the thin electron microscopy section cuts through synapses at arbitrary locations, statistical corrections of size and shape must be made. Corrections of apparent synapse density (counts on photomicrographs) must also be made to account for the synapse size distribution, for failure to recognize synapses just barely in the section plane, and for other distorting factors. Mathematically unbiased procedures termed stereological methods must be used to provide accurate estimates of synapse numbers. The result of these corrections, an accurate density estimate (e.g., number of synapses per unit volume), may still be misleading, since various manipulations may change the overall size of the brain region being investigated, such as the visual cortex. When this so-called “reference volume”
cannot easily be determined, for comparison with Golgi data the best measure using electron micrographs is probably the number of synapses per neuron (obtained using the ratio of synapses per unit volume to neurons per unit volume). Electron microscopic estimates of the number of synapses per neuron correspond well to estimates obtained from quantitative studies of Golgi material (Turner and Greenough, 1985; Sirevaag and Greenough, 1987). The electron microscope can also be used as a tool to investigate developmental and experience-induced changes in astrocytes, oligodendrocytes, myelin, and cerebrovasculature.

The technique of two-photon laser scanning fluorescent microscopy was first developed by Denk, Strickler, and Webb (1990). In recent years a number of investigators have employed this technique to engage in the repeated imaging of dendritic spines in the live rodent. Two-photon imaging involves the excitation of a fluorescent marker (termed a fluorochrome) by two photons of longer-wavelength light, rather than a single photon of shorter wavelength (as in one-photon fluorescence imaging). The value of the technique lies in the fact that the excitation of the fluorochrome is limited to a small focal point (rather than the entire sample), thereby greatly reducing the photobleaching and photodamage that have been the major drawbacks of one-photon fluorescence imaging, preventing longitudinal studies. Additionally, the longer wavelength scatters less, allowing for much deeper optical sections to be obtained through the tissue than was previously possible. Cells in cultured brain slices can be labeled with a special protein, such as GFP (green fluorescent protein), which will fluoresce when excited by the proper wavelength (Mainen et al., 1999). To achieve imaging in the live animal, neurons can be labeled in the cortical region(s) of interest while the animal is anesthetized or, alternatively, transgenic mice that express GFP in a small percentage of neurons can be used. In either case, neurons residing in layer II/III or V are typically labeled, because their apical dendrites extend up to layer I (layer I is conveniently located on the outer surface of the cortex but does not itself contain pyramidal neurons), where they can be viewed through a coverslip carefully secured in place of a portion of removed skull (Chen et al., 2000).

IV. NEUROBIOLOGICAL CORRELATES OF THE LEARNING PROCESS

Use of these techniques following early sensory deprivation, general enrichment of the environment, and training tasks has provided evidence that changes in both numbers of synapses and structural characteristics of synapses, as well as changes in nonneuronal components of the nervous system, may be involved in the storage of experiential information. Remarkably similar but still distinct effects have been seen with manipulations directed at the experience-expectant and experience-dependent types of information storage.
A. Early Sensory Deprivation

Because experience-expectant information storage occurs during a sensitive developmental time window, or critical period, the processes underlying such storage can be investigated by withholding the relevant experience during this window. For instance, behavioral and structural effects of early light and visual pattern deprivation have been reported in most mammalian species tested. The effects are most pronounced with monocular deprivation in species with binocularly overlapping visual systems, such as cats and monkeys, but significant effects also occur in largely nonoverlapping species, such as rats.

Animals raised in total darkness are impaired in behavior requiring vision. For example, Walk and Walters (1973) showed that dark-reared animals have long-lasting deficits on a shallow visual cliff, where animals can unwisely choose to step off a small platform. Cats and rodents reared in darkness or unpatterned illumination are slower to learn complex visual discriminations, such as an X versus an N (Riesen, 1965; Tees, 1968). This effect is related to difficulties in visual processing rather than to some general learning disability, since dark-reared animals have no problem associating two auditory stimuli but do have difficulty with an auditory-visual stimulus pair (Tees and Cartwright, 1972). These studies suggest that visual learning of adult animals is impaired if early visual experience has not established effective information-processing schemes.

Depriving a cat of light to one eye (monocular deprivation) during the critical period for visual cortical development results in structural and functional changes in the visual cortex (Wiesel and Hubel, 1965a, 1965b). If this deprivation is maintained for long enough, then even after the animal’s eye is opened it will remain blind in that eye, even though its retina is still capable of transmitting visual information. How does this happen? Prior to deprivation, there is an equal number of cells in the visual cortex that “expect,” or are programmed to respond to, visual input from the left and right eyes. However, for an animal that is monocularly deprived, the expected experience of visual input from both eyes does not occur. Visual cortical organization thus gradually begins to shift in favor of cells responding to the nondeprived eye, and synapses carrying information from the nondeprived eye are maintained, at the expense of synapses from the deprived eye. By the time the deprived eye is allowed to open, few neurons remain capable of responding to the information it is sending.

1. Synapses

What are the neurobiological correlates of impaired visual processing observed in light-deprived animals? Monocular deprivation studies in animals with extensive binocular overlap of the visual fields have indicated involvement of competitive processes in establishing synaptic connections (LeVay et al., 1980).
For instance, cells that respond to input from the left versus the right eye are spatially segregated into neighboring "ocular dominance columns" in the adult animal. When an animal experiences monocular deprivation during the critical period for the development of these columns, however, the functional blindness of the deprived eye is associated with a narrowing of its cortical columns, whereas the cortical columns of the nondeprived eye expand in width (LeVay et al., 1980). This is due to the enhanced regression of axon terminals in columns of the deprived eye coupled with attenuated regression of axon terminals in the columns for the other eye (Antonini and Stryker, 1993). Furthermore, the average axon from a deprived eye has fewer synaptic terminals and smaller pre- and postsynaptic components than those from an experienced eye (Tieman, 1984, 1985). Findings such as these have been attributed to competition between the axon terminal fields, with the more active connections winning out over the deprived. Activity-dependent synaptic competition also underlies the normal development of ocular dominance columns, as visual cortical neurons initially receive inputs from both eyes, and the columns develop over time (LeVay et al., 1978; LeVay et al., 1980). Thus inappropriate connections are routinely made early in development of the visual system that, with continued normal visual experience, are eventually pruned (reviewed in Katz and Shatz, 1996).

Other changes in the visual cortex of light-deprived animals include a reduction in the dendritic fields of pyramidal neurons (Coleman and Riesen, 1968; Valverde, 1971) and potentially delayed development of dendritic spines. Freire (1978) examined the spines of apical dendrites in layer IV of occipital cortex of 19-day-old dark-reared and normal mice with serial-section electron microscopy. The three-dimensional reconstructions indicated that spine development progressed from small spines with no spine apparati (a characteristic structure typically more common in larger spines) to large spines with extensive spine apparati. Dark-reared mice had more of the small-type spines, while normally reared animals had more of the large-type spines. Electron microscopy studies also suggest substantial changes in the number of synapses after visual deprivation. Cragg (1975) found that light-experienced kittens had about 40% more synapses per neuron than binocularly deprived kittens. These findings are compatible with the aforementioned Golgi studies, which suggest a reduction in the number of synapses per neuron with visual deprivation. It is possible that deprivation causes a maturational lag in the formation of synapses for which compensation can later occur. For example, Winfield (1981) showed that binocularly sutured cats eventually catch up with normally reared cats in synapses per neuron, and others have similarly shown that differences between dark- and normal-reared animals become smaller as the animals get older (Cragg, 1967; Valverde, 1971).

