Ontogenesis of Cortical Connectivity

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AN UNDERSTANDING of the neuronal mechanisms underlying visual perception has played a major role in shaping our understanding of how the brain extracts information concerning the world. In turn, insight into the development of this process is enormously conditioned by existing theories of brain function.

Over the last century, anatomical and physiological studies have provided a description of the point-to-point connectivity of the visual system where neighboring relations in the retina are conserved in their central projections. Hence, the primary visual cortex has been shown to contain a retinotopic map of the visual world in which each point is represented by neurons with specialized receptive fields that encode basic visual features. Initially, the primary visual cortex was thought to function as the cortical retina before relaying its activity to additional areas in the surrounding association cortex. It was in the association cortex that the important but highly mysterious business of seeing was thought to take place. Increasingly, the system came to be construed as being hierarchically organized, and successive levels were thought to subserve progressively higher functions. Essentially, the visual system was seen to be a highly passive system, whereby information in the retinal image was extracted in central structures, as reflected in the changing receptive field organization at successive levels (Hubel, 1995). The understanding of corticogenesis, which evolved in parallel, was that the early development of the cortex was dictated by the peripheral sense organ (Van der Loos and Woolsey, 1973), whereas later stages were dictated by the sensory experience of the animal (Blakemore and Cooper, 1970).

Recently, our understanding of the neuronal mechanisms underlying vision has shifted from a passive role of analysis of the retinal image to one of inference and the construction of constancy. Increasingly, the visual system is seen as a dynamic one where the individual stations are involved together in a computational process aimed at determining the probabilities of feature constellations in the visual environment (Scannell and Young, 2002; Young, 2000; Young, 2001; Zeki, 1993). In some ways, this modern synthesis has much to do with the Platonic understanding of the brain, in which the sensory impressions were compared to ideas of the world. In parallel to the release of central states from the dictatorship of the sensory periphery, developmental biologists are increasingly detecting the intrinsic constraints, largely of a molecular nature, which determine early neuronal development of higher levels of the visual system (Rakic, 1988).

One can argue, therefore, that our understanding of the development of the brain being environmentally driven or, alternatively, determined by internal constraints has largely been influenced by theories of brain function. The intrinsic and extrinsic control of cortical development has been epitomized in protomap and protocortex theories (O'Leary, 1989; Rakic, 1988). Although these theories have been considered by some to be antagonistic, it is becoming increasingly clear that normal development of the cortex involves a synthesis of both intrinsic and extrinsic control (Yuste and Sur, 1999).

Early studies of cortical connectivity tended to be dominated by a view that immature connections were imprecise and that mature patterns of connections were largely the consequence of pruning of exuberant, early-formed connections (Innocenti et al., 1977). More recently, it has become clear that, although some early-formed connections undergo pruning, others exhibit precise connectional features from the onset of their formation (Dehay et al., 1988a). Distinguishing between these two possibilities is important because different contributions of each might be expected to underlie the development of very different functions. In this chapter, the authors shall attempt to give an up-to-date account of where development of the connectivity of the visual cortex is thought to be driven by activity in the periphery and where connectivity is prespecified, presumably by molecular cues that lay down the basic structure of the system.

It is becoming increasingly evident that the physiology of the visual cortex can be interpreted usefully in terms of feedforward and feedback mechanisms underlying the hierarchical organization of the cortex (Felleman and Van Essen, 1991; Lamme and Roelfsema, 2000; Shao and Burkhalter, 1996). This approach provides a conceptual framework for interpreting the feedforward input from the thalamus to the cortex in terms of the recurrent excitation provided by the intracortical and intercortical processing (Douglas et al., 1995; Shao and Burkhalter, 1996). This approach turns out to be highly innovative because instead of viewing the role and development of geniculostriate and corticocortical connectivity as separate entities, it shows that these two sets of connections, in fact, share key features. Hence, the
development of feedforward projections from the thalamus to the cortex exhibits common features with the development of feedforward corticocortical connections; moreover, there are fundamentally different developmental mechanisms operant in the feedback and feedforward pathway.

**Development of thalamocortical pathways**

When Torsten Wiesel presented his Nobel address, he pointed out that his and David Hubel's pioneering work on the effects of sensory deprivation on the development of the functional architecture of the visual cortex had been inspired by early eighteenth century speculation that congenital blindness resulted in defective visual perception, presumably as a result of malformation of the brain (Von Senden, 1960).

Earlier, Hubel and Wiesel had demonstrated that adjacent territories of area 17 receive input alternatively from the right and left eye. These so-called ocular dominance columns had been explored both by single-unit recording in the cortex, as well as by injections of anterograde tracers in the eye, followed by transsynaptic transport of the tracer to area 17 (Hubel and Wiesel, 1962, 1977). To investigate the role of the sensory periphery, they carried out similar experiments in normal kittens as well as kittens having one eye, closed throughout development. These experiments suggested that, during normal development, the ocular dominance columns emerge from an immature stage in which input from the two eyes overlaps. If one eye is closed during development, then only the experienced eye drives most neurons in the adult (Wiesel and Hubel, 1963). Transsynaptic labeling has shown that, following monocular deprivation, there is an expansion of the columns receiving input from the open eye and a reduction of the columns receiving input from the deprived eye (LeVay et al., 1980). These experiments gave rise to the concept that, during normal development, the geniculocortical afferents conveying responses to the right and left eye initially are extensive and, therefore, overlap. Because binocular deprivation allowed segregation of the two sets of inputs while segregation was prevented by blockade of activity (Stryker and Harris, 1986), it was thought that the formation of ocular dominance columns depended on competitive interactions between inputs from both eyes during a so-called critical period. These experiments emphasized the role of sensory experience in shaping neuronal connections and defined a time window during which sensory experience has a profound effect on the development of the brain.

Although experiments involving monocular deprivation clearly show that the brain is susceptible to deprivation, they fail to elucidate the role of experience during normal development. Further, they have failed to provide insight into the normal process of development at the cellular level. It was assumed for many years that the segregation of ocular dominance columns was attributable to pruning of the thalamic axonal arbor in layer 4 of the primary visual cortex. However, few experiments have actually attempted to examine this issue. When single geniculostriate axonal arbor has been examined at different developmental stages, the main event that is documented is the progressive increase in total axon length and complexity of axonal arbors (Antonini and Stryker, 1993; Friedlander and Martin, 1989). Pruning of extended branches either does not happen, or happens very rarely.

The role of visual experience in constructing ocular dominance columns was challenged by a study involving newborn monkeys. After prolonged in utero development and, therefore, in the absence of stimulation of the retina, the left and right eye inputs to the cortex were found to be well segregated into ocular dominance columns (Horton and Hocking, 1996; Rakic, 1976). This led to the suggestion that spontaneous activity, possibly originating in the periodic waves of excitation in the immature retina, drove segregation (Galli and Maffei, 1988; Meister et al., 1991; Mooney et al., 1993; Wong et al., 1993). However, a number of studies have suggested that the development of ocular dominance columns can actually proceed relatively normally in the total absence of the retina. Cytochrome oxidase–rich blobs in layers 2/3 in the monkey relate to the ocular dominance columns (Hendrickson, 1985; Hendry and Yoshioka, 1994) and have been shown to develop in the absence of the retina (Dehay et al., 1989; Kennedy et al., 1990; Kuijjs and Rakic, 1990) and to show a normal periodicity (Kennedy et al., 1990). More direct evidence for the formation of ocular dominance columns in the absence of the retina has recently been obtained from studies of neonatal enucleated ferrets in which tracer injection in the lateral geniculate nucleus (LGN) revealed alternating stripes of label in the striate cortex (Crowley and Katz, 1999). Not only did ocular dominance columns appear to develop independently of the retina, but they were shown to appear well before the critical period and the period in which geniculostriate axons had been thought to undergo refinement and retraction (Crowley and Katz, 2000). Further, geniculocortical axons formed ocular dominance columns shortly after innervation of layer 4, and at this early stage, imbalance caused by removal of one eye fails to change the periodicity of the input to the cortex (Crowley and Katz, 2000). Although these results do not exclude a role for the sensory periphery in central development, they suggest that molecular cues play a major role in the formation of ocular dominance columns.

The influence of visual experience on the development of orientation columns has a long and controversial history. Unlike ocular dominance columns, where experimental manipulations quickly establish developmental plasticity, the effects of deprivation on the development of orientation columns has been a highly disputed issue (Blakemore and Cooper, 1970; Hirsch and Spinelli, 1970; Stryker and Sherk,
The issue has recently been reexamined using optical imaging of orientation columns during development in normal and deprived animals. This work shows that, in agreement with earlier observations, orientation selectivity develops independently of visual experience, but neuronal activity is required to fine-tune and maintain the orientation map into adulthood (Crair et al., 1998; Sengpiel et al., 1999; White et al., 2001; Wiesel and Hubel, 1974). Although activity may not be necessary for the development of orientation columns, cross-modal rewiring experiments strongly suggest that visual afferents can create orientation columns in the auditory cortex (Sharma et al., 2000).

The emerging consensus on the development of the functional architecture of the cortex is that the construction of both orientation and ocular dominance columns is largely activity independent, but that the fine-tuning and maintenance of columns during the critical period depends on activity. These findings imply that early development of the columnar organization relies on molecular markers that may tag columns in the early phase of thalamic innervation of the cortex and that may share certain features with molecules known to be expressed in the embryonic cortex (Donoghue and Rakic, 1999; Flanagan and Vanderhaeghen, 1998).