Using a more selective type of visual deprivation, it has been found that kittens exposed only to horizontal or vertical stripes during development have
visual cortex neurons that respond selectively to visual stimuli of the exposure orientation (Hirsch and Spinelli, 1970). Layer IV stellate cells of visual cortex from horizontal- and vertical-stripe-raised kittens were not found to differ in dendritic length or number of branches, but the angular distribution of distal dendritic segments were at approximately 90° from each other, just as their stimuli were at right angles (Coleman et al., 1981). Tieman and Hirsch (1982) similarly reported that stripe-rearing modifies the dendrite orientation of Layer III pyramidal cells of kitten visual cortex — horizontal-stripe-reared cats and vertical-stripe-reared cats had approximately perpendicular distributions of dendritic orientation, suggesting a specific relationship between the morphology of Layer III pyramidal cells, their physiological orientation, and their early experience.

Visual deprivation studies collectively indicate that early visual experience substantially affects experience-expectant neural plasticity and that these effects impair later experience-dependent visual learning. Functional and neurobiological changes in the brain in response to sensory deprivation are not limited to the visual system. In fact, it appears that competition for survival between synapses is the rule rather than the exception in brain development and that such competition is what drives the initial formation of mature neural circuits in the normal individual. In addition to the work conducted in the auditory and olfactory systems (e.g., Coss et al., 1980; Feng and Rogowski, 1980), a large literature also exists using somatosensory deprivation as a model system in which to investigate experience-expectant plasticity, primarily using the whisker system of rodents and the corresponding barrel cortex (in which each whisker is represented by a highly specialized functional unit, termed a *barrel*) (Fox, 2002). The topographic map linking one whisker to a single barrel in the somatosensory cortex that is maximally responsive to it develops early, perhaps before postnatal day 5, and is resistant to disruptions after this time (O'Leary et al., 1994; Shepherd et al., 2003). Other aspects of barrel cortical development continue to mature at later time points; in fact, most of the synaptic circuitry in this cortical region occurs after the barrels are formed (see Shepherd et al., 2003). For instance, synaptic responses of layer II/III neurons to whisker deflection are undetectable at postnatal day 12; however, two days later, on day 14, receptive fields of these cells are of a mature organization (Stern et al., 2001). Sensory deprivation prior to day 14 disrupts receptive field organization. The same study reported that receptive field properties of layer IV neurons, on the other hand, are mature by day 12 and are actually unaffected by deprivation at this time. This example illustrates that multiple critical periods exist in the barrel cortex, the timing of which can be cortical layer dependent.

Recently, it has become possible to monitor changes in dendritic spine shape and mobility over relatively long periods of time following sensory deprivation, both in the live animal and in cultured tissue, through the use of two-photon
fluorescent imaging. On the whole, these studies confirm the findings obtained using traditional neuroanatomical quantification techniques regarding deprivation-induced neuronal plasticity. Just as with the studies discussed earlier, when approaching this literature it is important to keep in mind when the deprivation occurred—whether it was prior to, during, or after the critical period for the development of that system (whether visual or somatosensory).

In the mouse visual cortex, Grutzendler and colleagues (2002) have repeatedly imaged the same dendritic segments over a number of weeks and found that highly transitory, filopodia-like dendritic protrusions are abundant in young animals but nearly absent in the adult. During the critical period for visual cortical development, the majority of observed changes were due to spine elimination, consistent with activity-dependent refinement of circuitry, which is known to occur during this time. In contrast, the overwhelming majority (about 96%) of spines in adult mice were stable over the one-month period. Similarly, Majewska and Sur (2003) found that spine motility was high at young ages, decreased between postnatal days 21–28 [the height of the critical period (Gordon and Stryker, 1996)], and then remained stable through day 42. Binocular deprivation before eye opening (which occurs on day 13) greatly increased the motility of spines during the critical period (day 28) but not at the beginning of (day 21) or after (day 42) the critical period. On the other hand, they did not observe high rates of spine turnover during the study's two hours of observation, and there was no difference in this measurement between groups, although it is not clear if the trend toward increased turnover in deprived mice at the critical period might have reached statistical significance had the imaging observation window exceeded two hours (Majewska and Sur, 2003).

Similar to the pruning of spines during the critical period in the visual cortex (Majewska and Sur, 2003), a recent study using in vivo imaging of spines also found spine turnover on the apical tufts of layer II/III somatosensory neurons, but a net loss of spines between postnatal days 16–25 (Holtmaat et al., 2005). The degree of spine turnover gradually reduced over development, and the proportion of stable spines (those with a lifetime of 8 days or more) gradually increased over development (Holtmaat et al., 2005). Zuo et al. (2005b) confirmed this net loss of spines (13–20% reduction compared to 5–8% formation) over two weeks in the barrel cortex and also in motor and frontal cortices, indicating that synaptic pruning occurs in many different cortical areas during this time. In comparison, only 3–5% of spines were either eliminated or formed over a two-week period during adulthood; and after an amazing 18 months, only 26% were observed to be eliminated and 19% formed in adult barrel cortex (Zuo et al., 2005b). It is important to remember that these mice were housed in relatively uninteresting environments during their lifetimes and did not experience any sensory deprivation; manipulations of either sort would be expected to alter spine numbers considerably over time.
Interestingly, the results of a recent in vivo imaging study suggest that sensory deprivation may impair activity-dependent pruning in the somatosensory cortex. In adolescent mice, whisker trimming prevented the loss of spines on layer V apical dendrites, which normally occurs during this time period, by reducing the rate of spine elimination (rather than increasing the formation of new spines) (Zuo et al., 2005a). In another study, Trachtenberg and colleagues (2002) repeatedly imaged dendritic spines on layer V pyramidal neurons of mice aged 34–74 days, both prior to and following selective unilateral whisker trimming. Deprivation increased the proportion of spines that were transient (present for a single day or less) and decreased the proportion of spines that remained stable over several days, indicating that, in addition to spine motility, spine turnover is heavily influenced by sensory experience. At the conclusion of the imaging portion of the experiment, they processed the brain tissue for electron microscopy and confirmed that the observed growth of new spines corresponded to synaptogenesis and that the retraction of spines corresponded to synapse elimination (Trachtenberg et al., 2002). The authors interpreted the deprivation-induced increased turnover of spines as a response to the destabilization of previously stable synapses.

Collectively, these findings indicate that spine motility is greatest when input is either immature or not present and that synaptic activity stabilizes spines. In support of this, in vitro work has shown that spines are stabilized by calcium influx into the spine head following synaptic activation by either AMPA or NMDA receptors (Fischer et al., 2000; Korkotian and Segal, 2001). It is important to note, however, that not all studies find that deprivation increases spine motility. For instance, Lendvai and colleagues (2000) also found that spines and filopodia of layer II/III neurons imaged in vivo were highly motile. However, in this case, trimming of all major whiskers (on one side) between postnatal days 11–13 was found to reduce the motility of spines by approximately 40% but was without effect in younger (days 8–10) or older (days 14–16) animals. The differences between Lendvai et al. (2000) and Trachtenberg et al. (2002) may be due to methodological discrepancies: Different cortical layers were imaged, different ages were examined, and the pattern of whisker deprivation differed, with Lendvai et al. trimming all major whiskers and Trachtenberg et al. trimming approximately half of the whiskers, in a "chessboard" pattern of deprivation. Future studies are necessary to resolve these differences. Regardless of the details, the in vivo imaging studies to date are generally compatible with static anatomy results, in that they suggest activity-dependent mechanisms regulate aspects of synapse development, especially during a critical period. Static anatomical measurements do appear to have underestimated the degree of synapse elimination/turnover that may be involved in generating and maintaining such activity-dependent connections. Nevertheless, findings resulting from both traditional neuroanatomical techniques and in vivo imaging support a model in which synaptogenesis early in
FIGURE 2-4  Electron micrographs taken from the splenium of the rat corpus callosum (bar = 0.6 μm). At postnatal day 15 (a), just prior to the onset of myelination, many unmyelinated axons can be observed. At day 25 (b), myelination is under way and myelin rings can be observed around several large axons here. By two months of age (c), which corresponds to young adulthood, the amount of area occupied by myelinated axons rivals or exceeds that occupied by unmyelinated axons, although the difference is due to the greater size of myelinated axons rather than number. [From Kim and Juraska (1997). Reprinted with permission from Developmental Brain Research.]

development is followed by activity-dependent pruning of connections, with spine synapses become permanent through transformations that are evident in morphology.