**Development of corticocortical pathways**

During phylogeny, there is a progressive shifting of visual processing to more central structures of the brain. One consequence is that, in the mammal, more than 90% of the synapses in the brain occur in the cerebral cortex. Quantitative investigation of the cortex shows that 95% or more of synapses in the cortex are of cortical origin (Kennedy and Fulcher, unpublished). One consequence of these figures is that developmental processes of the corticocortical pathways could have a major impact on the development of visual function.

It is now firmly established that the connectivity of infragranular-layer neurons is determined during the last round of mitosis in the ventricular zone prior to migration to the cortical plate (Polleux et al., 1998a; Polleux et al., 2001). Hence, commitment to a corticothalamic, corticocortical, and corticospinal fate is made independently of environmental influences in the cortical plate (Arimatsu et al., 1999; Koester and O'Leary, 1993; Polleux et al., 1997). There is evidence that specification of neuronal phenotype in the supragranular layers occurs at a later stage, so one might expect the determination of corticocortical neurons to be influenced more by epigenetic factors than is the case with other cortical connections. (McConnell, 1988). For many years, the callosal pathway linking the two hemispheres was the major model for the study of the development of cortical connectivity. Because the callosal connections in the adult are largely concentrated at the areal boundaries of area 17, the demonstration that these connections are initially more widespread suggested that elimination of so-called exuberant connections is largely responsible for forming the adult connectivity pattern (Innocenti et al., 1977). Although the elimination of exuberant callosal connections has been demonstrated in a wide range of species, its generality is nevertheless questioned by the fact that this developmental phenomenon does not occur in area 17 of primates and rats (Dehay et al., 1988b; Chalupa et al., 1989; Hernit et al., 1996). This further raises the question of how important axon retraction is in the development of other types of cortical connections.

The connections of the cortex can be subdivided into two broad categories in which developmental control seems to differ. Intrinsic cortical connections (also known as intrareal, horizontal, lateral, or tangential connections) link neurons within a cortical area, whereas extrinsic (or interareal) cortical connections link neurons in different cortical areas. In numerical terms, intrinsic connections constitute the vast majority of cortical connections, whereas extrinsic connections have been demonstrated to have much sparser connectivity (Kennedy et al., 2000).

**Horizontal Intrinsic Connections**

Horizontal intrinsic connections have been implicated in the elaboration of receptive field properties of individual neurons, including mediating inhibitory and excitatory effects from outside the classical receptive field (Das and Gilbert, 1999). Long-distance horizontal connections in superficial layers originate from pyramidal and spiny stellate neurons and form periodic clusters (Gilbert and Wiesel, 1979; Martin and Whitteridge, 1984; McGuire et al., 1991; Rockland and Lund, 1982). These connections have been shown to preferentially link columns with similar orientation preferences (Gilbert and Wiesel, 1989; Ts'o et al., 1986; Welisky and Katz, 1994), and similar or dissimilar ocular dominance columns (Livingstone and Hubel, 1984; Malach et al., 1993; Yoshioka et al., 1996).

Although developmental studies agree that the clustered connectivity that is characteristic of the adult emerges from a nonclustered juvenile situation, there is some uncertainty as to what extent this involves the elimination of inappropriate connections or the addition of appropriate connections. Studies that have focused on the maximum extent of connectivity have claimed tangential extensions of 10 mm in the kitten, compared to the adult configuration of 2 to 4 mm (Luhmann et al., 1986). This contrasts with later studies showing that the maximum extent of connections does not change significantly during development (Callaway and Katz, 1990, 1991; Lubke and Albus, 1992a, 1992b). These
later studies agree that, in kittens, clusters are formed by the end of the second postnatal week, shortly after eye opening. The studies by Callaway and Katz (1990) claim that crude clusters are present at the end of the second postnatal week and are later refined. However, the idea of a prolonged period of cluster refinement has been challenged by Lubke and Albus (1992a, 1992b), who showed that adult-like clusters are already present at the end of the second week. Lubke and Albus argue that the apparent late refinement of clusters seen by Callaway and Katz could be the consequence of using relative large tracer injections. Studies of the formation of clustered horizontal connections further differ on the reported effects of visual deprivation. Whereas dark rearing appeared to delay cluster refinement in some studies (Callaway and Katz, 1991; White et al., 2001), the rapid formation of clustered tangential connections was not influenced by dark rearing in the study of Lubke and Albus (1992a, 1992b).

The consensus remains that it is the emergence of clusters, rather than the restriction of tangential extent, that characterizes the formation of horizontal connections. However, the size of the tracer injection may govern whether one observes late refinement of cluster formation and an effect of dark rearing (Lubke and Albus, 1992b). Although one cannot exclude the possibility that increasing injection size introduces an experimental artifact, there is another possibility. Using paired injections of two distinguishable tracers with known interinjection separation, it is possible to examine the topographic organization of connections of large populations of neurons in the visual cortex. Such experiments show that, during development, the divergence and convergence values of intrinsic connections in area 17 remain constant for most area-17 neurons. However, a small contingent of neurons, less than 2%, do show much higher divergence values in the immature cortex than in the adult cortex (Kennedy et al., 1994). Thus, small injections or injections of single cells tend to emphasize the connectivity of the majority of neurons and may fail to detect the refinement of a subpopulation of neurons with widespread connections. The developmental significance of this subpopulation of immature corticocortical neurons with large divergence values remains to be explored.