2. Glia and Cerebrovasculature

In addition to synaptic changes, there is good evidence that the myelination process, whereby axons are covered in a fatty insulating sheath, is also sensitive to experience during critical periods of development. Myelinated axons can conduct action potentials at rates that are 50–100 times greater than those accomplished by their unmyelinated counterparts; thus proper myelination is an important part of information processing in the nervous system. The number of myelinated axons increases greatly over development and continues (at a slower rate) into adulthood (Figure 2-4), raising the possibility that the myelination process may be sensitive to experience across the life span. Visual deprivation results in reduced myelination of the optic nerve (Gyllensten and Malmfors, 1963), whereas, conversely, premature eyelid opening accelerates the onset of myelination in this structure (Tauber et al., 1980). Although not all studies have found a relationship between early visual experience and myelination in the optic nerve (see Moore et al., 1976; Fukui et al., 1991), there is additional evidence using other techniques for a relationship between axonal activity and myelination. For example, Demerens and colleagues (1996) have shown that experimentally increasing axonal activity promotes optic nerve myelination, and blocking of action potentials with tetrodotoxin inhibits myelination. Furthermore, cortical axon branch formation as well as proliferation of precursors to oligodendrocytes (the glial cells responsible for myelin
formation) are both activity-dependent processes (Barres and Raff, 1993; Uesaka et al., 2005). A number of signaling molecules are known to be released by axons; however, the molecular mechanisms responsible for inducing activity-dependent myelination are essentially unknown. Potential activity-dependent initiators of myelination include the neurotransmitters glutamate and γ-aminobutyric acid (GABA), receptors for which are expressed by oligodendrocytes (Butt and Tutton, 1992), adenosine triphosphate (ATP) (Fields and Stevens, 2000), and neuregulin (Taveggia et al., 2005). Regardless of the mechanism, the fact that early experience can influence myelination indicates that oligodendroglia are sensitive to manipulations of expected features of the environment during a critical window of development.

Maturation of astrocytes, another type of macroglial cell in the brain, is also disrupted in response to sensory deprivation during a critical period. Dark rearing reduces the size and number of astrocytes in the visual cortex (Gabbott et al., 1986; Stewart et al., 1986; Muller, 1990; Argandona et al., 2003). Hawrylak and Greenough (1995) also found that monocular deprivation during the critical period reduces the surface density of astrocytic processes as well as the ratio of these processes to neurons; however, deprivation during adulthood was without effect on these measures. Although the plasticity of astrocytes in response to visual deprivation has received less attention than that of neurons, astrocytic plasticity in fact appears to play an important role in the formation of ocular dominance columns. The evidence for this comes from studies in which astrocytic function was impaired, either by a suppression of metabolic activity of these cells (Imamura et al., 1993) or by a disruption of the astrocytic-specific protein S-100β (Muller et al., 1993), both resulting in an unusually high proportion of cells that stubbornly maintained binocular responses following monocular deprivation, indicative of a failure in activity-dependent selection of connections. Similarly, astrocyte-neuron interactions appear to be crucial to the maturation of the somatosensory system. For instance, whisker stimulation-induced neuronal uptake of glucose (an indication of activity) is greatly impaired in the barrel cortex of mice lacking a functional gene for either of the two glial glutamate transporters (GLT-1 orGLAST knockout mice) (Voutsinos-Porche et al., 2003a). Glial glutamate transport is involved in triggering enhanced glucose utilization by neurons and in other aspects of metabolic crosstalk between neurons and glia (Voutsinos-Porche et al., 2003b).

Finally, there are other changes that occur in response to the reduction in metabolic demands that accompany the loss of sensory experience in the brains of light-deprived or whisker-trimmed animals. Argandona and Lafuente (1996) have observed that dark rearing reduces the density of cerebrovascular elements in the visual cortex. They also report that normal vascular development in the visual cortex involves the early presence of a number of vertically oriented vascular “trunks,” the density of which is greatly decreased after birth in
light-reared rats but remains elevated in dark-reared rats (Argandona and Lafuente, 1996). More recently, these authors have employed unbiased stereological techniques to determine that, despite the reduced density of blood vessels, the ratio of vessels per neuron to vascular area per neuron remains stable in the visual cortex of dark-reared animals (neuronal density was also reduced in the dark-reared animals, possibly due to increased apoptosis in these animals) (Argandona and Lafuente, 2000). Thus the changes in the vasculature in the visual cortex in response to deprivation are consistent with reduced metabolic demand.

It is clear that experience-expectant mechanisms have a complex role. For example, in the mammalian visual system, the simple presence of light does not trigger complete maturation of the visual system. Rather, coherent experience of a range of features and relationships is required. The requirement for early coherent experience ultimately affects experience-dependent processes, since, as discussed earlier, young animals deprived of sensation in a given modality are impaired as adults in learning situations requiring that modality.

B. Manipulation of the Complexity of the Environment

The initial overproduction of synapses that occurs in many brain areas during development and that is part of the brain's experience-expectant plasticity allows for subsequent experience-dependent remodeling of synaptic architecture that is exquisitely tailored to allow optimal functioning in the individual's own unique environment. A large body of work has been conducted to elucidate the morphological changes in the brain that accompany such experience-dependent information storage. Much of this work has come from studies conducted on rodents raised in a complex (also called "enriched," relative to standard lab cages), stimulating environment. 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 together in a large cage containing numerous toys, such as balls, tunnels, and ladders, which are changed daily to provide a continuously stimulating environment (Figure 2-5). Comparisons of both behavior and brain structure are then made between animals housed in a complex environment (EC), those housed in a normal cage in isolation (IC), and those housed in a normal cage but with other animals (SC, or social condition). Importantly, as we will discuss, components of the nervous system other than synapses (such as glial cells and vasculature) are also sensitive to experience.

The behavioral effects of differential rearing are profound. It was Hebb (1949) who first reported that rats raised as pets at home were cognitively superior to laboratory rats. In general, rats raised in complex laboratory envi-
environments have been found superior to isolated or socially raised rats on memory tests, including the Hebb–Williams maze (Mohammed et al., 1986; Galani et al., 1997), the Morris water maze (Whishaw et al., 1984; Mohammed et al., 1990; Leggio et al., 2005), and the radial arm maze (Galani et al., 1998). Greenough, Wood, and Madden (1972) argued that the information-processing capability of EC mice was superior to that of IC or SC mice because they were uniquely capable of mastering the difficult Lashley III maze when trials were run immediately after one another. While learning a Hebb–Williams maze, ECs make fewer errors than IC rats. But that superiority vanishes if the maze is rotated, effectively disrupting extramaze cues (Hymovitch, 1952; Brown, 1968). Ravizza and Herschberger (1966), however, found EC rats better at maze learning even if extramaze cues were hidden by a curtain, suggesting that intramaze cues can also be better utilized by ECs in the absence of extramaze cues. Interestingly, the superior performance of EC animals is not due simply to greater visual experience, since Krech, Rosenzweig, and Bennett (1962) found blinded EC rats superior to blind IC rats in maze performance.