**Vertical Intrinsic Connections** How information flows through the cortex is determined by the specific set of connections linking the cortical layers (Gilbert, 1983; Martin and Whitteridge, 1984). Throughout development, interlaminar connections show high target specificity, and the linking of cortical layers appears not to involve elimination of inappropriate connections (Callaway and Katz, 1992; Callaway and Lieber, 1996; Dantzker and Callaway, 1998; Katz and Callaway, 1992). These results suggest that layer-specific cues enable developing axons to form appropriate connections at early stages of axonal outgrowth. The role of intrinsic influences has been examined in in vitro experiments. Immature cortical slices maintained in culture show that layer-specific connections of layers 5/6, but not 2/3, develop in the absence of extrinsic influences (Bolz et al., 1992; Butler et al., 2001; Dantzker and Callaway, 1998; Yamamoto et al., 1992).

The intrinsic cortical cues, which are used by growing axons interconnecting the cortical layers, are thought to be both molecular and activity dependent in origin. Axons from presumptive layer 6 precursors preferentially grow on membranes prepared from layer 4, the laminar targets of layer 6. This finding supports the notion that molecular surface bound cues promote axonal growth of layer 6 neurons on layer 4 (Castellani and Bolz, 1997). However, layer 6 neurons apparently require activity to contact their targets in layer 4 correctly. This has been demonstrated experimentally by maintaining cortical slice in the presence of the sodium channel blocker tetrodotoxin (TTX), which blocks spontaneous activity. In normal development, layer 6 axons grow through layer 5 and form axonal branches in layer 4. However, in the presence of TTX, layer 6 neurons form extensive branches in layer 5 (Callaway and Lieber, 1996; Dantzker and Callaway, 1998). In contrast, layer 5 neurons show normal targeting and specifically contact layers 2/3 in the absence of spontaneous activity (Butler et al., 2001).

The ability of infragranular layers to form layer-specific connections is independent of extrinsic cortical factors, and these connections, therefore, develop normally in vitro. This contrasts with the supragranular layers, which apparently fail to form layer-specific connections in vitro, suggesting that neurons in layers 2/3 depend on extrinsic factors for their appropriate development (Butler et al., 2001). Layers 2/3 are the major source of feedforward projections to higher cortical areas, and feedforward neurons in these layers receive monosynaptic input from feedback projections from these same areas (Johnson and Burkhalter, 1997). One possibility is that the feedback projections from higher areas are required for the normal development of the layer 2/3 interlaminar connectivity.

**Interareal Connectivity** In all mammals, the visual cortex is divided into multiple distinct areas that are organized into a hierarchical network (Coogan and Burkhalter, 1993; Felleman and Van Essen, 1991; Krubitzer and Huffman, 2000). Individual areas often show different cytoarchitectures, have distinct inputs and outputs, and emphasize the representation of specific attributes of the retinal image (Van Essen and Gallant, 1994). These functional specializations are reflected in receptive field properties that, in the primary visual cortex, encode simple features, such as the orientation of light bars or edges, and
that in higher areas represent increasingly more complex aspects of the visual scene (Felleman and Van Essen, 1991). The neuronal activity at different levels of the cortical hierarchy critically depends on ascending connections linking lower with higher visual cortical areas (Vanduffel et al., 1997). These all-important inputs are provided by a surprisingly small number of feedforward connections that account for only 1% to 3% of asymmetric synapses in the target region (Anderson et al., 1998; Johnson and Burkhalter, 1996). Feedforward inputs terminate principally in layer 4 in a topographically organized fashion (Maunsell and Van Essen, 1983; Price et al., 1994; Rockland and Pandya, 1979; Rockland and Virga, 1990). It is thought that these inputs contribute to the construction of progressively larger and more complex receptive fields at successive levels of the hierarchy (Felleman and Van Essen, 1991). In addition to these functions, feedforward connections channel inputs into the ventral and dorsal processing streams that are specialized for the representation of visual objects and spatial relationships (Maunsell and Newsome, 1987).

Pathways leading from higher to lower areas are provided by feedback connections. There is every indication that these connections are at least as numerous as the feedforward connections (Johnson and Burkhalter, 1996; Kennedy et al., 2000). Unlike feedforward pathways, feedback connections terminate outside of layer 4 and distribute their outputs over more extensive parts of the visuotopic map than do feedforward projecting neurons (Perkel et al., 1986). Widespread feedback connections not only provide input from noncorresponding regions of the map, but also are thought to combine signals from distinct processing streams (Rockland et al., 1994; Rockland and Virga, 1989; Zeki and Shipp, 1988).