1. Synapses

Bennett, Diamond, Krech, and Rosenzweig (1964) first reported that several cortical regions were heavier and thicker in EC rats than in IC rats, particularly
FIGURE 2-6 Numerical density of synapses and neuronal nuclei and ratio of synapses to neurons in upper occipital cortex (layers I-IV) of rats reared from 23 to 55 days of age in environmental complexity (EC), in pairs in social cages (SC), or in individual cages (IC). Synapses were counted in conventionally stained (osmium-uranyl-lead) electron micrographs and the counts corrected for differences in size using stereological formulae. Neuronal nuclei were estimated by point counting in toluidine blue-stained light microscopic sections. Reduced neuronal density in more experienced animals reflects the greater volume of neuronal processes, glia, vasculature, neuronal somata, etc. that accompanies the new synapses. [Data from Sirevaag and Greenough (1985); figure from Turner and Greenough (1985). Copyright 1985, Elsevier Science Publishers, reprinted with permission.]

Since then it has been shown that 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 Markham & Greenough, 2004). Holloway (1966) first reported that ring analysis (Figure 2-2) of visual cortical neurons indicated larger dendritic fields in rats reared in EC. Greenough and Volkmar (1973) placed rats in the EC, SC, and IC conditions at 23–25 days of age for 30 days. Pyramidal neurons from Layers II, IV, and V and Layer IV stellates in visual cortex had more ring intersections in ECs than in ICs. These effects were most pronounced in higher-order branches — the outer part of the dendritic field. To confirm that these dendritic differences reflected synapse number differences, Turner and Greenough used electron microscopy to show that EC rats exceeded ICs in synapses per neuron in upper visual cortex by roughly the amount predicted from the Golgi studies, with SCs intermediate but somewhat closer to the ICs (Figure 2-6) (Turner and Greenough, 1983; 1985). Thus quantitative Golgi procedures appear to accurately indicate differences in synaptic numbers. Dendritic elaboration (e.g., Greenough et al., 1973; Kolb et al., 2003; Leggio et al., 2005) and increased spine density (Moser et al., 1994) as a result of EC also occurs in other neocortical areas and in the dentate gyrus and area CA3 of the hippocampus, although interestingly the direction of the
changes in dendritic arbor in the latter has been found to vary by sex (Juraska et al., 1985, 1989). The increase in dendritic length can be detected after as few as four days in EC in the visual cortex (Wallace et al., 1992), and it contributes to the greater thickness of the visual cortex among EC animals that was initially reported (Bennett et al., 1964). The fact that EC induces plasticity in nonvisual cortical areas indicates that the effects do not merely result from visual stimulation. On the other hand, EC-induced dendritic elaboration is not a ubiquitous phenomenon, for Greenough and colleagues (Greenough et al., 1973) did not find this effect in frontolateral (sensorimotor) cortex (but did in visual and auditory cortices), suggesting that general hormonal or metabolic factors, which would be expected to affect all cortical areas, do not play a significant role in mediating EC-induced neuronal plasticity. The lateralized effects of training on the cortex (discussed later) also argue against general hormonal and metabolic effects. (For a more comprehensive review of this topic, see Grossman et al., 2002.) Finally, reports of EC–IC differences in cerebellar cortex (Floeter and Greenough, 1979; Pysh and Weiss, 1979), superior colliculus (Fuchs et al., 1990), and striatum (Comery et al., 1995) indicate that the experience-dependent effects of rearing complexity are not restricted to phylogenetically newer brain structures.

In addition to inducing the formation of new synapses, manipulations of environmental complexity can also modify the morphology of existing synapses (or, alternatively, induce the formation and/or loss of synapses exhibiting particular characteristics) (reviewed by Greenough and Chang, 1988). The morphology of dendritic spines and synapses has been shown to be important for their function, including synaptic efficacy, conducive properties, and biochemical compartmentalization (Sorra and Harris, 2000; Tsay and Yuste, 2004; Noguchi et al., 2005); thus interest in these factors stems from the idea that they may ultimately play a role in learning and memory. EC animals have, on average, larger pre- and postsynaptic components, including postsynaptic densities (PSD) and cross-sectional area of presynaptic vesicle aggregate profiles (West and Greenough, 1972; Diamond et al., 1975; Sirevaag and Greenough, 1985, 1987; Turner and Greenough, 1985). Reflective of the maturational state of a spine on a cortical neuron, spine shape changes in similar ways (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) over development (Galofre and Ferrer, 1987) and in response to EC (Sirevaag and Greenough, 1985) and long-term potentiation (LTP, a synaptic model for memory) (Chang and Greenough, 1984). The proportion of perforated synapses (those in which the PSD has enlarged and assumed a more complex shape, such as a horseshoe or doughnut) also increases in response to EC (Greenough et al., 1978; Jones and Calverley, 1991) as well as in the hippocampus in response to kindling or LTP induction (Geinisman et al., 1990, 1991). Perforated synapses are
characterized by the incorporation of greater numbers of glutamate receptors (both AMPA and NMDA subtypes) in the PSD, which may enable them to evoke larger postsynaptic responses (relative to nonperforated synapses) and thereby enhance synaptic plasticity (Ganeshina et al., 2004a, 2004b).

On the presynaptic side, boutons in animals exposed to EC are more concave than those in IC rats, with SC rats being intermediate (Wesa et al., 1982). EC also increases the number of multiple synapse boutons (MSBs; two postsynaptic contacts innervated by the same presynaptic varicosity). Comery et al. (1996) reported 60% greater density of multiple-headed dendritic spines on spiny neurons in the striatum of EC as compared to IC rats. Similarly, the number of MSBs per neuron that contacted both a dendritic spine and a dendritic shaft were greatly increased in layer IV of the visual cortex of rats exposed to EC for 60 days as compared to 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 or varicosities is a common form of experience-driven synaptic plasticity, one that would seem to alter the efficacy of a preexisting pathway rather than creating novel connections (further discussed later, in subsection C: Skill Learning).

2. Glia and Cerebrovasculature

The differences in synaptic number and morphology in response to a complex environment are accompanied by differences in supportive tissue components, such as glial cells and blood vessels. Some early studies indicated that EC resulted in changes in astrocytic morphology (Diamond et al., 1964; Szeligo and Leblond, 1977); since that time, EC-induced increases in astrocytic cell size (hypertrophy) and number (hyperplasia) have been confirmed using unbiased stereological techniques (Sirevaag and Greenough, 1987, 1991) (reviewed in Jones, 2002). In general, it has been found that morphological plasticity of astrocytes in response to EC occurs on a time scale that is comparable to neuronal changes observed in this paradigm (Jones et al., 1996; Sirevaag and Greenough, 1985; Jones and Greenough, 1996), raising the possibility that experience-dependent changes in nonneuronal components of the nervous system could contribute to the behavioral changes observed in animals exposed to EC.