The physiological role of feedback connections has been most effectively studied by reversible cooling of higher cortical areas and recording of the effects on neuronal responses in lower areas. Experiments in monkeys have shown that feedback inputs from both V3 and V2 to V1 facilitate responses to stimuli moving within the classical receptive field (Sandell and Schiller, 1982) and enhance suppression from outside the receptive field (Hupé et al., 1998). These effects suggest that feedback connections are involved in the discrimination of objects relative to the background. However, indirect evidence strongly suggests that feedback connections mediate top-down influence that result from attention, memory retrieval, and imagery (Chelazzi et al., 2001; Ishai and Sagiv, 1995; McAdams and Maunsell, 2000; Motter, 1994; Naya et al., 2001).

In a widely held view, perceptual abilities are, to a large extent, determined by the organization and processing capabilities of feedforward and feedback circuits interconnecting areas at different hierarchical levels. Networks of such enormous complexity must vary in their detailed synaptic organization. These differences may account for the wide range of cognitive abilities among individuals. Variability in hierarchical networks may arise from both the implementation of genetic programs and the patterns of activation during development. To determine the role of each of the factors in the development of feedforward and feedback connections, a number of studies have investigated when and how inter-areal pathways acquire a lamina specific and topographic organization, segregate into functionally distinct channels, form specific hierarchical relationships, and acquire distinct balances of excitation and inhibition.

**Inter-areal Connections of Preplate Neurons** The earliest inter-areal connections are formed before the cortical plate by neurons of the marginal zone. A major population of cells in the primordial layer 1 are Cajal-Retzius (CR) neurons that constitute a diverse group of neurons composed of derivatives of the subpial granular layer of the preplate and the neuroepithelium behind the olfactory bulb (Meyer and Wåhle, 1999). Some CR neurons send out axons that are completely confined to layer 1, extend for millimeters, and are long enough to interconnect different cortical areas (Derer and Derer, 1990; Marin-Padilla, 1984). Unlike inter-areal projections of cortical plate neurons that branch mainly at the end of the pathway, axons of CR neurons branch near the cell body, mainly issuing boutons of passage and distributing outputs over large parts of the cerebral cortex (Derer and Derer, 1990). This projection pattern indicates that connections of CR neurons are not point-to-point and are not designed to preserve the spatial relationship of sensory maps. Instead, they may provide a network for synchronizing activity of pyramidal neurons in different areas (Schwartz et al., 1998). The most fascinating feature of these connections is that they are not permanent. In fact, in the rat and monkey, they are eliminated in the first 1 to 3 weeks after birth owing to the death of CR neurons (Derer and Derer, 1990; Huntley and Jones, 1990). The reason for their demise remains unknown, but some of the consequences have been documented. One of these is that ablation of CR neurons in newborn mice arrests the migration of neurons to superficial layers of cerebral cortex (Super et al., 2000). Similar experiments in the developing hippocampus have further shown that CR neurons are necessary for the development of the lamina-specific innervation by afferents from the entorhinal cortex (Del Rio et al., 1997). In the mature cerebral cortex, layer 1 is thought to be strongly innervated by inter-areal feedback connections (Coogan and Burkhalter, 1993; Rockland and Virga, 1989). By contrast, feedforward inputs to layer 1 are sparse (Coogan and Burkhalter, 1993; Felleman and Van Essen, 1991). These distinct laminar innervation patterns emerge during development. Interestingly, feedforward connections acquire
their lamina specificity during a period in which layer 1 is occupied by CR neurons (Knutsen et al., 1997; Patel et al., 1999). However, as soon as CR neurons begin to vacate the marginal zone, feedback connections start to innervate layer 1. Whether these events are causally related is not known. However, the synchrony in which they occur suggests that CR neurons may play a gatekeeper role for the innervation of layer 1.

Support for this idea derives from experiments in cortical slices that show that high levels of a chemorepellant near the marginal zone keep pyramidal cell axons away from layer 1 (Polleux et al., 1998b). The postulated repulsion of feedforward axons may be caused by a factor that is secreted by CR neurons. At first glance, this scenario seems to contradict the growth-promoting activity attributed to hippocampal CR neurons (Del Rio et al., 1997). However, the recent demonstration that attraction and repulsion depend on the intracellular amount of cyclic nucleotides (Song et al., 1998) suggests that the different responses may be attributable to diverse concentrations of second messengers in neocortical and paleocortical projection neurons.