Plasticity of astrocytes is gaining more attention because there is increasing evidence for their role in synaptic function (see Volterra et al., 2002). Interestingly, the degree of synaptic ensheathement by astrocytic processes is increased by exposure to EC (Figure 2-7) (Jones and Greenough, 1996). This experience-dependent enhancement of astrocytic-synaptic communication is an important finding in light of the fact that perisynaptic astrocytes modulate synaptic transmission in response to synaptically released neurotransmitters and, in fact, themselves release neurotransmitters (Oliet et al., 2001; Zhang
and Haydon, 2005). Astrocytes are also involved in GABA and glutamate reuptake 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). Astrocytic coverage of synapses may thus also serve to enhance the input specificity of information, thus facilitating learning and memory.

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. The influence of developmental
experience on oligodendrocytes is not limited to the visual cortex. An electron microscopic study (Juraska and Kopcik, 1988) found that raising rats in EC increases the number of myelinated axons in the splenial portion of the corpus callosum, which contains axons of visual cortical neurons that carry information between the two cerebral hemispheres. The positive effect of a complex rearing environment on the size of the corpus callosum (size of a fiber tract is typically correlated with the degree of myelination) has also been demonstrated in rhesus monkeys (Sanchez et al., 1998).

Finally, the brain's vasculature is also quite responsive to manipulations of environmental complexity. Animals raised in EC have larger and more elaborately branched capillaries in the visual cortex, compared to both IC- and SC-raised animals (Black et al., 1987; Sirevaag et al., 1988; Black et al., 1991). Also, the plasticity of cerebrovasculature in response to behavioral demands appears to be far greater than that of synapses — volume fraction of capillaries (which combines diameter and density effects) nearly doubles following EC exposure (Black et al., 1987; Sirevaag et al., 1988). This effect is likely more related to satisfying the increased metabolic demands of neurons (undergoing dendritic arborization and activity-dependent synaptogenesis) and glial cells (undergoing hypertrophy, hyperplasia, and, in some cases, myelin synthesis) in response to new behavioral demands than to accomplishing new memories per se. The permanence of EC-induced angiogenesis has not been examined, but the effect is likely to be transient, for exercise-induced alterations in the brain's vasculature do not persist for very long beyond the period of increased physical activity (Rhyu et al., 2003). Because, like exercise, experimentally induced hypoxia induces rapid angiogenesis (Harik et al., 1995), indicators of blood oxygen levels and/or a related metabolic demand may serve as physiological signals that trigger vascular proliferation. Although the precise signals are unknown, it is known that, similar to synaptogenesis, angiogenesis in response to experience is greatest during development, also occurs during adulthood, and remains present, although diminished, during aging (Black et al., 1989).

3. Plasticity in the Adult Brain

The neocortex retains considerable structural plasticity in response to differential housing into adulthood, as we would expect for experience-dependent mechanisms designed for learning. EC/IC branching differences of 10% or more, nearly equivalent in magnitude to those described in animals exposed as weanlings, have been found in the adult visual cortex (Greenough and Volkmar, 1973; Uylings et al., 1978; Juraska et al., 1980). Briones et al. (2004) have observed that rats exposed as adults to EC for either 30 or 60 days had significantly more synapses per neuron in layer IV of the visual cortex than did IC animals of the same age, as revealed by electron microscopy (Figure 2-8). The increased synapse number was not diminished by a subsequent
FIGURE 2-8 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 a complex environment (EC). ICIC animals were individually caged (IC) for 60 days and 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 from Briones et al. (2004), with permission.]

period of 30 days of IC housing. Increases in dendritic branching, synapse number, and number of synapses per neuron have also been demonstrated to occur in response to EC in aging rats (Green et al., 1983; Greenough et al., 1986). Thus, in contrast to the greatly diminished effects of sensory deprivation during adulthood, the environmental complexity studies in adult animals clearly suggest that this kind of experience alters the neurons in the adult neocortex in a similar way to that seen in young animals. On the other hand, the production of new blood vessels appears to be substantially impaired in middle-aged rats, and continuing progressive failure of new blood vessel production may restrict their capacity for storing information in the form of new synapses when they reach old age (Black et al., 1989).

4. Adult 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, 1963; Kaplan, 1981), these were largely ignored until several key studies were published within the last decade. These studies confirmed the occurrence of adult neurogenesis in the
dentate gyrus of the hippocampus of both rodents and primates (Kuhn et al., 1996; Kempermann et al., 1997; Eriksson et al., 1998; Kornack and Rakic, 1999). Whether neurogenesis occurs in the adult neocortex remains controversial, with some reports of substantial neurogenesis (Gould et al., 1999a, 2001; Dayer et al., 2005) and others reporting little or no detectable production of new neurons, although proliferation of astrocytes and other non-neuronal cell types was detected (Kornack and Rakic, 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 have been shown to influence this process. In general, stress, glucocorticoids, and alcohol exposure — experienced either during pre- or postnatal development or during adulthood — and the aging process all decrease the number of new neurons added to the adult brain, whereas antidepressants, estrogen, environmental stimulation, and exercise all increase it (Kuhn et al., 1996; Gould et al., 1997; Cameron et al., 1998; Tanapat et al., 1999; van Praag et al., 1999; Malberg et al., 2000; Mirescu et al., 2004; Crews et al., 2006; Redila et al., 2006; Wong and Herbert, 2006).

Housing in a complex environment is one experience that can robustly induce neurogenesis in the adult dentate gyrus. Kempermann, Kuhn, and Gage (1997) were the first to demonstrate that adult mice housed in EC have an approximately 15% increase in the number of new cells in the dentate gyrus of the hippocampus (an effect that is reflected in an increased size of the granule cell layer in this structure). This finding has since been replicated by this laboratory and others in both mice (Kempermann et al., 1998b; Kempermann and Gage, 1999; J. Brown et al., 2003) and rats (Nilsson et al., 1999; Bruel-Jungerman et al., 2005). Although a decline in neurogenesis occurs during normal aging (Kuhn et al., 1996), the dentate gyrus remains sensitive to environmental stimulation, and EC housing can still increase the number of new hippocampal neurons during senescence (Kempermann et al., 1998a). Conversely, social isolation (IC) during adulthood reduces basal levels of neurogenesis and prevents the normal exercise-induced increase in neurogenesis (Lu et al., 2003; Stranahan et al., 2006). Importantly, the mechanisms by which environmental factors result in greater numbers of new cells added to the dentate gyrus of the adult rodent can vary: Some factors, such as exercise, tend to increase the rate of neurogenesis directly, whereas exposure to EC and learning tend to increase the survival of newly generated cells rather than affecting the rate at which cells are born (Kempermann et al., 1998a; van Praag et al., 1999; Ambrogini et al., 2000; Olson et al., 2006).

Because rodents exposed to EC exhibit superior learning and memory ability as compared to IC rats, and because EC exposure during adulthood increases neurogenesis in the dentate gyrus of the hippocampus but not in the olfactory bulb (J. Brown et al., 2003), a learning-specific role for neurons added to the adult brain is suggested. There is evidence that adult neurogenesis
and learning are indeed correlated. IC animals, in which hippocampal neurogenesis is reduced, also show a reduction in LTP in the hippocampus and impaired performance on Morris water maze spatial learning (which relies on the hippocampus); all three phenotypes are reversible by subsequent group housing (Lu et al., 2003). Conversely, EC-induced neurogenesis is associated with an improvement in water maze learning (Nilsson et al., 1999). In aged rats, a reduced survival of newly proliferated hippocampal neurons is associated with impairment in another form of learning that relies heavily on the integrity of the hippocampus, called contextual fear conditioning (Wati et al., 2006). Similarly, performance of aged rats on the spatial version of the Morris water maze is predictive of the level of hippocampal neurogenesis (Drapeau et al., 2003). Removal of the olfactory bulbs or developmental lead exposure both result in decreased adult hippocampal neurogenesis and impaired contextual fear conditioning (Jaako-Movits and Zharkovsky, 2005; Jaako-Movits et al., 2005).

Sleep deprivation, which (in rats, as in humans) disrupts memory, has also been shown to profoundly reduce adult hippocampal neurogenesis (Guzman-Marin et al., 2005). In fact, sleep deprivation can prevent neurogenesis from being induced by spatial water maze learning (Hairston et al., 2005). Interestingly, the same study found that performance on the spatial version of the task suffered as a result of sleep deprivation, whereas learning a version of the task that is not dependent on the hippocampus was not affected.

More direct evidence for a link between adult neurogenesis and learning comes from studies that induce hippocampal neurogenesis by training on hippocampus-dependent learning tasks as well as from those that prevent or reduce neurogenesis in the hippocampus directly and then observe the consequences of this for memory. An important study by Gould and colleagues (1999b) found 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. Subsequently, this group found that hippocampal-dependent learning enhanced the survival of newly born cells in the adult dentate gyrus long beyond the time when the hippocampus was required for learning of the task (Leuner et al., 2004). Ambrogini and colleagues (2000) have also found that training on a hippocampal-dependent learning task (spatial version of the Morris water maze) increased the survival of newly generated cells in the adult dentate gyrus. Rampon's group confirmed the benefit conferred on both memory performance and hippocampal neurogenesis by EC housing and furthermore reported that blocking adult neurogenesis (using the antimitotic agent methylazoxymethanol acetate) abolished the EC-induced improvement in hippocampal-dependent memory (Bruel-Jungerman et al., 2005). Mild irradiation, which inhibits adult neurogenesis, has also been found to impair hippocampal-dependent fear conditioning and performance on a delayed nonmatch-to-sample task in which longer delays were imposed (also hippocampal dependent) (Winocur et al., 2006). Two other groups have also
found irradiation to reduce neurogenesis and to impair performance on hippocampus-dependent (but not hippocampus-independent) learning tasks (Madsen et al., 2003; Raber et al., 2004; Rola et al., 2004), further strengthening the link between hippocampal neurogenesis and hippocampus-dependent learning. Interestingly, it may be the neurons born prior to the learning experience, and not those generated by the learning experience itself, that are critical for memory performance. Irradiation disrupted performance on the spatial (hippocampal-dependent) version of the Morris water maze (but was without effect on performance of the hippocampal-independent, visible platform version of the maze) when administered 4–28 days prior to maze training but not when administered just prior to or immediately following maze training (Snyder et al., 2005). This finding is perhaps not surprising 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. Whatever the subtleties may be, what is clear is that adult hippocampal neurogenesis is an additional form of experience-dependent brain plasticity that appears to contribute to the learning process.

5. Conclusions from Environmental Complexity Studies

The complexity of the rearing environment can profoundly affect the structure of the brain. However, the changes resulting from differential rearing complexity are probably not a simple extension of those found in the visual deprivation studies. While the deprivation studies demonstrated that a drastic but simple manipulation of experience can modify connectivity and subsequent learning ability, visual experience is definitely “expected” during ontogeny. The types of visual experience of which the animals are deprived are normally quite uniform, for all species members, in their timing (i.e., after eye opening) and quality (e.g., all visual angles present). Visual deprivation at later ages, once the animals have had experience, has minimal lasting effect. On the other hand, the modification of experience in the environmental complexity research has a character that is much less “expected,” from the phylogenetic perspective. The timing and character of individual experience in the EC environment cannot be uniformly predicted for all species members, such that synaptic plasticity must remain able to capture new information from experience whenever it becomes available.

The connectivity modifications observed in the EC animals appear more related to how neural activity is processed than to how much is processed. For example, both EC and IC animals use approximately the same amount of light (average intensity on the retina) quite differently, one with self-initiated activity and its visual consequences, the other with dull routine. The importance of active involvement is evident in the finding that there is essentially no brain
effect of rearing rats within a small cage inside the EC environment (Ferchmin and Bennett, 1975). The fact that enhanced visual experience does not solely explain the effects is further highlighted by the finding that differences between blinded EC rats and blinded IC rats are comparable in magnitude to those between intact EC versus IC rats (Krech et al., 1962). Combined with the findings of widespread similarity of effects noted in the brains of EC animals and those trained in learning paradigms (discussed later), the available evidence to date indicates that EC-induced brain plasticity is related specifically to the learning process.

C. Skill Learning

1. Synapses

If the dendritic and synaptic alterations seen after EC experience are related to experience-dependent mechanisms such as learning, then we would expect to observe similar structural changes after training on traditional psychological learning tasks. Greenough et al. (1979) used the Hebb–Williams maze, which has movable barriers, allowing a large variety of problems. Adult rats received extensive training for 25–26 days on a new problem plus several old problems each day for water reward. Littermate control rats were allowed to drink water several times daily while held by the investigator. Trained animals had more dendrite branches along distal apical dendrites of Layers IV and V pyramidal cells in occipital cortex. Similarly, Bennett, Rosenzweig, Morimoto, and Hebert (1979) exposed rats to complex mazes in their cages, which were changed daily for 30 days, while their littermates were kept in IC. The maze-reared animals had heavier visual cortices. The complexity of the environment is a factor in the effect, since rats housed with a single, simple maze for 30 days had brain weights between those of EC and IC rats. Another group similarly found that extensive Hebb–Williams maze training of 500-day-old rats otherwise kept in IC increased forebrain weight and cortical area relative to baseline groups remaining in the IC cages (Cummins et al., 1973). However, littermates from an EC condition showed no effect of maze training, suggesting that additional effects were small or were obscured by the neural effects of EC exposure.

To further examine the specificity of training effects, Chang and Greenough (1982) studied monocular maze training effects on visual cortex of split-brain rats. Since about 90% of visual afferents in the rat cross to the contralateral cortex, use of an opaque contact lens over one eye of a split-brain rat can effectively isolate one occipital area from the other. Split-brain littermate triplets were assigned to one of four groups: (1) the left and right eyes were occluded on alternate days during successive training periods, (2) the same eye
was occluded during all training periods, and (3) alternating or (4) unilateral occlusion of the eyes with no training at all. The occluders were worn for about four hours daily during the training period. There was no effect of unilateral versus alternating occluder position in the nontrained rats (group 3 versus group 4), indicating that occluder insertion alone did not affect the brain measures. The apical dendrites of cells in trained visual cortex were more extensive both within the fixed occluder rats (group 2, comparing adjacent hemispheres) and between the alternating occluder and nontrained rats (group 1 versus groups 3 and 4, comparing two trained hemispheres to two nontrained). This indicates that the effects of training are relatively restricted to the side of the brain most involved in learning the task. Thus the effects appear to be related to where memory for the task may be stored rather than to general metabolic or other activity.

A similar interpretation arises from experiments in which rats are trained to reach food pellets through a thin slot in their cage using one or both forepaws. Dendritic changes occurred in several neuronal populations in the sensory motor cortex region that governs forelimb activity (Greenough et al., 1985; Withers and Greenough, 1989). Using this skilled reaching task, Kleim and colleagues have continued to study the structural and functional correlates of motor learning. They found that, in response to motor skill learning, the area of motor cortex controlling forelimb movements expands and that in this region but not adjacent regions, the number of synapses per neuron increases (Kleim et al., 1998, 2002). Upon further testing they found that the addition of synapses precedes expansion of these motor maps and that both occur during the late phase of training (Kleim et al., 2004), prompting the authors to speculate that synaptogenesis and its functional correlates may play a role in the consolidation of motor learning. Some of the changes mentioned in the foregoing studies were lateralized with respect to the particular forelimb that had been trained, while others were more general, occurring on both sides of the brain, even in unilaterally trained animals. There are at least two possible interpretations of this result: (1) Some changes (bilateral) could be due to general activation of the tissue by the training experience (e.g., motor activity, sensory input), while others (unilateral) reflect changes associated with memory storage. (2) All of the changes reflect memory-associated brain reorganization, which must occur on both sides of the brain for the task to be learned.