During late fetal or early postnatal development, subplate neurons, a different, perhaps better known, population of short-lived preplate neurons (Luskin and Shatz, 1985), form connections with subcortical structures (De Carlos and O'Leary, 1992; McConnell et al., 1989). Intracortical connections of subplate neurons also have been observed within the subplate, as well as to the cortical plate, and have been reported, in one study, to extend to the homotopic areas of the opposite hemisphere (Antonini and Shatz, 1990; Friauf et al., 1990). There is currently no evidence that subplate neurons form inter-areal connections. Thus, the postulated role of subplate neurons as pioneer neurons that provide a scaffold for the development of cortical pathways might only apply to descending pathways (De Carlos and O'Leary, 1992; McConnell et al., 1989), but some researchers dispute that such a scaffold role ever applies in descending pathways (Clasca et al., 1995).

**Feedforward Connections**

In vivo retrograde tracing experiments in the visual cortex of fetal monkeys and DI tracing studies in the fixed brains of monkeys and humans have demonstrated that feedforward connections from lower to higher cortical areas emerge prenatally during the third trimester of gestation (Barone et al., 1996; Burkhalter et al., 1993; Coogan and Burkhalter, 1988). In the cat, feedforward connections from area 17 to area 18 are well established at birth (Kennedy et al., 1994; Price and Blakemore, 1985a; Price et al., 1994), suggesting that they emerge at late fetal stages. In rodents, the earliest connections between V1 and V2 were observed at postnatal day 1 (Coogan and Burkhalter, 1988; Knutsen et al., 1997; Patel, 1994; Patel et al., 1999). Relative to the time point when neuronal migration ends (Ignacio et al., 1995; Rakic and Nowakowski, 1981; Shatz and Luskin, 1986), the onset of the formation of inter-areal connections in cats is 2 to 3 weeks earlier than in rodents and primates.

In newborn kittens, feedforward connections from area 17 to area 18 originate from superficial and deep layers (Price and Blakemore, 1985a; Price et al., 1994). As development proceeds, the number of projection neurons in upper layers increases to a peak, after which it declines to the adult level (Price et al., 1994). Quantitatively similar developmental processes have been observed in forward pathways of the monkey visual cortex. Here, feedforward projections from V1 to V4, V3 to V4, and V3A to V4 in both the adult and the fetus principally originate from supragranular layers (Barone et al., 1996; Batardière et al., 2002; Coogan and Van Essen, 1996).

In the adult cat, feedforward projecting neurons from area 17 to area 18 form distinct clusters (Gilbert and Kelly, 1975) that contain cells with similar receptive field properties (Gilbert and Wiesel, 1989). Retrograde tracing studies have shown that, at birth, projection neurons in superficial and deep layers are distributed uniformly and that the clusters emerge during the first 3 postnatal weeks (Bullier et al., 1984; Price and Blakemore, 1985b). Although these findings suggest that inappropriate connections are eliminated, the demonstration in newborn kittens that anterogradely labeled feedforward connections from area 17 form distinct clusters in deep layers of area 18 (Price and Zumbroich, 1989) clearly indicates that feedforward connections develop with a high degree of spatial precision. A complementary retrograde study has shown that this is true for the bulk of feedforward connections, and has further demonstrated that inappropriate feedforward connections in newborn kittens are the result of a small population of neurons with widespread axonal connections (Kennedy et al., 1994). Most of these connections are formed by supragranular neurons (Caric and Price, 1996). This mode of development is not a peculiarity of cat area 17 to area 18 connections. In the monkey, the earliest (embryonic day 112) feedforward projecting neurons from V2 to V4 are clustered and are in register with regions of high acetylcholinesterase activity (Barone et al., 1996). Similar clusters, albeit weaker, have been observed after white matter injections of E129 fetuses. Clusters in older animals were more distinct than in animals at an early stage of development. Together, these findings indicate that feedforward axons grow to roughly the correct location and then enter gray matter, after which axon arbors undergo minor remodeling. In the monkey, this process is largely complete at birth. Although these results strongly argue for precise target selection by feedforward axons, it remains unknown whether similar mechanisms guide the development in the vertical dimension to form connections with the correct layers.
Feedback Connections Retrograde and anterograde tracing studies have shown that cortical feedback connections emerge at about the same time as feedforward connections. In the monkey, feedback connections to V1 begin to form at E108 (Fig. 11.1; Barone et al., 1995; Coogan and Van Essen, 1996). In humans, they are present at 37 weeks of gestation (Burkhalter et al., 1993). In the cat, feedback connections to areas 17 and 18 are present at birth, indicating that they emerge at late fetal stages (Batardière et al., 1998; Dey et al., 1984; Kato et al., 1991). In mice and rats, sparse feedback connections are present at postnatal day 1 (Knutsen et al., 1997; Patel et al., 1999).