In a series of studies designed to tease apart morphological changes associated with learning from those associated with general physical 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 rats trained on the acrobat course had more synapses per neuron (Black et al., 1990; Kleim et al., 1996) (Figure 2-9), more perforated synapses (Jones, 1999), and more multiple synapse boutons (MSBs; see earlier)
Exercise/Learning Condition

FIGURE 2-9 Data from cerebellar cortex indicate that new patterns of neural activity associated with motor learning, rather than the stereotyped patterns associated with repetitive exercise, affect synaptic connectivity. The paramedian lobule of cerebellar cortex was examined in four groups of adult rats: acrobatic conditioning (AC), which were trained to traverse a complicated elevated obstacle course that became progressively more difficult over the 30-day training period; voluntary exercise (VX), which had free access to a running wheel; forced exercise (FX), which were subjected to a treadmill exercise routine; and individual condition (IC), which were kept in standard cages without opportunity for additional exercise. In terms of distance traveled, the AC group covered much less than the two exercise groups; whereas in terms of opportunity for learning, the AC condition offered new skills to be acquired each day while the exercise conditions quickly became dull routine. Relative to the other three groups, the AC group had a lower density of Purkinje somata. Since the density of molecular layer synapses did not differ, the number of synapses per Purkinje neuron was substantially higher in the learning group than in the others. [From Black et al. (1990).]

(Federmeier et al., 2002) in regions of the brain involved in the control of fine motor movements, such as the motor cortex and the cerebellum, as compared to animals that exercised without the opportunity for learning and as compared to animals that were sedentary during the course of the experiment. The learning-induced changes in synapse number have been found to persist for at least four weeks after training has finished (Figure 2-10A) (Kleim et al., 1997). Motor skill learning (and not exercise) also increases the number of parallel fiber synapses and climbing fiber synapses per unit of cerebellar Purkinje cell reference volume (Anderson et al., 1996). Purkinje cell morphology is also altered by classical eye-blink conditioning (Anderson et al., 1999). Olfactory learning has also been found to increase spine density along apical dendrites of pyramidal neurons in the piriform (olfactory) cortex (Knafo et al., 2001). Finally, still others have found that associative memory formation induces synaptogenesis and the formation of multiple synapse boutons in the
FIGURE 2-10  The increase in synapses per neuron in the motor cortex 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 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, or Delay). [Modified from Kleim et al. (1997b) and Kleim et al. (in press) with permission.]
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hippocampus, a region with a known major role in this type of memory (O’Malley et al., 2000; Geinisman et al., 2001; Leuner et al., 2003). Thus it appears that learning, and not merely a general enhancement of brain activity, is required to induce synaptogenesis.

2. Glia and Cerebrovasculature

Astrocytic hypertrophy has also been found to accompany skill learning (but not physical exercise in the absence of learning). And because astrocytic and synaptic changes in the cerebellar cortex are correlated on an animal-by-animal basis, increased astrocytic volume can be inferred to arise in association with learning-specific synaptogenesis and not merely to constitute a response to a general increase in neural activity (Anderson et al., 1994). There is some evidence that learning-induced changes in astrocytes may be transient. When animals were first trained on a motor skill learning task for 10 days and then left idle for the following 28 days, synaptogenesis that had occurred during learning remained clearly evident in these “delay” animals, whereas training-induced effects on astrocytes were reduced and no longer statistically significant (Figure 2-10A and B) (Kleim et al., 1997; Kleim et al., in press). Therefore it is tempting to speculate that astrocytic changes may be necessary to induce and enhance, but not to maintain, adaptive changes in the brain’s “wiring diagram” in response to experience. Learning-induced responses of oligodendrocytes and/or the myelination process have not been examined. Motor skill learning induces angiogenesis. However, blood vessel changes are also induced by physical exercise in the absence of learning (Black et al., 1990), further indicating that changes in cerebrovasculature are driven more by the repeated performance of movements rather than by the learning process itself.

D. Experience-Expectant and Experience-Dependent Plasticity in the Human Brain

The first demonstration of developmental overproduction and subsequent pruning of synaptic connections in a primate was accomplished by Boothe et al. (1979), who showed that synaptogenesis early in monkey visual cortical development was followed several months later by a reduction to adult levels of connectivity. Although technically more difficult to accomplish in human subjects, influential early work conducted by Huttenlocher (Huttenlocher, 1979; Huttenlocher and Dabholkar, 1997) suggested that a similar pattern of synaptogenesis followed by synaptic pruning occurs during human development. Potentially reflective of this underlying cellular pattern, volumetric increases in cortical gray matter followed by postpubertal decreases to adult
values are observed in pediatric neuroimaging studies (e.g., Giedd et al., 1999). Although scientists cannot ethically conduct experiments aimed at manipulating the experience-expectant and experience-dependent processes that undoubtedly are at work here, there are some studies that nevertheless strongly suggest that the human brain responds with similar plasticity to early sensory deprivation, manipulations of environmental complexity, and skill learning.

Critical periods in human development have been characterized. For example, children who have amblyopia due to a misalignment of one eye will permanently have impaired vision if surgical correction does not occur prior to the age of 7; surgical correction of the eye's alignment is without effect on the brain later in development, so vision remains impaired (Daw, 1998). In congenitally blind individuals, the occipital cortex reorganizes in the absence of visual information and, far from being functionally silent, appears to actually respond to nonvisual information (Theoret et al., 2004). In fact, such reorganization in response to visual deprivation may underlie the superior auditory and tactile processing of blind individuals (Van Boven et al., 2000; Doucet et al., 2005). Although some changes result if blindness occurs during adulthood, they are far less than what is observed in the congenitally blind (Sathian, 2005). A similar phenomenon has been observed for those born congenitally deaf—in order to maximally benefit from a cochlear implant, in terms of both nonlinguistic sounds and the development of normal speech, this intervention must occur at a very young age (Harrison et al., 2005). In contrast, individuals who have developed normal hearing and speech prior to deafness can benefit from cochlear implantation technology even in adulthood (Harrison et al., 2005). The development of phoneme distinction is another example of an early window of plasticity: Infants initially respond to a large number of distinct phonemes, but within the first year of life their responsiveness to phonemes present in their native language is enhanced, whereas their responsiveness to phonemes not present in their native language is reduced. This effect was first observed behaviorally (Kuhl et al., 1992) and has since been observed in event-related potentials recorded from the brain (Cheour et al., 1998). Such findings indicate that, similar to the animal models discussed earlier, the human brain has critical periods of maximal responsiveness to visual, auditory, and linguistic experience.

Studies of Romanian orphans have made it clear that the human brain is terribly sensitive to deficiencies in environmental complexity. Due to social policies and economic problems in Romania in the 1980s, these children were subjected to early, global, and serious environmental deprivation. Neuropsychological assessment of these children revealed that they suffer from remarkable deficits in cognitive and social functioning (Kaler and Freeman, 1994). Furthermore, a neuroimaging study revealed that regional cerebral blood flow in these children was greatly below that of normal children's in areas crucial for learning and memory, including the prefrontal cortex, the amygdala, and
the hippocampus (Chugani et al., 2001). These studies are reminiscent of the impaired mnemonic abilities of IC animals in comparison to EC-raised animals. Fortunately, just as in rodent models, childhood enrichment programs are able to produce a long-lasting increase in IQ, especially among children from disadvantaged backgrounds (Campbell et al., 2001).

Experience-dependent remodeling of the brain has also been shown to occur in primates. For instance, training a monkey to use a specific fingertip increases the area devoted to representation of that finger in the somatosensory cortex (Recanzone et al., 1992). Interestingly, the same phenomenon of altered cortical representation occurs for the fingers used by Braille readers (Pascual-Leone and Torres, 1993) and for the left hand of string musicians (Pantev et al., 2003). Such experience-dependent brain reorganization occurs in other sensory modalities; for example, enlarged cortical representation of tones of the musical scale (as compared to pure tones) were found in skilled musicians. Furthermore, the degree of enlargement is correlated with the age at which musicians began to practice (reviewed in Pantev et al., 2003). Very recently it was found that the structure of white matter (measured using diffusion tensor imaging and believed to correlate with degree of myelination) in the brain of adult pianists is correlated with the amount of time spent practicing or playing the piano during adolescence (Bengtsson et al., 2005). Studies such as these indicate that both neuronal and glial components of the human brain can reorganize in an experience-dependent fashion. These studies validate the continued use of animal models of experience-dependent plasticity.

V. IMPLICATIONS FOR THE NEUROBIOLOGICAL STUDY OF MEMORY

The evidence is strong for learning-induced changes in the number of synapses, glial cell morphology, and neuron–glia communication in many brain regions of both young and mature animals. The fact that such effects (1) are seen across a number of species, including nonmammalian (e.g., Coss et al., 1980; Patel and Stewart, 1988; Withers et al., 1995), and across types of experience, from light deprivation to maze learning; and (2) are correlated with changes in neuronal function and behavior suggests that evolution may not have established one simple mechanism for developmental plasticity and another for memory. Rather, there may be a set of cellular mechanisms on which the organism can draw for the incorporation of information from a wide variety of experiences, and it seems likely that we have not discovered all of the cellular mechanisms involved in encoding experience. Some mechanisms may not even be structurally detectable, at least with currently available techniques. At this point, however, there are consistently reported effects of various experiences on the number, and presumably the pattern, of synaptic connections,
changes in glial cells and cerebrovasculature, and alterations in the number of new neurons and glial cells added to the adult brain. The consistency of these effects across visual deprivation, environmental complexity, and training paradigms may lead one to question whether our distinction between experience-expectant and experience-dependent neural plasticity is biologically meaningful, despite differences in the timing of susceptibility, response pattern across brain regions, and relative magnitude of effects. We argue here that the distinction is meaningful and that different neural mechanisms underlie these two forms of neural plasticity.

Specifically, we suggest that species survival may be facilitated by information storage processes anticipating an experience with identical timing and features for all juvenile members. A structural correlate of "expectation" may be a temporary overproduction of synapses during the sensitive period, with a subsequent pruning back of inappropriate synapses. The neuromodulatory event that triggers this synapse overproduction may be under maturational control or may be activity dependent (as after eye opening), but it is diffuse and pervasive. The expected experience produces patterned activity of neurons, effectively targeting which synapses will be selected. For example, Cragg (1975) reported that the number of synapses per neuron in cat visual cortex reached a peak at about 5 weeks of age and then fell to lower levels in adulthood. Similarly, Boothe and colleagues (1979) reported that spine frequency on some types of monkey visual cortex neurons reached an early peak and later declined to adult values. The peak values are reached, in both cases, at about the time that sensitivity to gross manipulations of visual experience, such as monocular pattern deprivation, is also maximal. In both species, afferent axonal terminal fields overlap during early development, segregating through the elimination of overlapping synapses as development progresses (e.g., LeVay et al., 1980); and in both species, occlusion of one eye causes more of its connections to be lost and more of the open eye's connections to be preserved (LeVay et al., 1980; Tieman, 1984). Similar phenomena are seen in other developing sensory projection systems, and entire dendritic or axonal branches regress in some cases (Falls and Gobel, 1979; Feng and Rogowski, 1980; Mariani and Changeux, 1981a, 1981b; Greenough and Chang, 1988). This overproduction of synapses followed by pruning appears to occur during normal development of the human cortex as well (Huttenlocher, 1979). It thus appears that the nervous system may become ready for expected experience by overproducing connections on a sensory-systemwide basis, such that experience-related neural activity can select a functionally appropriate subset of them. If not confirmed or stabilized, these synapses regress according to a developmental schedule and/or due to competition from confirmed synapses. Even when an aggregate overproduction is not observed (e.g., Valverde, 1971), it is quite possible that the process is being masked by the concomitant generation of some synapses and the loss of others in approximately equal magnitude.
The neural basis of experience-expectant information storage thus appears to be (1) the overproduction of potentially permanent synaptic connections paired with (2) the selective survival of connections deemed valuable by use during a critical window in development.

In contrast, for experience-dependent neural information storage, which includes traditional forms of learning, neither the timing nor the specific nature of the information can be anticipated by the nervous system, and there is little evidence for systemwide overproduction in these cases [although low-level systemwide turnover cannot be ruled out (Greenough and Chang, 1988)]. Because the phylogenetic adaptations of neural plasticity cannot anticipate the timing or specific features of such idiosyncratic experience, synapses are generated locally, on demand. Metabolically, the most efficient way to generate synapses locally within the system would be for activity to trigger local synaptogenesis, from which activity-dependent stabilization might further select an appropriate subset. Thus new synapses would be formed only when they were needed for incorporation of new information. A wonderful illustration of the specificity of experience-dependent synaptic plasticity was provided by Knott and colleagues (2002), who showed that, in adult mice, a single 24-hour period of whisker stimulation was sufficient to increase synaptic density by 36% in the barrel corresponding to the stimulated whisker, whereas there was no change in synapse density in a neighboring barrel corresponding to an unstimulated whisker. We suggest that active participation by the animal is necessary for it to obtain the necessary coherent relationship between responses and their consequences. This experience-dependent localized shaping of connectivity suggests that very general experience (as in EC) would produce a widespread increase in synaptic frequency but that relatively specific experience (as in training tasks) would produce more localized increases. In describing information storage mechanisms, we have tried to break down some of the distinctions between maturation, experience-sensitive development, and learning. Some aspects of experience (such as juvenile EC/IC rearing) may influence both experience-expectant and experience-dependent processes. In fact, these processes probably cannot be entirely isolated, since they have substantial interactive consequences for how the brain processes information and they share mechanisms at the cellular level. Thus we propose that learning results in the formation of new synapses as well as new neurons (in the dentate gyurs) that are involved in the permanent encoding of the memory as alterations in the circuitry of the brain systems in which the memory is stored.

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REFERENCES


Ferchmin PA, and Bennett EL (1975) Direct contact with enriched environment is required to alter cerebral weights in rats. *J Comp Physiol Psychol* 88:360–367.


Geinisman Y, Morrell F, and de Toledo-Morrell L (1990) Increase in the relative proportion of perforated axospinous synapses following hippocampal kindling is specific for the synaptic field of stimulated axons. Brain Res 507:325–331.


Mosser MB, Trommald M, and Andersen P (1994) An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. *Proc Natl Acad Sci USA* 91:12673–12675.