**Figure 11.1.** Schematic representation of the development of inter-areal feedforward and feedback connections in monkey visual cortex (Barone et al., 1995; Barone et al., 1996; Batardière et al., 2002). The development of the laminar and tangential distribution of axon arbors is inferred from the distribution of retrogradely labeled neurons; it has not yet been shown directly by anterograde tracing of axonal connections. The diagram illustrates a highly dichotomous strategy of the development of feedforward and feedback connections. Feedforward connections are complete early in prenatal life, and their development largely depends on directed growth and target recognition mechanisms that do not involve large-scale elimination of inappropriate axons. In contrast, the development of feedback connections requires prolonged remodeling, massive postnatal pruning of early-formed axon connections, and elaboration of branches. **AchE**, acetylcholinesterase-rich bands in area V2 (Barone et al., 1995).
In vivo retrograde tracing studies in the cat and monkey have shown that, early in development, feedback connections are formed by both supragranular and infragranular neurons (Barone et al., 1995; Batardière et al., 1998; Kato et al., 1991; Kennedy et al., 1989; Meisirel et al., 1991). For unknown reasons, DiI tracing in fixed tissue of monkey and human visual cortex has failed to reveal early feedback connections from upper layers (Burkhalter et al., 1993; Coogan and Van Essen, 1996), and similar results have been reported in the developing rat visual cortex (Coogan and Burkhalter, 1988). Recent experiments in mouse visual cortex with biotinylated dextran amine, however, have revealed feedback projecting neurons originating from upper layers (Q. Wang and A. Burkhalter, unpublished observations), suggesting that technical reasons may account for the failure to label upper-layer neurons in some earlier studies. Regardless, it is clear from experiments in the cat and monkey that many feedback connections from upper layers are transient and gradually eliminated over a prolonged period that begins at fetal stages and ends about 1 to 2 months after birth (Barone et al., 1995; Batardière et al., 1998; Batardière et al., 2002). The elimination of upper layer neurons leads to both a reduction in the overall strength of the connection and a small increase in the topographic precision (Barone et al., 1995; Batardière et al., 1996; Batardière et al., 2002). In contrast, during maturation, deep-layer connections become stronger and more widespread, presumably by delayed ingrowth of axons into the target (Barone et al., 1995). All of these insights have been obtained by retrograde tracing experiments; it will be critical to see with anterograde labeling techniques whether, and in what way, the present concepts need to be revised.

Studies in rodents show that feedback axons reach their final laminar targets several days after feedforward connection (Knutsen et al., 1997; Patel et al., 1999). This is consistent with the notion that feedback connections mature over a longer period than do feedforward connections. At this point, the most important fact is that shaping of the feedback pathway takes longer than that of the feedforward pathway and involves more extensive remodeling of early connections, which, to a significant degree, occurs after birth. Comparison of rodent and primate development shows that essentially similar processes are involved, with the first formed connections stemming from the infragranular layers (Batardière et al., 2002; Coogan and Burkhalter, 1988). This suggests that the major role of pathfinding resides in the axonal outgrowth of infragranular-layer neurons when distances to be covered between cortical areas are relatively short compared to those in later stages, particularly in the monkey. This is followed by a later overshoot of the numbers of supragranular-layer neurons projecting in feedback pathways and the subsequent massive reduction of projections from supragranular layers.

Quantitative studies of the visual cortex in adult monkeys have shown that feedforward connections have a higher percentage of supragranular projection neurons than do feedback connections, and that, in each type of pathway, the percentage of supragranular neurons is correlated with the relative hierarchical rank of a given area (Barone et al., 2000). Recently, this framework has been used to interpret results from retrograde tracing of connections of V4 in the developing monkey visual cortex (Batardière et al., 2002). These studies have revealed that the hierarchical relations of extrastriate areas are invariant during development.

Clearly, feedforward and feedback pathways develop according to different strategies. Feedforward pathways are created largely through directed growth and target recognition and do not involve large-scale axon elimination. Feedback pathways, in contrast, require substantial and prolonged refinement of early-formed connections. Most interestingly, the relative rank of areas in the cortical hierarchy is established prenatally, weeks before the interareal connections have acquired their mature laminar, compartmental, and topographic organization. This implies that the identity, in terms of connectivity, of individual areas in monkey visual cortex is already specified at the time when feedforward and feedback connections first emerge. Therefore, it seems unlikely that interareal connections play a major role in the specification of cortical areas.

Effects of Visual Deprivation The role of visual experience in the development of interareal connections has been studied mainly in the feedforward pathway. Studies of kittens raised with both eyes sutured closed—from before natural eye opening to up to 80 days postnatally—have revealed no apparent effects on clustering of neurons projecting from area 17 to 18 (Price and Blakemore, 1983a) or on the size and shape of axonal projections in area 18 (Zufferey et al., 1999). Furthermore, the retraction of early-formed connections from deep layers is independent of visual stimulation. However, in monocularly deprived kittens, the clusters of feedback projecting neurons in area 17 are ~10% larger, suggesting a minor role of binocular competition in the refinement of topographic mapping (Price et al., 1994). These findings are consistent with the notion that, at the present level of resolution, feedforward connections are largely specified prenatally and that the development and maintenance are largely independent of patterned visual stimulation. Whether similar rules apply to the development of feedback connections is an interesting question. The protracted development that in monkey continues for weeks after birth certainly offers the opportunity for epigenetic influences on these connections.

Synaptic Organization So far, the issue of connectivity at the global level has been addressed. However, the actual
response of neurons to visual stimuli is the consequence of the activation by excitatory projection neurons of local excitatory and inhibitory neurons in the projection target. Based on the cortical processing of thalamocortical inputs, it was proposed that the local excitatory and inhibitory interactions are performed by a canonical circuit that is similar throughout cortex (Douglas and Martin, 1991). The key features of the model are that cortical activation is produced by a small number of afferent synapses whose excitatory effects are opposed by weak intracortical inhibition that, in turn, controls access to large numbers of excitatory, recurrent, intracortical connections that amplify the primary response. It has been shown recently that the basic structure of the canonical circuit applies to inter-areal feedforward and connections (Johnson and Burkhalter, 1996). In rodent, cat, and monkey visual cortex, mature inter-areal feedforward and feedback connections form asymmetrical synapses on pyramidal and nonpyramidal neurons (Anderson et al., 1998; Johnson and Burkhalter, 1996; Lowenstein and Somogyi, 1991). In adult rats, about 10% of both feedforward and feedback inputs between areas V1 and V2 are sent to nonpyramidal cells that all belong to the same family of parvalbumin expressing GABAergic neurons (Gonchar and Burkhalter, 1999). The remaining 90% of connections are formed on pyramidal neurons.

Stimulation of feedforward or feedback inputs to pyramidal cells elicits monosynaptic excitatory responses followed by disynaptic inhibition (Dong et al., 1999; Shao and Burkhalter, 1996; Shao et al., 1996). Although postsynaptic excitation is similar in both pathways, inhibitory responses in the feedback pathway are smaller and less frequent than in the feedforward pathway (Dong et al., 1999; Shao and Burkhalter, 1996). One factor that might contribute to this difference is that feedback inputs terminate on thinner GABAergic dendrites than do feedforward inputs (Gonchar and Burkhalter, 1999). During development, this pathway difference emerges, after animals open their eyes 14 days postnatally, by selective elimination of feedback synapses from thick GABAergic dendrites (Yamashita et al., 2000). In parallel with these structural changes, during the third and fourth postnatal week, inhibition generated by feedback inputs becomes weaker, whereas inhibition in the feedforward circuit increases (Dong et al., 1999; Shao and Burkhalter, 1998). In mice raised with one eye closed for 36 days, this transformation of the feedback circuit is arrested, and inhibition of pyramidal neurons remains as strong as in the feedforward circuit (Dong et al., 2001). The effect is specific to the binocular region of area 17, suggesting that the development of intracortical inhibition is influenced by competitive mechanisms. The transformation of inhibition in the feedback pathway from a level that is similar to the feedforward circuit to a significantly lower amount fails to occur in BDNF-overexpressing mice (Dong et al., 2000).

This suggests that the pathway-specific balance of excitation and inhibition in inter-areal circuits is determined by the activity-dependent competition for a neurotrophic factor. Further studies are needed to determine whether, in BDNF-overexpressing mice, feedback synapses fail to retract from thick GABAergic dendrites (Yamashita et al., 2000).

Conclusion

Distinguishing between feedforward- and feedback projections in the cortex suggest that they follow different sorts of developmental control. The feedback connections appear to undergo prolonged maturation in which inappropriate connections are eliminated so that activity-mediated processes can be implemented. Feedforward projections show directed growth and target selection and are precociously formed, suggesting that molecular guidance mechanisms may be more predominantly used in these pathways. Studies suggest that similar differences in the development of feedforward and feedback pathways are to be found in the ascending pathways. The terminal arbors of retinal feedforward projection to the LGN show progressive increases in size and target appropriate territories (Kennedy et al., 2001; Snider et al., 1999). As discussed earlier, similar findings characterize the feedforward projections from the LGN to the visual cortex. Not much is known about the development of feedback projections from the cortex to the thalamus, but interestingly, in the adult, corticothalamic arbors span several layers of the LGN (Murphy and Sillito, 1996). This is in sharp contrast to the feedforward input from the retina to the LGN. These results suggest similar developmental mechanisms in feedback and feedforward projections at different levels and hint that they may share common physiological functions. In this respect, the role of feedback projections to the LGN in controlling mode of firing (Sherman, 2001) may prove to be significant for understanding the role of corticocortical feedback projections onto feedforward neurons (Johnson and Burkhalter, 1997). At the level of the cortex, the development and maintenance of feedback projections that perform the processing of retinal inputs necessary for the segmentation and interpretation of the visual scene (Lamme and Roelfsema, 2000) may depend on visual experience.

REFERENCES


